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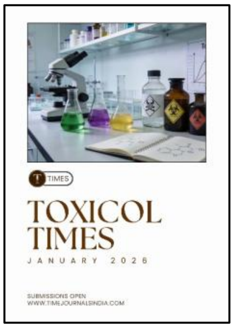

Magnetic Nanomaterial-Assisted Enzymatic Detoxification of Organophosphate Pesticides: Toxicological Relevance, Mechanisms, Risk Pathways and Future Directions

Jatinder Singh Randhawa 1*, Robin 2 and Pardeep Kaur 3

1 Department of Biotechnology, School of Applied & Life Sciences, Uttaranchal University, Dehradun, Uttarakhand-248007, India
2 Sant Singh Sukha Singh Khalsa Educational Institutions, Amritsar, Punjab-143001, India
3 P.G. Department of Biotechnology, Khalsa College, Amritsar, Punjab-143002, India

ABSTRACT

Chlorpyrifos (CPF) is a widely used organophosphate insecticide that induces toxicity primarily through acetylcholinesterase inhibition, resulting in pronounced neurotoxic effects in humans and non-target organisms. Its persistence, high lipophilicity, and extensive environmental distribution contribute to significant contamination of soil, water, and food systems. This review critically evaluates magnetic nanomaterial-assisted enzymatic detoxification as an emerging and sustainable strategy for the remediation of organophosphate pesticides. It highlights the potential of enzyme immobilization on magnetic nanoparticles as an advanced and sustainable approach to enhance enzymatic stability, catalytic efficiency, and reusability. The synthesis approaches and key physicochemical properties of magnetic nanomaterials are discussed in relation to their effectiveness as immobilization supports. Recent studies demonstrating efficient CPF degradation using immobilized enzymes, including laccase and organophosphorus hydrolase, are highlighted. Furthermore, the toxicological implications of CPF degradation products, particularly 3,5,6-trichloro-2-pyridinol are also presented. The review also identifies key challenges, including scalability, environmental safety, and long-term impacts of nanomaterials, and emphasizes future directions for the development of multifunctional nanozyme systems for effective and sustainable bioremediation.

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***Corresponding author:**
Dr. Jatinder Singh Randhawa
E-mail:
jatinderbiotech@gmail.com

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1. INTRODUCTION

Chlorpyrifos (CPF) is a widely used organophosphorus insecticide known for its broad-spectrum biological activities (Perry et al., 2020). This substance is used to treat biting and stinging pests in a variety of agricultural and horticultural crops, as well as in households. CPF exerts its insecticidal action through multiple exposure pathways, including contact, ingestion, and inhalation, enabling effective control of pests both on plant surfaces and within plant tissues (George et al., 2014). The primary mode of action of CPF involves the inhibition of acetylcholinesterase (AChE), a key enzyme that affects the neurological systems of pests. It may have a comparable effect on the human nervous system, potentially disrupting normal biochemical and physiological processes (Perry et al., 2020).

The extensive agricultural application of chlorpyrifos (CPF) contributes to its widespread distribution across multiple environmental compartments, including air, soil, surface water, and groundwater (Mackay et al., 2014). Owing to its pronounced lipophilic nature, CPF readily penetrates biological membranes, facilitating its accumulation in animal tissues, organs, milk, and other bodily fluids. Furthermore, detectable levels of CPF residues have been reported in various food commodities such as fruits, cereals, and vegetables, raising concerns regarding dietary exposure. The occurrence of CPF residues in environmental matrices (soil and water), food systems, and biological samples from animals and humans has been extensively documented. Nevertheless, ongoing research continues to focus on its environmental persistence, biodegradation pathways, and ecotoxicological impacts (Lu et al., 2020; Bernal-Rey et al., 2020). In addition to agricultural applications, it has been widely used in residences for the control of common household pests, including flies, cockroaches, and ants, thereby contributing to chronic low-level human exposure (Casida and Quistad, 2004). Prolonged exposure to CPF has been associated with a range of adverse health effects, primarily affecting the nervous, cardiovascular, and respiratory systems (Bootharaju and Pradeep, 2012). Notably, residues of CPF and its metabolites have been detected in human tissues and urine samples from both occupationally exposed individuals (e.g., agricultural workers) and the general population, indicating its pervasive nature and potential for bioaccumulation (Rezg et al., 2010; Akoto et al., 2015).

In this context, advanced remediation strategies are gaining increasing attention. Among these, enzyme immobilization using magnetic nanoparticles has emerged as a promising approach for the degradation of persistent pollutants such as CPF. This review provides a comprehensive overview of magnetic nanoparticles, including their synthesis, physicochemical properties, and suitability as matrices for enzyme immobilization. It further discusses current immobilization techniques, highlighting their advantages, limitations, and potential applications. Additionally, the review outlines future perspectives for their application in environmental bioremediation.

2. TOXICOLOGICAL PROFILE OF CHLORPYRIFOS

Despite being successful in reducing pests and thereby increasing crop yields, CPF has been demonstrated to be hazardous to human and animal health due to its toxicity, which has been connected to a number of physiological and lifestyle-related conditions, such as neurological, endocrine disruption, hematological, and reproductive abnormalities (EPA, 2000). The primary mechanism underlying CPF-induced neurotoxicity involves the obstruction of active sites of enzyme acetylcholinesterase (AChE). Following consumption, CPF is hydrolyzed to produce 3,5,6-trichloro-2-pyridinol (TCP) after undergoing oxidative desulfuration to oxon. Due to relatively short half-life in the body, its metabolites TCP, diethylthiophosphate (DETP), or diethylphosphate (DEP) serve as biomarkers for assessing CPF exposure in blood or urine, and measuring them can help determine how much CPF is absorbed in vivo (EPA, 2000). The toxicological effects of CPF are largely attributed to both the parent compound and its bioactivated oxon form, with CPF-oxon exhibiting a greater potency in inhibiting AChE activity (Shaffo et al., 2018). Studies have demonstrated that CPF exposure leads to significant inhibition of cholinesterase activity, particularly in erythrocyte cholinesterase (RBC-ChE) and plasma cholinesterase, often exceeding the degree of inhibition observed in central nervous system AChE (Kondakala et al., 2017; Shaffo et al., 2018). In addition to AChE inhibition, emerging evidence suggests that CPF-oxon can interact with multiple neuronal targets and modulate various neurobiological processes independent of cholinesterase inhibition, thereby contributing to its complex neurotoxic profile (Gao et al., 2017).

Toxicokinetic studies further indicate that CPF is efficiently absorbed following oral exposure. For instance, Timchalk et al. (2002) reported that co-administration of CPF with maize oil resulted in an absorption efficiency of up to approximately 80% in animal models. Additionally, the oral administration of CPF at a dosage of 50 mg/kg body weight resulted in peak blood levels of the CPF metabolites TCP, DETP, and DEP within three hours of treatment (Eaton et al., 2008). Moreover, Mattsson et al. (2000) demonstrated that oral exposure to CPF at a dose of 5 mg/kg body weight in pregnant rats resulted in peak blood concentrations of approximately 109 ng/g, highlighting its rapid systemic distribution.

3. ENZYME IMMOBILIZATION

Enzymes are extensively employed in diverse industrial sectors, including biofuel production, food processing, and pharmaceutical manufacturing, due to their high specificity and catalytic efficiency. However, the practical application of free enzymes is often limited by their low stability, difficulty in recovery, and lack of reusability. To overcome these challenges, enzyme immobilization on solid supports has emerged as an effective strategy. In particular, immobilization on magnetic nanoparticles (MNPs) has gained significant attention due to their ability to enhance enzyme stability, catalytic performance, and operational lifespan.

Magnetic nanoparticles are especially advantageous as immobilization matrices because of their large surface-to-volume ratio, tunable surface chemistry, and super-paramagnetic properties. These characteristics enable efficient enzyme loading, improved mass transfer, and facile separation of the biocatalyst from reaction mixtures using an external magnetic field, thereby facilitating enzyme recovery and reuse. Consequently, considerable research efforts are directed toward the synthesis and functionalization of nanomaterials tailored for specific enzymatic applications. Immobilization strategies are carefully optimized based on both enzyme characteristics and support properties to achieve maximum catalytic efficiency and stability.
Magnetic materials exhibit a wide range of chemical and physical properties depending on their composition and structural organization. Typically, materials containing elements such as iron, cobalt, nickel, or their oxides demonstrate strong magnetic behavior (Wang et al., 2018; Liu et al., 2020). Based on their magnetic response, materials can be classified as soft or hard magnets. Soft magnetic materials exhibit temporary magnetization in the presence of an external magnetic field, whereas hard magnetic materials retain permanent magnetization (Fan et al., 2020). Due to these versatile properties, magnetic materials are widely utilized in advanced technologies, including sensing devices, data storage systems, smart materials, biomedical applications, enzyme immobilization, and wastewater treatment processes. Moreover, these materials can be used at several scales, including macrometric, micrometric, and nanometric, further allowing for tailored applications (Li et al., 2019; Liu et al., 2020).

In recent years, magnetic nanomaterials have attracted considerable interest across multiple disciplines due to their unique physicochemical properties arising at the nanoscale level (Prozorov et al., 2013; Yammine et al., 2021). One of the most important features is superparamagnetism, which results from the influence of thermal fluctuations on ferromagnetic nanoparticles, allowing them to exhibit magnetization only in the presence of an external magnetic field without residual magnetism (Liu et al., 2020; Fan et al., 2020). These properties make magnetic nanomaterials highly suitable for applications requiring rapid magnetic responsiveness and easy dispersion. Additionally, they can serve as reinforcing agents to enhance the mechanical, chemical, and functional properties of composite systems (Nosrati et al., 2017). The synthesis of magnetic nanoparticles is a dynamic and evolving field, with various chemical and physical methods developed to control particle size, morphology, and surface properties according to the intended application (Cai et al., 2021; Stiufiuc and Stiufiuc, 2024). Common synthesis techniques include co-precipitation (Darwish et al., 2019), aerosol-based methods (Zhu et al., 2018), hydrothermal synthesis (Lassoued et al., 2018), oxidative precipitation, organic precursor methods (Maya et al., 2017; Khmara et al., 2019), sonochemical synthesis (Saffari et al., 2015; Almessiere et al., 2019), and sol–gel processes (Manzoor et al., 2018). The choice of synthesis route depends on the desired physicochemical characteristics and the specific application of the resulting nanomaterials.

4. MAGNETIC NANOMATERIALS

Enzymes are essential biocatalysts that play a central role in both biosynthetic and biodegradative processes, thereby supporting a wide range of biological and industrial activities. They facilitate metabolic reactions under mild physiological conditions with high specificity and efficiency, contributing significantly to cellular energy production and biochemical transformations (Wohlgemuth et al., 2018). Owing to these properties, enzymes have become integral to the bioeconomy, driving sustainable advancements across sectors such as energy production, biofuels, pharmaceuticals, biosensors, food processing, and textile industries (Ashkan et al., 2021).

Despite their advantages, the industrial application of free enzymes is often constrained by their inherent instability under non-physiological conditions, such as elevated temperatures, extreme pH, and the presence of organic solvents. Additionally, the difficulty in recovering and reusing free enzymes limits their economic feasibility. To address these challenges, enzyme immobilization has emerged as a robust strategy to enhance enzyme stability, reusability, and overall catalytic performance. Immobilization significantly improves resistance to thermal and chemical denaturation, enhances operational stability, and enables enzyme function in harsh environments (Rodríguez-Restrepo and Orrego, 2020).

Among various support materials, magnetic nanomaterials have gained considerable attention due to their unique physicochemical properties. Magnetic nanoparticles (MNPs), particularly those based on iron oxides such as magnetite, offer high surface area, tunable surface functionality, and superparamagnetic behavior. These features facilitate efficient enzyme loading, improved catalytic activity, and, importantly, easy separation and recovery of immobilized enzymes using an external magnetic field (Peng et al., 2023; Melo et al., 2023). The magnetization of such nanocarriers significantly enhances their applicability in recyclable and continuous biocatalytic systems.
Immobilized enzyme systems on magnetic supports often exhibit enhanced chemo-, regio-, and stereoselectivity, leading to higher product purity and improved reaction efficiency. Furthermore, they enable shorter and more sustainable synthetic routes by reducing the need for protection/deprotection steps and simplifying downstream purification processes, thereby improving overall atom economy (Zanuso et al., 2022). A variety of immobilization techniques have been developed to optimize enzyme-support interactions, including physical adsorption, covalent binding, cross-linking, and encapsulation. Among these, physical and chemical methods are widely preferred in industrial applications due to their cost-effectiveness, simplicity, and scalability (Parandi et al., 2023). The choice of immobilization strategy and support material is critical, as it directly influences enzyme loading capacity, activity retention, and long-term stability (Feng et al., 2023).

Nanostructured materials such as nanoparticles, nanofibers, nanotubes, and nanosheets have been extensively explored as support matrices for enzyme immobilization due to their high surface area and customizable surface properties (Mohammadi et al., 2023). In this context, nanomaterials not only serve as effective supports but also exhibit intrinsic catalytic properties, operational stability, and cost-effectiveness, making them comparable to or synergistic with enzyme systems in certain applications (Mulinari et al., 2023).

Overall, magnetic nanomaterials represent a highly promising platform for advanced enzyme immobilization, offering improved stability, reusability, and catalytic efficiency, thereby contributing significantly to the development of sustainable and efficient biotechnological processes.

5. EVIDENCE FOR CHLORPYRIFOS DEGRADATION USING IMMOBILIZED ENZYMES

S. No.	Immobilized enzyme	Material	Significant results	Reference
1.	LK. ex Fx	Sodium alginate	Immobilized enzyme exhibited its maximum activity	Xie et al. (2010)
2.	Organophosphorus hydrolase	Various immobilization strategies (membrane/support-based)	Proven ability to detoxify organophosphate insecticides in a laboratory scale	Kapoor and Rajagopal (2011)
3.	Organophosphorus hydrolase (OPH)	Immobilized on chitosan	Efficient breakdown of organophosphate insecticides; increased stability and reuse compared to free enzyme	Mulyasuryani and Prasetyawan (2015)
4.	Laccase	Immobilized on magnetic nanoparticles	Effectively degraded 99% chlorpyrifos	Das et al. (2017)
5.	Laccase	Iron magnetic nanoparticles (Fe_3O_4)	Improved catalytic efficiency and stability following immobilization; efficient chlorpyrifos breakdown	Srinivasan et al. (2020)
6.	Laccase	Immobilized on magnetic nanoparticles	Chlorpyrifos leaching was decreased in a soil column containing laccase immobilized nanoparticles	Das et al. (2020)

6. TOXICOLOGICAL INTERPRETATION OF DEGRADATION

Chlorpyrifos (CPF) and its primary metabolite, 3,5,6-trichloro-2-pyridinol (TCP), exhibit considerable environmental persistence, which can adversely affect soil microbial communities, promote bioaccumulation, and ultimately pose risks to both terrestrial and aquatic organisms within food webs. Their prolonged persistence in environmental matrices disrupts ecological balance and interferes with essential biogeochemical processes. Therefore, methods that concentrate on the on-site degradation of chlorpyrifos and are both sustainable and effective are highly anticipated.

In recent years, microbial remediation has emerged as a promising and eco-friendly approach for CPF detoxification. Various bacteria and fungi possess the enzymatic machinery required to degrade CPF into less toxic or non-toxic metabolites. These microbial processes typically involve hydrolysis and subsequent mineralization pathways, thereby reducing environmental toxicity. Although numerous studies have documented microbial-mediated degradation of CPF, current research increasingly emphasizes biotransformation pathways that yield less hazardous intermediates, minimizing secondary pollution risks. Furthermore, innovative approaches such as plant–microbe symbiotic systems (e.g., rhizoremediation) have demonstrated enhanced efficiency in CPF removal, offering synergistic benefits for sustainable environmental cleanup (Kiran et al., 2025).

The ecotoxicological effects of TCP have also been extensively investigated. For instance, Hou et al. (2022) evaluated the impact of TCP on the earthworm *Eisenia fetida* across different soil types over exposure periods of 7, 14, 28, 42, and 56 days using a biomarker-based assessment approach. The study revealed that TCP exposure induced significant oxidative stress, characterized by elevated levels of reactive oxygen species (ROS) and DNA damage in *E. fetida*. To quantify these effects, the Integrated Biomarker Response (IBR) index was calculated based on standardized enzymatic activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). The results indicated that TCP-induced oxidative stress was most pronounced in red clay soils compared to other soil types. Notably, temporal variations in toxicity were observed: after 28 days of exposure, CPF exhibited lower toxicity than TCP; however, by 56 days, CPF demonstrated greater toxicity than TCP. These findings highlight the dynamic and time-dependent nature of CPF and TCP toxicity, underscoring the importance of considering both parent compounds and their metabolites in environmental risk assessments.

7. RESEARCH GAPS AND FUTURE DIRECTIONS

The development of highly efficient and multifunctional nanozymes offers significant promise for advancing sustainable environmental remediation and monitoring technologies. However, several critical research gaps remain that must be addressed to enable their practical and safe application:

1. Development of dual-function nanozymes: Future research should focus on designing nanozymes that integrate both sensing (detection) and catalytic (degradation) functionalities within a single platform. Such dual-function systems would enable simultaneous real-time monitoring and in situ degradation of contaminants like chlorpyrifos, thereby improving process efficiency and reducing operational complexity. The rational design of surface-functionalized nanomaterials with high specificity and catalytic efficiency could lead to innovative multifunctional systems for environmental applications.
2. Evaluation of long-term environmental and health impacts: Despite their potential, the long-term fate, transport, and ecotoxicological effects of nanozymes in environmental systems remain insufficiently understood. Comprehensive risk assessments are required to evaluate their persistence, bioaccumulation potential, and interactions with biotic components, including microorganisms, plants, animals, and humans. Establishing standardized protocols for toxicity evaluation and life-cycle assessment is essential to ensure the safe and sustainable deployment of nanozyme-based technologies.
3. Scalable and cost-effective manufacturing strategies: A major challenge in the practical implementation of nanozymes is the lack of scalable and economically viable production methods. Future efforts should prioritize the development of robust, reproducible, and cost-effective synthesis techniques that maintain consistent physicochemical properties at industrial scales. Additionally, integration of these manufacturing processes with real-world applications such as wastewater treatment, agricultural systems, and food safety monitoring will be critical for their widespread adoption.

Overall, addressing these research gaps will facilitate the transition of nanozyme-based systems from laboratory-scale innovation to large-scale, real-world applications, thereby contributing to environmental sustainability and public health protection.

8. CONCLUSION

Magnetic nanoparticle-assisted enzyme immobilization represents a promising and sustainable approach for the detoxification of chlorpyrifos and related organophosphate pesticides. By improving enzyme stability, reusability, and catalytic performance, these systems overcome major limitations of free enzymes in environmental applications. Experimental evidence supports their efficiency in degrading CPF and reducing its ecological impact. However, challenges related to large-scale implementation, long-term environmental safety, and cost-effective production remain. Future research should focus on multifunctional nanozyme systems, standardized risk assessment protocols, and industrial scalability to enable real-world applications, ultimately contributing to environmental protection and public health sustainability.

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