

Remediation of Profenofos Pesticide Contamination: Mechanistic Investigations on Nano Adsorbent facilitated removal and Microbial degradation of Profenofos

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CERTIFICATE

This is to certify that the thesis entitled "**Remediation of Profenofos Pesticide Contamination: Mechanistic Investigations on Nano Adsorbent facilitated removal and Microbial degradation of Profenofos**", submitted to the Bharathidasan University, Tiruchirappalli, by **Mr. A. Arjunan**, for the award of the degree of **Doctor of Philosophy in Biotechnology**, is based on the studies carried out by him under my guidance at the Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India. This work has not been formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any University.

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DECLARATION

I, **A. Arjunan** hereby declare that the thesis, entitled "**Remediation of Profenofos Pesticide Contamination: Mechanistic Investigations on Nano Adsorbent facilitated removal and Microbial degradation of Profenofos**", submitted to the Bharathidasan University, Tiruchirappalli in partial fulfillment of the degree of **Doctor of Philosophy in Biotechnology** is a record of original and independent research work done by me during January 2012 to January 2022 under the supervision of **Dr. M. Muthuselvam**, Assistant Professor, Department of Biotechnology and it has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or other similar title to any candidate of any University.

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LIST OF ABBREVIATIONS

ACE	-	Acetylcholinesterase
AFM	-	Atomic Force Microscopy
ai/ha	-	Amount of active ingredient or acid equivalent per hectare
ANOVA	-	Analysis of variance
Ag	-	Argentum (silver)
As	-	Arsenic
Au	-	Aurum (gold)
BET	-	Brunauer, Emmett and Teller surface area analysis
BLAST	-	Basic Local Alignment Search Tool
Br	-	Bromine
CH ₃ COOH	-	Acetic Acid
CH ₂ N ₂	-	Diazomethane
C	-	Carbon
Cl	-	Chlorine
Co	-	Cobalt
Cr	-	Chromium
Cu	-	Copper
DLS	-	Dynamic Light Scattering
D-R	-	Dubinin-Radushkevich
EDX	-	Energy-Dispersive X-Ray Spectroscopy
EDTA	-	Ethylenediaminetetraacetic acid
EDDS	-	Ethylenediamine-N, N'-disuccinic acid

EPA	-	Environmental Protection Agency
FAO	-	Food and Agriculture Organization
Fe(0)	-	Zero valent iron
FeO	-	Iron (II) oxide
FeO(OH)	-	Iron oxyhydroxide
FeSO ₄ ·7H ₂ O	-	Ferrous sulfate heptahydrate
FT-IR	-	Fourier-Transform Infrared Spectroscopy
g	-	Gram
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography–Mass Spectrometry
h	-	Hour(s)
HCl	-	Hydrochloric acid
H ₂ O ₂	-	Hydrogen peroxide
HPLC	-	High Performance Liquid Chromatography
ICP-MS	-	Inductively Coupled Plasma Mass Spectrometry
INPs	-	Iron Nano Particles
JCPDS	-	Joint Committee on Powder Diffraction Standards
K ⁺	-	Potassium ion
kg	-	Kilogram
kJ	-	Kilo Joule
KMnO ₄	-	Potassium permanganate
L	-	Litre
LC-MS	-	Liquid Chromatography–Mass Spectrometry
mg	-	milli gram

mV	-	Milli volts
mg/L	-	milligrams per Liter
mL	-	Milli Litre
MSM	-	Minimal Salt Medium
N	-	Nitrogen
NaOH	-	Sodium hydroxide
Na ⁺	-	Sodium ion
Na ₂ SO ₄	-	Sodium sulfate
Ni	-	Nickel
nm	-	Nano meter
NO ₃ ⁻	-	Nitrate
O	-	Oxygen
P	-	Phosphorus
Pd	-	Palladium
PCI	-	Phenol: Chloroform: Isopropanol
PCR	-	Polymerase Chain Reaction
PDI	-	Polydispersity Index
PF	-	Profenofos
PHzpc	-	pH of Zero Point of Charge
PO ₄ ³⁻	-	Phosphate
ppm	-	Parts per million
Pt	-	Platinum
PXRD	-	Powder X-ray Diffraction
rDNA	-	Recombinant DNA

rRNA	-	Ribosomal ribonucleic Acid
SEM	-	Scanning Electron Microscope
SiO ₂	-	Silicon dioxide
SPR	-	Surface Plasmon Resonances
S	-	Sulfur
TEM	-	Transmission Electron Microscopy
TGA	-	Thermo Gravimetric Analysis
UV-visible	-	Ultraviolet–Visible Spectroscopy
WHO	-	World Health Organization
XPS	-	X-Ray Photoelectron Spectroscopy
XRD	-	X-Ray Diffraction Spectroscopy
ZnO	-	Zinc oxide

Chapter I

Introduction

1.1. Pests and Pesticides

The Food and Agriculture Organization (FAO) reports that plant pests and diseases cause up to 40% of food crop losses every year (Lopez-Sánchez *et al.* 2021). Agricultural pests are responsible for two main types of crop destruction. The first is the direct damage to the plants when they feed on the tissues, which includes a reduction of leaf surface area exploitable for photosynthesis, distortion of developing shoots, lessening in growing of plants and vitality, and wilting of the root, shoot, and branches caused by the insects' tunneling actions. Second, there is indirect injury, in which insects do no overt harm but instead spread or enable fungal, bacterial, and viral pathogens to enter. This causes devastation on agriculture and starvation of millions of people worldwide. The world's population is rapidly increasing, and it will exceed 10 billion by 2050 and it is inevitable to feed such a growing populace (Paarlberg, 2009). At this juncture, consumers and organizations are revolutionarily concerned about the global food safety crisis. As protecting agronomy from pests and weeds has been crucial, agriculturalists promoted the utility of pesticides to enhance the yield of food crops (Carvalho, 2006).

Pesticides, fertilizers, and genetically engineered crops have also become suitable options for increasing food production. Synthetic agrochemical materials, particularly pesticides were invented and practiced about 4500 years ago (Riyaz *et al.* 2022). Pesticides are chemical and biological substances employed to annihilate, counteract as well as control insect, weed pests, rodents, microbes, etc. that are affecting agricultural production. Pesticides are made up of two basic components: active and inert. The active component

executes the primary role of pest management, but inert substances, including edible oils, botanicals, spices, cellulose, and so on, are added to pesticides and play an important part in pesticide effectiveness and performance (Piwowar, 2021). Pesticides are unavoidable in modern agriculture, and their utility is rising day by day. They have been turned into a significant agricultural key to promote production, quality, and quantity of food for the world's growing population by controlling pests, preventing crop losses, vector-borne diseases, and so on (Cooper and Dobson, 2007). They are commonly used in farm areas to shield crops from numerous diseases and pests while also increasing crop yields. According to some reports, crop losses due to pests could rise by 30% in the absence of pesticides. Pesticides are mostly economically profitable and return a certain amount of money invested for their applications (Hazra and Purkait, 2019). As a result of the successful progress of these agricultural practices, especially pesticide uses, they have been further promoted and widely used in several domains like agriculture and public health and driving them widespread in the ecosystem.

Every year, such compounds are typically used in much larger quantities, approximately 2.5 million tons of pesticides, than are needed to destroy pests globally (Ecobichon, 2001). Though the green revolution has provoked a rise in food productions, due to the increasing use of agrochemicals it induces many environmental crises including soil productivity depletion, acidification, nitrate leaching, pesticide tolerance, and loss of biological diversity (Mahmood *et al.* 2016). Despite their benefits, their extensive, injudicious, and long-term utilization processes cause serious pollutions to water, air, soil, and agricultural products (Poudel *et al.* 2020). It is reckoned that just less than 0.1 % of pesticide administered reaches the target, with the balance of 99.9 % remaining in the environment, causing negative effects on human health, flora, and fauna. Further, it jeopardizes crop production by eventually being harmful to the environment, especially agriculturally beneficial microbes and posing risks to human health through food

chains (Neuwirthova *et al.* 2019). Some of the negative environmental consequences of these agrochemicals include a reduction in the population of insect pollinators, a threat to endangered species, marine animals, and the loss of bird habitat (Kohler and Triebkorn, 2013). The pollution rate of these pesticides in soil and water is exacerbated by higher doses. Since majorities of pesticides are lipid-soluble they are stored inside the bodies of animals (Riyaz *et al.* 2021). This is a key rationale for the biological enhancement of these agrochemicals as they go up the food chain. People who are regularly exposed to pesticides are more likely to have skin and eye disorders. These pesticides disrupt the nervous system, induce tumors, mimic hormones, and, in extreme cases, cause death (Rani *et al.* 2021). Every year, over a million agricultural workers experience pesticide poisoning symptoms (Rani *et al.* 2021).

1.2. Classification of Pesticides

Pesticide is a broad term that refers to a variety of insecticides, fungicides, herbicides, greenhouse pesticides, domestic disinfectants, and rodenticides that are used to both kill and protect against pests (World Health Organization, 2020). The chemical and physical properties of these pesticides differ from one another. Therefore, classifying the pesticides based on their properties and studying their specific groups is virtuous. A synthetic pesticide is a manmade compound by uses a variety of chemicals. They are divided into several classes depending on their intended use. Drum recommended three broadly accepted pesticide classifications (Yadav and Devi, 2017). These three typical pesticide classes include: (i) the pesticide's chemical composition, (ii) the pesticide's mode of entry, and (iii) the pesticide's activity and the organisms it kills. Chemical pesticides, among the various forms of pesticides, have a wide range of uses in agricultural fields. Chemical pesticides are categorized into four groups based on their source: organochlorine, organophosphate, carbamate, and pyrethroid pesticides (Figure 1) among which the first two pesticides are the most extensively used (Kaur *et al.* 2019). Organochlorine pesticides were

used for controlling the pest from the 1930s until they were banned in 1979. The metabolites of these pesticides were spotted in soil, groundwater, and also in humans. The recalcitrance, toxicity, mutagenicity, carcinogenicity, and susceptibility to bio-magnifications of chlorinated substances had posed public health issues. Organochlorine pesticides are stable compounds and persistent in the atmosphere and possess the ability to accumulate in adipose tissues (Qing Li *et al.* 2006).

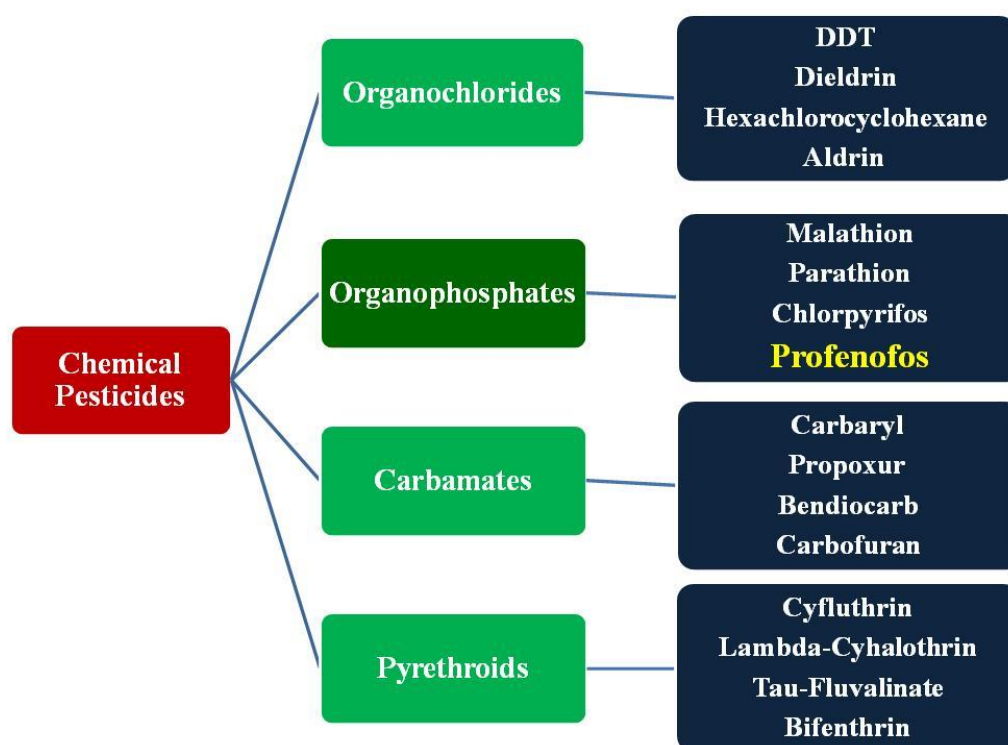


Figure 1. Categorization of chemical pesticides

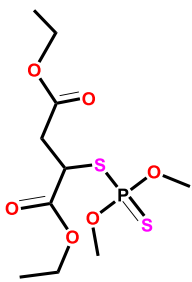
These compounds or their metabolites mostly act at the central nervous system, manipulating enzymatic nerves membranes and electrophysiological properties. It results in the alterations in the kinetics of Na^+ and K^+ ions flowing through the nerve cell membrane and potentially causing signs such as acute poisoning death and seizures from apnea in humans (Zaynab *et al.* 2021). These adverse effects directed the synthesis of alternate pesticides that comprise biodegradable organophosphorus and carbamate components (Kazemi *et al.* 2012). These organophosphorus and carbamate pesticides were developed to

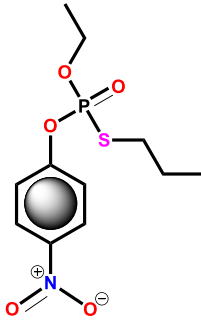
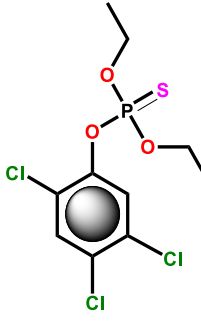
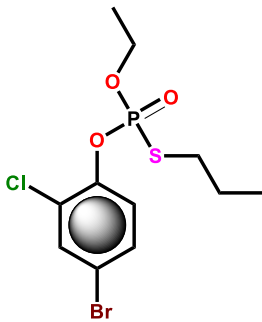
displace dangerous chlorinated pesticides, and they attained popularity across the world due to their low-cost, relatively quick biodegradation, and low environmental toxicity (Van Dyk and Pletschke, 2011).

1.3. Organophosphorus Pesticides

Owing to their wide spectrum of insecticidal efficacy, chemical durability, high performance, and cost-effectiveness, organophosphorus pesticides are the second largest class of most commonly used pesticides worldwide (Kazemi *et al.* 2012). Organophosphorus compounds are esters of phosphoric acid derivatives that serve as precursors to a variety of herbicides, insecticides, and nerve agents (Table 1). The organophosphorus insecticides scilicet profenofos ([O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate]), monocrotophos (dimethyl [(E)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate) and chlorpyrifos (O, O-diethyl O-3,5,6-trichloro 2-pyridinyl phosphorothioate) are present as residues in soil, environment, groundwater, and agricultural goods and have exceeded their maximum residue limit (MRL) as proposed by WHO in vegetables and crops. Monocrotophos is classified as class I (highly toxic) while profenofos and chlorpyrifos are classified as class II (moderately toxic) pesticides by the Environmental Protection Agency (EPA) (Barcelo, 1993).

Table 1. Properties of Organophosphorus Pesticides

Name (Chemical name)	Molecular structure	Uses	Disadvantages
Malathion (2- [(dimethoxyphosp horothioyl) sulfanyl]butanedi oate, diethyl)		It controls mosquitoes and a variety of insects that attack fruits, vegetables, shrubs, and landscaping plants. It is also be used indoors and on pets to control ticks and insects, such as fleas and ants.	It is highly toxic to non-target organisms, including humans. Long-term exposures to malathion can affect the nervous system causing a variety of side effects, including headaches, nausea, dizziness, weakness, cramps, diarrhoea, excessive

			sweating, blurred vision, and increased heart rate.
Parathion (O, O-diethyl O-(4-nitrophenyl) phosphorothioate)		It is primarily used as an insecticide on fruit, cotton, wheat, vegetables, and nut crops.	On absorption, it rapidly metabolized to paraoxon which results in headaches, convulsions, poor vision, vomiting, abdominal pain, unconsciousness, tremor, severe diarrhoea, dyspnea, and finally lung oedema as well as respiratory arrest.
Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate)		It controls Coleoptera, Diptera, Homoptera, and Lepidoptera in soil and on foliage in over a large number of crops including rice, cotton, oilseeds, vegetables, pulses, and plantation	Exposure to small amounts of chlorpyrifos can cause runny nose, tears, and increased saliva or drool. People may sweat, and develop headaches, nausea, and dizziness. More serious exposures can cause vomiting, abdominal muscle cramps, muscle twitching, tremors and weakness, and loss of coordination.
Profenofos (O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate)		Controls the tobacco budworm, cotton bollworm, armyworm, whiteflies, spider mites, plant bugs and Fleahoppers and also control lepidopteron species (the worm complex) at varying rates	Toxic small mammals, to birds, bees, fish, and aquatic invertebrates

1.4. Profenofos

Profenofos is an extremely active organophosphate pesticide that is commonly employed on various crop varieties (Kushwaha *et al.* 2016). Profenofos has phenolic and phosphate ester fragments in its molecular structure (Figure 2). It is one of the most generally used insecticides (Figure 3) due to its simple synthesis, low cost, and intense effectiveness in eliminating weed pests, insects, rodents, and microbes. Furthermore, the

particular structural motifs existing in profenofos can be linked together and quickly degraded by hydrolysis when exposed to the ecosystem, sunlight, air, and soil. It exhibits only less toxicity and a short-time environmental persistence (Talwar and Ninnekar, 2015).

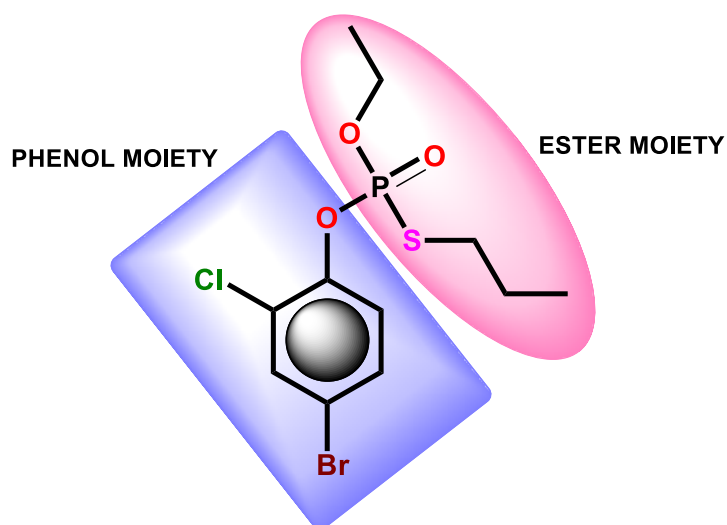


Figure 2. Chemical structure of profenofos

While profenofos has some advantages towards agricultural practices, it also has considerable drawbacks (Gotoh *et al.* 2001). Profenofos has a wide range of environmental impacts. Since profenofos is employed as an aerosolized liquid, it vaporizes and its particulate components can gradually be deposited back into the environment. Furthermore, discharges from profenofos-containing agricultural fields contaminate and intoxicate both surface and groundwater systems. As it is relatively nonvolatile from water surfaces, it persists in these reservoirs. The accumulation of these pesticide residues causes significant environmental damage due to their negative effects on the environment. As a result, people are almost undeniably subjected to profenofos residues and byproducts. An analysis, for example, found that profenofos and its metabolite, 4-Bromo-2-chlorophenol were present in vegetables, fruits, aquatic animals, and humans (Kushwaha *et al.* 2016).

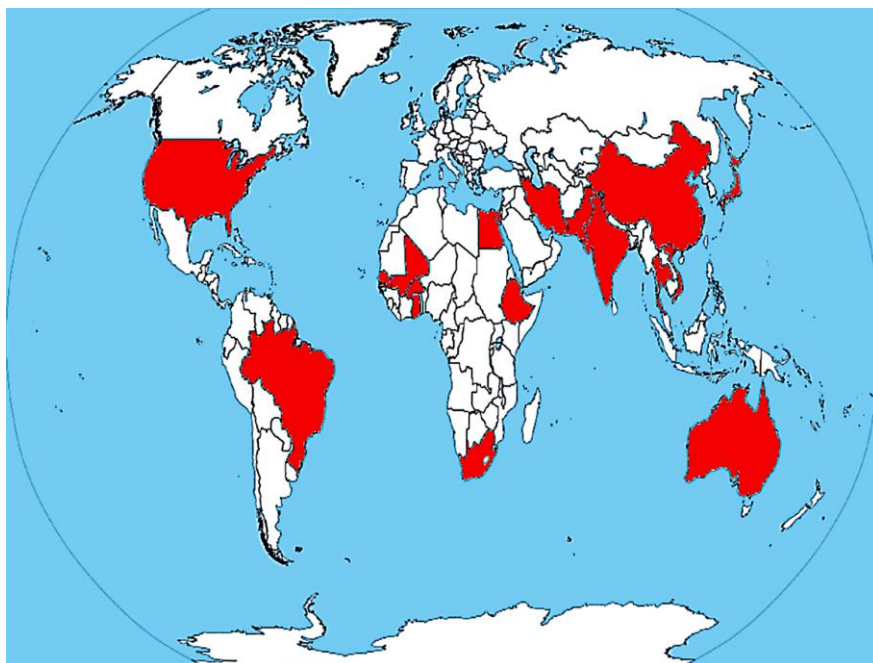


Figure 3. Profenofos use in various parts of the world (highlighted in red)

Another investigation has also proved the toxic nature of profenofos from the lymphocytes of peripheral blood samples collected from stable human donors. Further, profenofos is also very poisonous to marine animals (Rao *et al.* 2003; Maharajan *et al.* 2012) and invertebrates (Kumar *et al.* 2010; Rao *et al.* 2007). In addition, hepatocellular damage is caused by high doses of profenofos toxicity. Furthermore, high-level exposure of profenofos caused tissue haemorrhage, vacuolization, and kupffer cell hyperplasia in the liver. Besides, Bowman's capsule swelling and tubular dysfunction in the kidney were also reported (Amer *et al.* 2007). It also triggers oxidative stress which is an early diagnostic indicator of profenofos poisoning (Lin *et al.* 2003). Similarly the toxic impact of profenofos in the marine ecosystem, with a focus on morphological, behavioral, and target enzyme interactions, as well as toxicant bioaccumulation in different parts of a fish, *Oreochromis mossambicus* was also documented (Rao *et al.* 2003). Moreover, the impact of profenofos on chromosomes samples causing satellite links, chromatid defects, and gaps were also reported (Mulla *et al.* 2020).

Acetylcholinesterase (ACE) is an enzyme that hydrolyzes acetylcholine, a commonly dispersed neurotransmitter in both the central and peripheral nerves systems that is responsible for a broad range of functions including memory and motor function regulation (Nillos *et al.* 2007). Profenofos exerts its action by targeting the ACE and inhibits the enzymatic activity by phosphorylation. It results in acetylcholine neurotransmitters, accumulating in the body causing substantial impairment to the nerve system, cardiovascular system, and respiratory tract (Figure 4). Pesticides work by targeting systems or enzymes in pests that may be identical or quite equivalent to systems or enzymes in off-target species, primarily mankind, and so pose risk to human health (Omwenga *et al.* 2021). As the toxic action of profenofos is stemming from its inhibitory properties and much intoxication has been reported in farmers and field workers. The signs of intoxication are coma, dizziness, fatigue, headache, cramps, convulsions, lack of consciousness, etc. The permanent ACE inactivation leads to organ failure and subsequent death (Germany, 2012; Dawson *et al.* 2010).

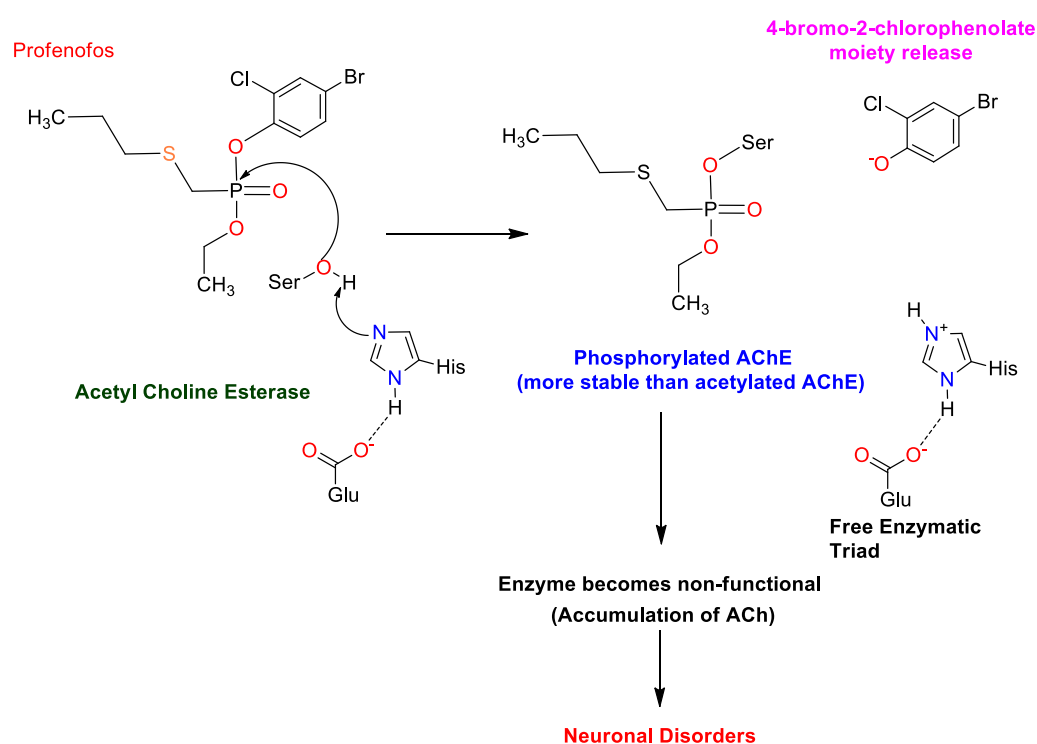


Figure 4. Plausible inhibition mechanism for acetylcholinesterase by profenofos

1.5. Environmental Distribution of Profenofos

Pesticides are widely used, resulting in their spread through the surrounding environment such as water bodies, soil area, and air, primarily in the regions near agricultural land. Parent chemicals for the synthesis of pesticides and their residues provide a significant concern that must be resolved. Meticulously screening the proportion of pesticides in the environment would assist in evaluating ambient pesticide levels for the identification of possible risks. Diverse investigations on the distribution and toxicity of organophosphates in multiple ecological sectors have been undertaken around the world. Pesticide residues in water and soil samples obtained from different cotton-growing locations in West Africa showed the presence of 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene], its toxic metabolites, endosulfan derivatives, as well as profenofos, and the contamination rate of about 80 % of the soil samples (Abaga *et al.* 2011). The dispersion of pesticides among different environments in Thailand varies depending on the season. The levels of various organophosphates, particularly profenofos were significantly higher within soil areas than in water and air environment. It was observed that the overall quantity of profenofos in the soil area was 40 mg/kg in the summer and 20 mg/kg in the winter, but the normal level of profenofos in water and air in the summer has been 1 mg /L and 0.04 mg/m³, contrasting to 0.3 mg/L and 0.002 mg/m³ in the winter correspondingly (Harnpicharnchai *et al.* 2013). An investigation carried out at *Solanum melongena* farms in Pangasinan revealed that approximately 42 % of soil samples were polluted by different pesticides including profenofos, but the amount of the pesticide present in water samples taken from the farm was negligible (Del Prado-Lu, 2015). Cotton was grown in Burkina Faso, West Africa, with the presence of cypermethrin, acetamiprid, and endosulfan, as well as profenofos. These pesticides have been measured in soil samples taken from both customarily planted and newly planted zones. Their concentrations ranged from 30 mg/kg in the traditionally planted locations to 80 mg/kg in a newly planted cotton area. The

quantity of profenofos residue has been somewhat higher (up to 0.1 mg /kg) than the number of other pesticide residues in the soil samples taken from the newly planted areas (Abaga *et al.* 2011). Pesticides can also contaminate water sources when discharged from sewage treatment plants or when untreated sewage is discharged into rivers or seas. A study of some wastewater treatment plants in Spain found that most of the pesticides are only partially eliminated after treatment, implying that these harmful substances can pollute waterways (Masia *et al.* 2015). Many of these of the most harmful pesticides are not eliminated by biological treatment methods in sewage treatment plants. Partially treated sewage dumped into rivers can damage the sea via river transit to the sea. Pesticide usage in farmlands is another source of river pollution. A study of the Seta River discovered a few pesticides, including molinate and isoprothiolane, with maximum rates of 0.5-1.1 g/L in the river's effluent (Sudo *et al.* 2002). The utilization of sewage treatment plant sludge on agricultural land may extend the pesticide contamination cycle. This is a method of reprocessing the by-products from wastewater treatment that contain pesticides and can be spread into the ecosystem or infused into crops. Chlorpyrifos was detected at a concentration of approximately 200 ng/g in sewage sludge employed during agricultural land fertilization. Pesticide fragments in water bodies, sedimentation, and muscle fibers of *Cyprinus carpio* taken from various locations on the river area at Balloki Headworks were also documented. Around 14 mg/kg of endosulfan and profenofos were the most common pesticides identified in the tissues of fish. In contrast, approximately 1.4 ng/L of profenofos and cypermethrin were discovered in river water samples. Profenofos was discovered at the greatest proportion of 23 mg/L of any pesticide identified in sediment (Mahboob *et al.* 2013).

1.6. Strategies for the Removal of Profenofos

While profenofos possesses less toxicity and low environmental persistence, overconsumption and exploitation have developed in residual issues that could endanger

human health (Prabhavathy Das *et al.* 2006). Profenofos residues have been discovered in fruits, crops, fresh and processed vegetables. As a result, metabolites of profenofos have been discovered in human blood, serum, urine, and breast milk. Because profenofos is toxic and widespread in the environment, it is crucial to eliminate its existence in the environment using highly sensitive and effective assays for decontamination, detoxification, and mineralization to secure the ecosystem and ensure social safety (Kushwaha *et al.* 2016). A wide range of traditional techniques such as incineration and open combustion, chemical-coagulation, sedimentation, filtration, disinfection, chemical precipitation, catalytic photo-degradation, ultrasound-mediated photo-Fenton treatment, oxidation, aerobic degradation, ozonation, ion exchange, membrane filtration, and electrochemical methods were used to remove profenofos from various environments each with its own set of benefits and drawbacks. Therefore, there is an increasing demand for simpler, cleaner, environmentally friendly, and commercially sustainable methods to eradicate profenofos and its traces. Researchers all over the world are actively developing newer strategies for effectively removing pesticide residues (Jatoi *et al.* 2021).

1.7. Adsorptive Removal of Profenofos

Adsorption is a surface process that occurs when adsorbate aggregates on the adsorbent's surface. It is based on the mass transfer of a certain or set of compounds at the interface during the interaction of adsorbate and adsorbent. The effect is known as physisorption when the nature of the interaction is physical and predominantly dominated by weak van der Waals forces, and chemisorption when chemical bonds predominate in the interactions (Rashid *et al.* 2021). Adsorption of a pesticide depends on several variables in the adsorptive elimination of pesticides by various adsorbents, such as adsorbent dosage, interaction time, temperature, pH of the medium, particle sizes, etc. Pesticide detoxification by different adsorbents is often influenced by adsorbent characteristics and also the chemical constitution of the adsorbate. Several strategies for pesticide removal from water were

presented in the literature (Cosgrove *et al.* 2019). Van der Waals interactions, electrostatic attractions, hydrophobic properties, hydrogen bonding, pi-pi stacking, ion exchange, and complexation may occur upon the interaction of adsorbent and the adsorbate. Some of these factors trigger the adsorptive removal of hazardous chemicals. The selected pesticide profenofos is constituted with the electronegative atoms including bromine, chlorine, oxygen, sulfur, and phosphorus atoms that may interact with the adsorbent. Furthermore, the coordinative or complexing capacity of oxygen, phosphorus, and sulfur atoms with metal-based adsorbent may be involved in its adsorption.

Exposure to toxic contaminants on the well-being of humans and other organisms can lead to the use of a variety of methods to mitigate the harmful effects of pollutants. Water contamination is one of the utmost severe environmental problems that has resulted in pollution entering freshwater sources or groundwater (Mojiri *et al.* 2020). Adsorption has significant advantages for water treatment, including cost-effectiveness, versatility, easy implementation, profitability, reliability, and high sensitivity towards the targeted harmful pollutants from aqueous solution while preventing the formation of secondary pollutants. Adsorption is becoming more popular in comparison to traditional methods. Adsorption is an efficient method for removing anionic, cationic, or organic pollutants from one phase and transporting them to the external surface via complexation or ionic exchange. Many adsorbents such as activated carbon, clays, resin, and biomass have been reported for the removal of agricultural waste and industrial excrements. Because of the reversible nature of the adsorption technique, the adsorbents can be reused multiple times by employing the proper desorption procedures. Furthermore, adsorbents utilized for pollutant removal possess the following properties. It has a low environmental impact, excellent sorption capacity, high selectivity for pollutants even at low concentrations, easy elimination of adsorbed contaminants, minimal time requirement of adsorption equilibrium attainment, and recyclability. Various Adsorbent materials such as activated carbon, zeolites, clay

minerals, industry by-products, and biomaterials were routinely used for decades to remove various contaminants from aqueous solutions. However, the adsorption effectiveness of these traditional materials is insufficient. As a result, the demand for alternative adsorbents has risen exponentially (Ponnuchamy *et al.* 2021).

1.8. Nanotechnology-mediated adsorption for the Remediation of Pesticides

Nanotechnology is a discipline that can help with the remediation of pesticides (Dhir, 2021). Nanotechnology is the engineering of atoms and molecules to create structures of nanometer-scale measurements (1-100 nm). They can be fabricated in two strategies: (i) top-down and (ii) bottom-up. The top-down method involves breaking down bulk materials into nanoscale particles. The bottom-up approach engages the accumulation of atoms of the bulk materials to construct nanoparticles. As compared to their bulk counterparts, these nanoscale particles have distinct physical, chemical, and biological properties. The size, structure, and increased surface area of nanoparticles can account for such properties. Various categories of nanomaterials, such as nanoparticles, nanotubes, and nanocomposites have resulted in extensive studies in this field using a variety of different techniques. Especially, metal nanoparticles, bimetallic nanoparticles, and metal oxide nanoparticles are the three main types of nanoparticles that have been employed for the detection, degradation, and elimination of toxic pesticide residues and attained gained global recognition. Nanoparticles of various metals, particularly precious metals such as gold (Au), silver (Ag), platinum (Pt), and palladium (Pd) have established widespread applications in environmental remedies. Besides these noble metal nanoparticles, other studies have also been conducted using other non-precious transition metal nanoparticles such as iron (Fe), copper (Cu), and zinc (Zn). The main benefits of these non-precious nanoparticles include simple synthetic protocol using relatively low-cost reagents. All these materials compact nanoscale size, complexity, large surface-to-volume ratio, lesser flocculent formation, physicochemical properties, and high target precision capacity drive highly precise pesticide

detection. Numerous nanomaterials have found use in the development of biosensors for detecting pesticides in various sample vectors (Jamkhande *et al.* 2019). In addition to detecting pesticides, the overt surface characteristics and surface area of nanoparticles perform a key role in pesticide destruction. The redox reactions that occur at the surface of nanoparticles play a significant role in the oxidation of toxic pesticides to smaller, less volatile organic compounds (Rawtani *et al.* 2018).

Nanomaterials including Au nanoparticles, Ag nanoparticles, silica nanoparticles, carbon nanotubes, and halloysite nanotubes have been employed to determine the extremely low level of pesticide residues. The accuracy and precision of these nanoscale materials for pesticide detection were enhanced by various surface changes. The nanocomposite of graphene oxide and Au nanoparticles, as well as nanoparticles including iron nanoparticles, Fe/Ni nanoparticles, titania nanoparticles, ZnO nanoparticles, and halloysite nanotubes, have demonstrated rapid decomposition of diverse pesticides present in water, with efficiencies greater than 90 % in all cases (Prasad *et al.* 2014). Pesticide degradation by various nanomaterials is accomplished through photocatalytic activity, dechlorination, and catalytic reduction. Adsorption or magnetic separation has been used to eliminate these agrochemicals from various matrices using metal oxide nanomaterials, nanocomposites, carbon nanotubes, and halloysite nanotubes. The removal efficiencies of ZnO nanomaterials and the nanotubes were about 100 %. Enzyme-based biosensors customized with various nanomaterials have also demonstrated rapid response, high selectivity, and sensitivity, as well as detection limit for several pesticides. The suppression of cholinesterases and laccase in the vicinity of pesticides is the strategy behind pesticide sensing. Nanotechnology-based materials and biosensors have several benefits over traditional approaches, including a large surface area with customizable chemistry, small size, great selectivity and sensitivity, and rapid response (Khan *et al.* 2021).

1.7.1. Iron Nanoparticles

Iron is the fourth most prevalent metal in Earth's crust and the most ubiquitous transitional metal. Extensive applications have been reported by using magnetically active nanoparticles. In water treatment, nanoscale Fe(0) and FeO particles are the most broadly adopted iron-based magnetically active nanoparticles. Because the nanoparticles display super-paramagnetic behavior, magnetic characteristics are only present when they are subjected to an external magnetic force (Zhang, 2003). The magnetic features allow for the isolation of the material following water treatment, allowing for subsequent recovery and reuse. Furthermore, iron nanoparticles are abundant in nature, inexpensive, non-toxic to the ecosystem, and have high adsorption capacity due to the productivity of functionalizing or covering the interface with other materials such as polymers, graphite oxide, and SiO₂. The effectiveness of iron nanoparticles in the sorption of organic and inorganic materials from contaminated water has been demonstrated. The pathway of pollutant elimination by iron nanoparticles will be determined by the oxidation states of iron, which include adsorption, electrochemical treatment, and reductive precipitation. Adsorption and precipitate formation also arise on the surfaces of iron oxides or hydroxides (Leonel *et al.* 2021). Hydrogen bonding, pi-pi stacking interactions, complex formation, electrostatic attraction, chemical adsorption, and ion exchange are all part of the adsorption mechanism. The interactions are caused by the surface -OH groups and surface ionization of FeO. Physisorption and chemisorption of iron oxide nanoparticles were observed for the elimination of contaminants from water and wastewater, including pilot projects (Yan *et al.* 2013).

1.7.2. Zero-Valent Iron Nanoparticles

Because of the enhanced specific surface area and the potential nanoscale effects, Fe(0) nanoparticles have been demonstrated to be more reactive than traditional iron powder

and filings. One of the most often used metals to remediate harmful contaminants from wastewater and the recovery of polluted soil and groundwater is Fe(0) nanoparticles (Pasinszki and Krebsz, 2020). It possesses several distinguishing characteristics, including an extremely small size and a distinct molecular structure. Nanoparticles contain notable qualities such as magnetic, optoelectronic, electric, and chemical features, which have promoted their widespread use in the treatment of environmental toxins. The chemical properties of Fe(0) nanoparticles are influenced by their unusual core-shell structure. The core is made of Fe(0) and is surrounded by an iron oxide/hydroxide shell, the thickness of which increases as iron oxidation progresses. The Fe(0) nucleus can be converted to Fe^{2+} and Fe^{3+} and then to FeO, resulting in a chemical reduction of contaminants. Nanoscale Fe(0) acts primarily on the deterioration of a broad range of pollutants, such as chlorinated organic pollutants and heavy metals, because it is a potent reducing agent (Tang *et al.* 2021). Fe(0) is the finest and most robust for extracting numerous elements including chromium (Cr), nickel (Ni), cobalt (Co), copper (Cu), as well as arsenic (As) from wastewater while meeting waste-treatment criteria with a 99 % removal rate (Zou *et al.* 2016). The decomposition of the dye crystal violet in sewage water was investigated using a zero-valent iron nanomaterial reinforced with kaolin. It was found that nanoparticles were the most effective at removing crystal violet by more than 97 %. Zero-valent iron nanoparticles were employed in a physical technique to remove nitrate from water (Mukherjee *et al.* 2016). Within 3 h, the iron nanoparticle with an average width of 25 nm with a high specific surface area eliminated around 95 % of the nitrate. In general, depending on the reduction potentials and extraction conditions, Fe(0) nanoparticles have the potential to treat hazardous metals, dyes, phosphates (PO_4^{3-}), and nitrates (NO_3^-) from wastewater via adsorption processes, photo-degradation, and redox precipitation (Li *et al.* 2006).

1.8. Eco-friendly Synthesis of Iron Nanoparticles

Physical and chemical methods are routinely used to produce nanoscale zero-valent iron. Each of these processes has drawbacks, including high costs, the creation of flammable and toxic gases during the process, and the harshness of the reducing agent (Huber, 2005). Rapid aggregation of nanoparticles happens due to van der Waals forces and electrostatic attraction, which reduces their surface area per unit volume in general. Organic agents or capping agents can be employed to increase inter-particle electrostatic repulsions and overcome the aggregation barrier. As a consequence of these constraints, the adoption of green technologies for the fabrication of zero-valent iron has risen dramatically (Hasany *et al.* 2012). Green synthesis, as an alternate and eco-friendly method of producing iron nanoparticles. Extracts from greener materials were utilized as a reducing agent in these processes to reduce trivalent iron to zero-valent iron. This approach has many advantages: i) harmless to living creatures ii) Use of non-toxic solvents such as water and natural products iii) polyphenols of extract serve as reducing and capping agents to reduce aggregation iv) stabilize the Fe(0) nanoparticles and increase the activity of nanoparticles; iv) The extract of these compounds as a nutrient supplement increases biodegradation (Bolade *et al.* 2020). The leaves of vine and pomegranate plants, *Crataegus pentagyna*, oak tree, zinger, tea leaves, mint, eucalyptus, mint, and hippophae have all been employed in the eco-friendly fabrication of iron nanoparticles. The findings of an investigation revealed that the produced nanoparticles differed in shape, structure, and size. Furthermore, nanoparticle analysis shows that the iron surface is entirely covered by proteins and polyphenols, which prevents nanoparticle oxidation. Furthermore, the nanoparticles are well disseminated in the medium and there is no nanoparticle aggregation (Mondal *et al.* 2020). According to one study, the structure and shape of green-synthesized nanoparticles differed from those of chemically synthesized nanoparticles. These nanoparticles demonstrated a significant

efficiency in terms of removal rate and kinetics. According to the research, each of the green nanoparticles generated from different plant extracts varies in size and shape and hence has a distinct ability to remove contaminants from landscapes and water bodies (Bolade *et al.* 2020). Furthermore, surface sorption and reductive precipitation of pollutants, primarily inorganic elements, may occur via the outer layer of iron oxides or hydroxides. Due to the formation of oxide or hydroxide layers, the efficiency of iron nanoparticles sometimes decreased (Biftu *et al.* 2020). It can be avoided by the formation of bimetallic nanoparticles upon the addition of a second metal (Sharma *et al.* 2019). As a result, there is a growing demand for the development of safe, convenient, environmentally beneficial, and economically viable biological pesticide remediation approaches such as microbial degradation, biomineralization, enzymatic degradation, bioaccumulation, phytoremediation, and biotechnological solutions. Microbes-mediated disintegration has proven to be the most potent technique of bioremediation (Adams *et al.* 2015).

1.9. Bioremediation – Microbial Degradation of Profenofos

Among these remediation strategies, the degradation of profenofos has also emerged as a key protocol of the scientific community. Pesticides may be degraded using physical, chemical, or biological approaches, with biological methods receiving intensive attention (Andreu and Pico, 2004). Bioremediation has the potential to be an efficient and low-cost method of cleaning damaged ecosystems and destroying nerve toxins. Bioremediation is a technique that uses microbes and plants as natural mediators to detoxify hazardous organic and inorganic substances into less dangerous smaller molecules. It is an effective, eco-friendly, and cost-effective tool for decontaminating polluted environments as well as an innovative technology for dealing with a variety of persistent contaminants (Bose *et al.* 2021). Microorganisms are employed for the degradation or elimination of organophosphate

pollutants in laboratory settings. The degradation speed is faster than chemical-induced hydrolysis, which is about 10 times quicker than photo-decomposition (Kumar *et al.* 2011). Organophosphates are mainly degraded by microorganisms by consuming compounds as carbon (C), phosphorus (P), nitrogen (N), or as sources of energy. *Flavobacterium sp.* was found as the first bacteria capable of degrading organophosphate pollutants in 1973 (Kumar *et al.* 2018). Likewise, *P. aeruginosa* extracted from profenofos-polluted soil area in China, was observed to degrade profenofos up to 87 % in liquid media within 48 hours. In another analysis, different strains of *Bacillus subtilis* strains extracted from grape origins were found to degrade 90 % of profenofos in the vicinity of other carbon sources (Parte *et al.* 2017). Since then, various bacterial and fungal species that can decompose a wide spectrum of organophosphate pesticides in liquid cultures, as well as soil environments, have been established (Singh and Walker, 2006). Organophosphorus insecticides may be rapidly decontaminated by hydrolysis when exposed to the environment, which is significantly affected by abiotic and/or biotic processes (Mahajan *et al.* 2022). Bacterial cultures extracted from numerous locations are key environmental entities with the unique ability to break down various organophosphorus insecticides for their growth (Verma *et al.* 2014). The biology of organophosphorus compound breakdown by most bacteria appears to be the same, with the first step catalyzed by a structurally related enzyme known as organophosphate hydrolase or phosphotriesterase (Sarker *et al.* 2021). The organophosphate hydrolase encoding gene was already extracted from regionally and taxonomically diverse organisms (Abdullah *et al.* 2016).

Pesticide production is increasing and their use is becoming unavoidable as a result of urbanization and the world's rapidly rising population. Their prevalent, unregulated, and improper use, on the other hand, has resulted in serious challenges to both habitats and living

things (Edwards, 2013). Although organophosphorus pesticides have shorter environmental durability, their metabolites are found in water bodies, soil areas, vegetables, fruits, and other food items all over the world. Aside from that, significant amounts of their metabolites were discovered in human fluids such as blood, breast milk, and urine. They often play the main role in the annihilation of off-target species and the depletion of biodiversity (Tudi *et al.* 2021). Hence, the risk posed by pesticide residues prompted people to consider the safe destruction of pesticides in an environmentally benign and cost-effective method, which resulted in the advancement of new protocols with safer and more low-cost approaches than traditional treatment approaches without environmental damage (Marican and Duran-Lara, 2018). Adsorption techniques employing iron nanoparticles are very effective, simple to operate, and may be used to remove organic and inorganic toxins. It appears extremely likely that these kinds of adsorbents will find widespread commercial use in wastewater remediation shortly (Ahmed *et al.* 2021). Further, pesticide-contaminated wastes and areas have been treated using biological processes. Several experiments have shown that microbial degradation of xenobiotic compounds is beneficial. The bioremediation of toxic pesticides provides a primary, environmentally compassionate as well as an efficient remedy for the clearance of excessive pesticides for farm soil management, water pollution, or contaminated ecosystems. All over the world, a broad range of microorganisms has been extracted and recognized with the potential of degrading or eliminating organophosphorus pesticides from various fields by using them as C, N, or P or as sources of energy (Kumari *et al.* 2022). Nevertheless, additional research is compulsory for dynamic field applications to diminish their aggregation possibilities and associated health problems (Jiang *et al.* 2021).

Motivated by the opportunities and challenges, we have described in this thesis, the eco-friendly fabrication and complete characterization of iron nanoparticles. The use of

fabricated iron nanoparticles as adsorbent for profenofos pesticide removal was demonstrated via the isotherm and kinetic studies and evaluated the maximum adsorption capacity of the adsorbent. The plausible mechanism behind the efficient removal of profenofos by the adsorbent iron nanoparticles was proposed. Further, the isolation and identification of a bacterial culture from profenofos contaminated soil was carried out. The bioremediation characteristics of the isolated indigenous bacterium were examined. The metabolites derived from profenofos degradation were identified by using GC-MS and a probable mechanism has been proposed. Furthermore, the ability of the isolated bacterial strain towards the growth of the *Vigna mungo* plant was evaluated.

Aim and Objectives

Aim

Remediation of profenofos contaminated soil and aquatic systems by nano-adsorbent facilitated removal and microbial degradation of profenofos

Objectives

- ❑ Green synthesis and characterization of iron nanoparticles from tea waste
- ❑ Green synthesized iron nanoparticles as an efficient adsorbent towards the removal of profenofos pesticide from the aquatic environment
- ❑ Isolation, screening, and identification of potential profenofos degrading bacteria from pesticide-contaminated agricultural soil
- ❑ Effect of *Bacillus subtilis* on profenofos degradation and plant growth

Chapter II

Review of Literature

2.1. Introduction

Pesticides are one of the crucial components of today's modern agriculture. The use of pesticides on a routine basis is widespread in contemporary agriculture to meet the rising need for yield and food. Pesticides are used in millions of tons per year all over the world. Pesticides' genotoxicity, mutagenicity, and impact on ecosystems are major global concerns. When a genotoxic substance has bio-accumulative qualities and enters the ecosystem's food chain, it becomes much more deadly. Among all pesticides currently in use, organophosphorus insecticides are the most commonly utilized across the globe. Organophosphorus pesticides are the most common kind, accounting for 38 % of the global market. Because of their broad-spectrum insecticidal effectiveness, chemical stability, high efficiency, and low cost, organophosphorus pesticides account for one-third of all pesticides used globally. Several insecticides, herbicides, and nerve agents are made from organophosphorus esters of phosphoric, phosphoric acid derivatives. Profenofos is one of the most widely employed organophosphorus pesticides on crop varieties, vegetables, and fruits in the category of organophosphorus pesticides. It has also been designated by WHO as a highly hazardous (toxicity class II) pesticide, with a moderate degree of toxic effect upon oral and topical application. The use of pesticides on a large scale pollutes the environment, including water bodies such as groundwater, surface water, and soil. Water samples from agricultural areas, for example, had up to mg per liter of profenofos. As a consequence, people and animals are poisoned by the profenofos residues. The mechanism of action of pesticides is by targeting enzymes or systems in the pests which may be very

similar to enzymes or systems in off-target organisms principally human and hence, they pose risks to human health and the environment even at their minute concentrations. The strong toxic effects of organophosphates are attributed to their ability to irrevocably inhibit the function of acetylcholinesterase in the central and peripheral nervous systems, leading to the accumulation of the neurotransmitter acetylcholine in the body and thus causing damage to the human nervous system, respiratory system, and cardiovascular system, leading to organ failure and eventual death. Hence it is essential to selectively remove the existence of pesticides in the environment to concern public safety and establish a precise as well as an efficient method for detoxification of pesticide residues to protect the ecosystem.

A brief survey of literature concerning the toxic effects, adsorptive removal, detoxification, microbial degradation of pesticides prominently profenofos, and microbial mediated plant growth development is represented here.

2.2. Toxicological Effects of Pesticides

Habiba *et al.* determined the presence of profenofos metabolites in raw and processed potatoes. One month before harvest, profenofos was applied foliar to potato plants. For raw, iced, baked, oven-baked, and fried potatoes as well as potato skins, the measured amounts were 11, 0.2, 0.2, 0.2, 0.004, and 0.2 mg/kg, respectively. Profenofos residues increased transaminase levels and the ratio was reduced. The activity of acid phosphatase increased just slightly. Furthermore, the residues inhibited peroxidase activity while inducing polyphenol oxidase activity. They concluded that attention must be paid to using this pesticide to reduce the negative impacts of profenofos on living things and also environment protection by confining its usage to non-edible crops or regulating its use with severe safety intervals (Habiba *et al.* 1992).

Digrak and Kazanici evaluated the influence of organophosphorus insecticides such as isofenphos, phorate, and fonofos on various soil microbiota. During incubation, the sum of the viable microbial count in the isofenphos treated soil sample was observed to be greater than in untreated groups. Furthermore, it was discovered that this procedure did not affect the development of the other microbe groups. The insecticides fonofos and phorate were shown to have no adverse impact on the growth of soil microbiota (Digrak and Kazanici, 2001).

Profenofos and its derivatives have been discovered in a fatal poisoning case by Gotoh and co-workers. Flame photometry and gas chromatography identified minimal profenofos and a considerable amount of metabolites in blood and urine upon alkylation using CH_2N_2 . The metabolites were found to comprise phosphorus atoms. 4-Bromo-2-chlorophenol was also detected as free or conjugated metabolites in blood and urine using an HPLC technique (Gotoh *et al.* 2001).

Pehkonen and Zhang reviewed the distribution of organophosphorus insecticides in the aquatic systems using hydrolysis, sorption, oxidation, and photochemical decomposition. Furthermore, they addressed the breakdown products of organophosphorus pesticides and their toxic nature to the ecosystem (Pehkonen and Zhang, 2002).

Using two cholinesterase inhibiting organophosphate pesticides, fenamiphos, and profenofos, McDaniel and Katherine investigated the connection between cholinesterase inhibition and neurobehavioral alterations. Interestingly, fenamiphos elicited substantial neurobehavioral symptoms but not profenofos. Both pesticides reduced blood cholinesterase up to 98 % after a single oral dose, although total brain cholinesterase was only affected by 14 % at the highest levels. Fenamiphos has dose-dependent effects on a variety of behavioral tests. Despite having a comparable inhibition property, profenofos did not affect behavior (McDaniel and Moser, 2004).

Radwan's team investigated the residual effects of profenofos on some vegetables, as well as its clearance employing different washing solutions and domestic treatment. To investigate the concentration and rate of dissipation of profenofos in fruit samples after application, it was sprinkled on grown eggplant and pepper at the prescribed rate, and samples were analyzed for two weeks following application. The influence of several washing solutions, as well as some domestic processing on the elimination of deposits from pre-treated vegetables, was also tested. GC was used to measure profenofos metabolites. The findings demonstrate that the consumption safety period for sweet pepper fruits was 10 days and 14 days for hot pepper and eggplant fruits. Furthermore, the results showed that tap water, KMnO_4 , and CH_3COOH solution removed a high percentage of pesticide traces out of the fruits, however, no evident fragments were identified in eggplant following cleaning with soap and CH_3COOH solutions. Overall, the investigated washing methods removed more profenofos metabolites out of the eggplant fruit than the other pepper fruits. Frying and blanching the fruits caused the complete removal of accumulated profenofos. Furthermore, after one week and two weeks, the pickling procedure eliminated 93 and 96 % of the hot pepper fruit, respectively (Radwan *et al.* 2005).

Individual enantiomers of three organophosphorus pesticides namely profenofos, fonofos, and crotoxyphos were isolated and tested for enantio-selectivity in acetylcholinesterase inhibition by Nillos *et al.* The enantiomers and racemates inhibited acetylcholinesterase in the aquatic invertebrates *in vivo*, as well as *in vitro* using electric eels and human recombinant acetylcholine esterase. Overall, the results revealed that acetylcholineesterase enzymes from various species had varying degrees of sensitivity, as well as varying degrees of enantio-selectivity in enzyme inhibition. The inversion of enantio-selectivity between the *in vivo* and *in vitro* experiments shows that enantio-selectivity occurs during toxico-dynamic processes such absorption, biotransformation, or elimination. The results of this work show that acetylcholineesterase inhibition of chiral

organophosphorus pesticides in non-target species is enantioselective, implying that enantiomers should be considered separately when assessing the environmental danger of these chiral pesticides (Nillos *et al.* 2007).

Abass and colleagues investigated profenofos biotransformations *in vitro*. After incubating profenofos into human liver homogenates and other mammalian liver microsomes, LC-MS revealed the formation of desthiopropylprofenofos and hydroxyprofenofos metabolites. Based on intrinsic clearance, the order of desthiopropylprofenofos production in liver microsomes has been rat < human < mouse, whereas hydroxylation of profenofos, the order has been human < rat < mouse. The liver microsomes from humans have been the most effective with profenofos bioactivation based on the ratio of intrinsic desthiopropylation and hydroxylation clearance rates. The interspecies differences, as well as inter-individual variance, have been found within the limit of the chemical risk assessment's default uncertainty/safety parameters. Profenofos is substantially degraded by liver microsomal cytochrome P450 enzymes, and it has a high interaction potential with a variety of cytochrome P450 activities (Abass *et al.* 2007).

Reddy and Rao investigated the toxic effect, morphological changes, and histopathological implications of profenofos on the *Eisenia foetida* earthworm. Morphological and histopathological examinations revealed body fissures, bloody blisters, and internal overgrowth of glandular cell mass, as well as the destruction of circular and longitudinal muscles, that failed to balance inner coelomic pressure, resulting in earthworm fragmentation. Acetylcholinesterase activity was used to assess profenofos neurotoxic propensity. Morphological changes and histopathological impacts, in addition to acetylcholinesterase inhibition, should be evaluated to determine profenofos exposure in the soil environment (Reddy and Rao, 2008).

He *et al.* investigated the environmental functioning of 40 % profenofos in paddy field scenarios. After applying 40% profenofos EC at 900 g ai/ha, the early profenofos accumulation over paddy plants, soil, and water have been reported to be 33, 0.2, and 3.9 mg/kg, respectively. The half-lives of the pesticide on the targets reached 5, 4, and 3 days, respectively. The quantity and regularity of application had a substantial effect on profenofos residue levels on rice straw, ground, and rice grain. The results obtained may aid in recommending the appropriate dose and calculating the safety duration of profenofos application (He *et al.* 2010).

Kushwaha's research group chronicled the structure, dissemination in the ecosystem, uses, mechanism of acetylcholinesterase inhibition, toxicity, and bioremediation of profenofos pesticide (Kushwaha *et al.* 2016).

A review of the numerous types of organophosphate pesticides, their environmental consequences, analytical methodologies for quantification, and environmentally friendly biodegradation approaches to ensure successful bioremediation has been documented by Sidhu's research group (Sidhu *et al.* 2019).

Hassaan and El Nemr presented an overview of pesticide definitions, categorization, toxicity, variables impacting toxicity, persistence in the water as well as other environments, human health implications, and detection, disposal, and treatment procedures. The analytical techniques employed to evaluate the extremely low concentrations of pesticides and the separation techniques utilized for pesticides examination have been described. Further alternative methodologies for the detection of pesticide remnants and other highly dangerous contaminants have been suggested (Hassaan and El Nemr, 2020).

Elliott and Zhang focused on the methods of fabrication, characterization, and utilization of iron nanoparticles for the remediation of both organic and inorganic pollutants.

Key challenges connected to field applications, such as fate, transportation, and potential ecological consequences, were also investigated (Li *et al.* 2006).

Hoag's research group described an ecofriendly single-step fabrication of iron nanoparticles employing tea polyphenols that does not require any extra additives as encapsulating /reducing agents. At room temperature, the rapid interaction between *Camellia sinensis* polyphenols and ferric nitrate happens within a few minutes and is evidenced by color shifts from light yellow to dark black in the generation of iron nanoparticles. TEM, UV-vis, and XRD were used to evaluate the synthesized iron nanoparticles. The nanoparticles were used to catalyze H₂O₂ for the treatment of organic pollution, and the findings were matched to those obtained with Fe-EDTA / Fe-EDDS complexes (Hoag *et al.* 2009).

2.3. Adsorptive Removal of Pesticides by Nanomaterials

Ahmad *et al.* reviewed the usage of several inexpensive adsorbents for pesticide removal from various water sources. Pesticides emerge as pollutants in water sources, creating negative effects on human health due to their harmfulness, genotoxicity, carcinogenicity, or generating aesthetic issues including odor and taste sensitivity. The pesticide contaminants can be effectively removed by a variety of inexpensive adsorbents that exhibited excellent pesticide removal capabilities. A variety of parameters influence the adsorption process, including pH, contact time, temperature, dosage and particle size of adsorbent, stirring speed, as well as the ionic intensity of solution. In general, increasing the dose of adsorbent, contact time, and agitation speed increase the % adsorption. Information about adsorption using carbon-based materials is copious because a wide range of adsorbents have been accessible and are associated with many methods. However, commercial activated carbon, powdered activated carbon, and granular activated carbon are

the more prevalent adsorbents used in the typical laboratory and engineering columns for water and wastewater management (Ahmad *et al.* 2010).

Kushwaha and co-workers investigated the adsorption of two organophosphate insecticides, monocrotophos, and chlorpyrifos, over acid-treated palm shell powder adsorbent made from agro-waste. Adsorption was examined in terms of adsorbent dose, incubation time, as well as concentration and subjected with the Freundlich and Langmuir isotherms. The adsorbent's monolayer adsorption capabilities for monocrotophos and chlorpyrifos were 53 and 51 mg/g, respectively. The estimated thermodynamic characteristics demonstrated that adsorption of monocrotophos on the adsorbent was spontaneous, feasible, and exothermic under the tested conditions, but chlorpyrifos showed no discernible temperature change. To explain the kinetic data and evaluate rate constants, the pseudo-first, pseudo-second, and intra-particle diffusion kinetic systems have been employed. The findings of this study suggested that acid-treated palm shell powder adsorbent might be used as an inexpensive adsorbent for the elimination of chlorpyrifos and monocrotophos from wastewater and agricultural runoff water (Kushwaha *et al.* 2011).

Shahwan and co-workers used green tea leaf extracts to fabricate iron nanoparticles for the elimination of methylene blue and methyl orange dyes. FT-IR, SEM, TEM, XPS, PXRD, XPS as well as DLS techniques were used to characterize the materials, which were found to contain primarily FeO and FeO(OH). The nanomaterials have been used as Fenton-like catalysts to decolorize the selected solutions. The associated investigations focused on the elimination kinetics as well as the impact of concentration of both dyes. UV-vis spectroscopy was used to measure the amounts of dyes in the aqueous phase. The results showed that the dyes were quickly removed, with kinetic data for methylene blue followed second order and for methyl orange, it was first order. The loading studies revealed

that both dyes were almost completely removed from the water on a wide range of concentrations (Shahwan *et al.* 2011).

Valickova's research team has performed the removal of some hazardous pesticides namely pentachlorobenzene, heptachlor, hexachlorobenzene, hexachlorobutadiene, and lindane. The pesticides' adsorption on granular activated carbon, zeolite, and activated sludge was examined. The influence of interaction time upon the removal efficacy of the adsorbents was explored. The adsorption efficiency order was determined to be activated sludge > zeolite granular activated carbon (Valickova *et al.* 2013).

Huang's research team used oolong tea extract to create iron nanoparticles. EDS, SEM, PXRD, UV-vis, and FT-IR techniques were used to confirm their morphology, structure, and size. Iron nanoparticles are usually spherical, with sizes varying from 40-50 nm. The degradation of malachite green with green iron nanoparticles indicated that the kinetics suited well with the pseudo-first-order mechanism by eliminating 76 % of the malachite green. Their research also revealed that iron nanoparticles have the potential to be used as a green adsorbent for environmental clean-up (Huang *et al.* 2014).

A polymer-based approach was used by Dehaghi and colleagues to synthesize chitosan-based ZnO nanoparticles composite beads for adsorption applications to pesticide pollution remediation. SEM, PXRD, and FT-IR techniques were employed to characterize the fabricated adsorbent. The optimal parameters for pesticide adsorption by the adsorbent were examined, including adsorbent dose, agitation time, beginning pesticide concentration, and pH. 0.5 g of the bio-nano composite removed the complete pesticide from permethrin solution at ambient temperature and neutral pH. The adsorbent's application for pesticide elimination was then investigated in the column. The column was entirely regenerated with 0.1 M NaOH solution before being employed for adsorption. With 56 % regeneration after

three cycles, chitosan-based ZnO nanoparticles appear to be a potentially potential platform in water treatment applications (Dehaghi *et al.* 2014).

Iron nanoparticles are frequently manufactured using sodium borohydride with agglomeration, which is an expensive and environmentally hazardous technique. Huang *et al.* generated iron nanoparticles employing eco-friendly techniques using various extracts of green, oolong, and black teas, to address these difficulties. Green tea extracts were the best method for disintegrating malachite green because they include a great amount of caffeine and/or polyphenols, which can react as reducing and capping chemicals in the creation of iron nanoparticles. SEM, UV-vis, and BET surface area methods have been employed to corroborate these features. The iron nanoparticles generated were studied by SEM, EDS, and PXRD to better understand the production of iron nanoparticles using various tea extracts. Tea extracts produced diverse sizes and quantities of iron nanoparticles, resulting in distinct decompositions of malachite green. Furthermore, the kinetics of malachite green degradation exploiting these iron nanoparticles consistent with the pseudo-first-order reaction with 20 kJ/mol activation energy, indicating a chemical diffusion-controlled process. Adsorption of the dye to iron nanoparticles, iron oxidation, and cleavage of the bond connected to the benzene ring was all part of the decomposition processes using these iron nanoparticles (Huang *et al.* 2014).

Fairooz *et al.* investigated the potential of pomegranate peel to adsorb two insecticides, lambda-cyhalothrin, and diazinon, from aqueous solutions. The samples' equilibrium contact time was determined. The effect of several parameters on the adsorption process, as represented by the acidity function, was also investigated. It was determined that the most effective adsorption occurs at acidic pH. The impact of the various temperatures was also investigated. The amount of pesticide adsorption decreases as temperature rises,

indicating that the adsorption mechanism is exothermic. Finally, the adsorption follows the Freundlich model for adsorption (Fairooz *et al.* 2015).

Smedt and coworkers explored the elimination of mobile insecticides from an aqueous medium using various zeolites. Investigations have been performed to better comprehend the adsorption pathway of different pesticides onto zeolites and to identify the most effective adsorbent for the cleansing of pesticide-contaminated water. Imidacloprid, isoproturon, and metalaxyl-m, which are more immobile pesticides, tend to combine with the zeolites, but bentazon and clopyralid, which are more mobile pesticides, dispersed in water. As per the adsorption %, zeolite produced the greatest performance for pesticide adsorption. Pseudo-first order and pseudo-second-order systems were used to analyze the adsorption kinetics. The pseudo-second-order reaction best described the experimental data. The adsorption rate was likewise proportional to pesticide mobility. The Freundlich model, which represents this type of isotherm, provided a strong fit for pesticide adsorption. The findings concluded the adsorption process depends on the particle size of the zeolites, water adsorption, and crystal structural alterations (De Smedt *et al.* 2015).

Using a batch approach, the organophosphate insecticide dimethoate has been adsorbed into Au nanospheres and nanorods in water. UV-vis, TEM, AFM, and FT-IR analyses were used to confirm dimethoate adsorption into gold nanoparticles. Nanosphere adsorption resulted in agglomeration, however, nanorod adsorption did not. The adsorption characteristics of nanomaterials were studied using the Freundlich and Langmuir models. The Langmuir isotherm provided the best fit to equilibrium data for both types of adsorbents. Nanospheres have an adsorption capacity of roughly 450 mg/g, while nanorods have a capacity of around 55 mg/g. Furthermore, nanomaterials were proven to be more efficient than nanorods at removing dimethoate from contaminated drinking water (Momic *et al.* 2016).

Zhao's research team provided an update on the most recent developments in stable zero-valent iron nanoparticles for diverse environmental remediation. Stabilizers that are often used are compared, and the stabilizing mechanisms are addressed. The effectiveness and limitations of the *in situ* remediation technique based on zero-valent iron nanoparticles are summarized. Because of their high specific surface area and great reducing power, freshly fabricated zero-valent iron nanoparticles are extremely reactive. The conventional borohydride procedure for generating zero-valent iron nanoparticles has been continuously upgraded by the addition of various stabilizing agents or surface modifiers to produce the most stable and soil implementable Fe(0) nanomaterials to eliminate various contaminants in soil and water. While most studies have concentrated on evaluating Fe(0) nanomaterials for water purification, the greater efficacy or benefit of Fe(0) nanomaterials seems to be *in situ* remediations of polluted surface and groundwater by directly delivering stable zero-valent iron nanoparticles to polluted subsurface, as proposed from the commencement. In comparison to traditional remediation procedures, the *in situ* treatment technique employing stable zero-valent iron nanoparticles has some distinct advantages. They also identified significant information gaps and research priorities, such as interactions between supplied Fe(0) nanomaterials and local biogeochemical conditions (Zhao *et al.* 2016).

The Bimetallic iron/nickel nanomaterials have been produced by Mansouriieh *et al.* and used to eliminate the organophosphorus insecticide profenofos from water. SEM, EDS, PXRD, and FT-IR were used to characterize these new bimetallic adsorbent nanoparticles. Adsorption was examined using the parameters of initial concentration of pesticide, pH, adsorbent dose, temperature, and incubation time. The adsorbent was highly efficient for adsorption of profenofos, and equilibrium was reached within 8 minutes. The equilibrium was determined using the Langmuir, Freundlich, as well as Temkin isotherm systems. The Langmuir model fits the experimental data well. To assess absorption kinetics, pseudo-firs,

pseudo-second, and intra-particle diffusion systems have been examined. The best relationship with the results was provided by the pseudo-second-order model. The thermodynamic characteristics of Gibb's free energy (ΔG), entropy (ΔS), and enthalpy (ΔH) were also analyzed for the adsorption process. According to the thermodynamic parameters, profenofos adsorption utilizing iron/nickel nanoparticles is a spontaneous and endothermic process. The activation energy value validates the nature of profenofos chemisorption onto iron/nickel adsorbent (Mansouriieh *et al.* 2016).

Sahithya *et al.* focused on the adsorption-based removal of an organophosphate insecticide, monocrotophos by montmorillonite-CuO biopolymer composites varied with chitosan, polylactic acid, and gum ghatti. The solution pH, incubation time, temperature, initial adsorbate concentration, and adsorbent dosage were all modulated in optimization trials. Equilibrium and kinetic tests demonstrated heterogeneous and physical methods of adsorption with a capacity of 212 mg/g. This was validated further by SEM analysis. The Boyd plot and intra-particle diffusion revealed that diffusion may not be the only rate-limiting process. Thermodynamic investigations validated the spontaneous and endothermic nature of adsorptive removal. FT-IR studies revealed that $-NH_2$ as well as $-COOH$ groups operated a significant role in monocrotophos adsorption. The presence of carbon atoms, CuO nanoparticles, and Si, was confirmed by EDX analysis. AFM examination verified the homogeneous dispersion of CuO nanoparticles on the surface of adsorbent with polylactic acid, validating the potential of the adsorbents for monocrotophos remediation in aquatic environments (Sahithya *et al.* 2016).

Iron nanomaterials have been generated from *Rosa damascene*, *Thymus vulgaris*, and *Urtica dioica* plant extracts by Fazlzadeh's research group. The FT-IR results revealed that polyphenols and some organic components reacted as reducing/capping agents towards the generation of iron nanoparticles and minimize the likelihood of iron nanoparticle

aggregation when compared to chemical techniques of iron nanoparticle fabrication. The amount and type of chemicals in plant extracts have an effect on the structure and aggregation of iron nanoparticles following adsorption. According to the findings, the maximum removal effectiveness occurred at acidic pH. The % removal increases with increasing contact duration and dose. Inversely, increasing the initial concentration of adsorbate chromium reduces the contaminant's removal efficacy. The nanomaterials possess a greater adsorption efficiency. As a result, with a concentration of 0.2 g/L and an interaction period of 10 minutes, the iron nanoparticles removed more than 90% of the iron (Fazlzadeh *et al.* 2017).

The green fabrication of iron nanoparticles was accomplished by Gottimukkala and colleagues employing an environmentally friendly plant extract. At normal temperature, the extract from the leaves of *Camellia sinensis* was found to be capable of reducing Fe ions into Fe nanoparticles. The goal of this research is to create iron nanoparticles utilizing leaf extract in an environmentally friendly and sustainable manner. SEM and FT-IR analyses were used to characterize the produced Fe nanoparticles. The Fe nanoparticles can be fabricated using the leaf extract of *Camellia sinensis* as a reducing agent for environmental remediation (Gottimukkala *et al.* 2017).

Nanomaterials have now been widely researched in the elimination of a diverse range of contaminants such as pesticides, heavy metal ions, and dyes from wastewater. Silva's research team investigated the potential of nanoparticles as efficient adsorbents for wastewater remediation. Numerous innovative nano-adsorbents were developed in recent years to improve the efficacy and adsorption capabilities of eliminating pollutants from wastewater. The invention, innovation, and problems of low cost and environmentally compatible nanomaterials for water remediation are explored and reviewed. This review indicates that nanoparticles have several distinct morphological and structural features that

qualify them for usage as effective adsorbents to address a variety of environmental issues (Silva *et al.* 2017).

Eco-friendly fabrication of metal nanoparticles is a technology that reduces the production of byproducts, harsh chemicals, and hazardous solvents, among other things, which have harmful effects on not only the ecosystem as well as on public health. The capacity of tea waste extracts to act as novel reducing agents for the green production of iron nanoparticles was investigated by Gautam *et al.* The average particle size of the adsorbent generated from tea waste was 99 nm, according to SEM analysis. The existence of distinct functional groups accountable for the adsorption of phenol red was revealed by the FT-IR study of adsorbents from tea waste. The kinetics of adsorption of phenol red on the adsorbent from tea waste were examined in terms of initial adsorbate concentration, various pH, adsorbent doses, and temperature. The results demonstrate that raising the initial concentration of the phenol red dye and temperature lowered PR adsorption. In comparison to acidic and alkaline pH, the most favorable elimination of PR was accomplished at pH 8.0 (95 %). In comparison to the Freundlich isotherm model, the adsorption equilibrium data have been found to have a vest fitted with the Langmuir model. The results show that the pseudo-second-order kinetic system accurately describes the kinetic data. The particle size of adsorbent from tea waste was determined by the Zeta nanosizer to be in the range of 30–190 nm. With increasing phenol red starting concentration at pH 8, the adsorption efficiency of phenol red on adsorbent from tea waste increased significantly from 48 to 218 mg/g. The adsorption capability of the metal nanoparticles from tea waste towards is improved by waste tea extract. The removal effectiveness of phenol red with adsorbent from tea waste was found to be greater than that of chemically produced iron nanoparticles (Gautam *et al.* 2018).

The removal of cyanazine from tailored iron nanoparticles is reported by Ali and colleagues. The nano-adsorbent was generated using an environmentally benign process and functionalized with 1-butyl-3-methyl imidazolinium bromide. The adsorbent particles were studied using a variety of techniques, including UV-vis, FT-IR, PXRD, SEM, and TEM. The amount of cyanazine that remained in the water was determined using the HPLC technique. The batch experiments were conducted by varying parameters adsorbent concentration, interaction time, pH, adsorbent dosage, and temperature. Four distinct isotherms were used to analyze the experimentation results. These observations indicate that the elimination process is rapid and exothermic. The removal was carried out via a pseudo-first-order kinetic model and liquid-film diffusion method. Because it was acceptable in most circumstances involving water resources, the removal approach was rapid, eco-friendly, and inexpensive. The procedure can be used to remove cyanazine from various bodies of water (Ali *et al.* 2018).

Singhal and Lind have demonstrated a new approach for the one-step fabrication of cysteine-appended silver nanoparticles. Several techniques were used to characterize the particles, including UV-vis, FT-IR, PXRD, SEM, and TEM. These nanoparticles have been tested for their ability to adsorb two important water pollutant pesticides, chlorpyrifos and malathion (Singhal and Lind, 2018).

Dalal and co-workers demonstrated a biogenic material encapsulated with Fe nanomaterials via an eco-friendly approach in which biomass acts as both a reductant as well as capping agent. Fe nanomaterials contained in *Citrus limetta* skins have been evaluated using ICP-MS to determine metal loading, XRD, XPS to determine crystalline nature and redox states, TEM, and SEM-EDX to determine particle morphology and size. Nanoparticle sizes were determined to be in the 4–70 nm range. Batch tests have been carried out to investigate the impact of various factors including incubation period,

biomaterial quantity, and Cr solution volume for 2500 mg/ L. For that concentration, an interaction time of 5 minutes with 1.5 g of adsorbent resulted in a complete decrease. According to the results of the experiments, 1 g of adsorbent entirely converted 33 mg of Cr^{6+} to Cr^{3+} . XRD demonstrated that the Fe nanomaterials are amorphous, and XPS confirmed the zero-valent state. The conversion of zero-valent to iron (II)/iron (III) during chromium solution treatment verifies the conversion of Cr^{6+} to Cr^{3+} (Dalal and Reddy, 2019).

The main consequence of natural organic compounds in water is that they react with disinfectants, producing disinfectant-based by-products that are mostly cancerous. Rashtbari *et al.* investigated the best conditions for removing humic acid from aqueous solutions using zeolite loaded with iron nanoparticles. Various approaches were used to analyze the structural morphology of the manufactured adsorbent. Adsorbate concentration, composite composition, pH, and incubation time were all tested. The Langmuir and Freundlich isotherm models, as well as pseudo-first and second-order kinetic systems, have been employed to assess the experimental results. The thermal properties were then computed. The test findings confirmed the composite structure's correctness. It had a definite surface area of around $200 \text{ m}^2/\text{g}$ when estimated using the BET technique. In the ideal conditions of 50 mg/L dosages of humic acid, 2 g/L adsorbent dose, acidic pH (3), and reaction duration of one hour, the adsorbate removal efficiency was 93 %. The isotherm and kinetic studies revealed that the adsorption route matches the Langmuir model and pseudo-second-order kinetics. The Langmuir model determined the composite's maximal adsorption capacity to be 23 mg/g. Thermodynamic factors show that HA adsorption is endothermic and cannot occur spontaneously. After five recyclings, the zeolite tethered iron nanocomposite demonstrated a high removal efficiency (Rashtbari *et al.* 2020).

Bisphenol A in the aquatic systems is toxic to living things. Bisphenol A, even in tiny amounts, has been linked to heart disease, type II diabetes, and altered liver enzymes. Chitosan immobilized zero-valent iron nanoparticles were produced and employed as an adsorbent for effective Bisphenol A removal by Dehghani's research group. Because the efficacy of Bisphenol A removal is affected by independent process variables such as chitosan dose, initial Bisphenol A concentration, pH, and contact time, studies were carried out methodically. The experimental matrix is created using response surface methodology to evaluate the interaction impact of independent variables. Using differential evolution optimization, the isothermal and kinetic model coefficients are determined, and these values better characterize adsorption behavior. The batch adsorption tests revealed that the adsorption of Bisphenol A on the adsorbent follows pseudo-first-order kinetic models. As per the Langmuir isotherm model, the highest amount of Bisphenol A adsorption has been 65 mg/g. The renewability experiments show that the synthesized adsorbent may be employed for up to three cycles with a removal effectiveness of more than 50%. The thermodynamic studies revealed a process of spontaneous adsorption. Coexisting ions inhibited Bisphenol A adsorption in the following order: manganese > calcium > magnesium > iron for cations; and sulphate > nitrate > chloride for anions. The removal effectiveness of Bisphenol A from genuine pharmaceutical wastewater is 94 % for an ideal initial adsorbate dose of 6 mg/L, an adsorbent concentration of 1.5 g/L, a solution pH of 3, and an adsorption duration of 1 h, but it is 95 % for synthetic wastewater (Dehghani *et al.* 2020).

Silveira's research group employed a green synthesis approach to infusing the surface area of palm coconut-based activated carbon with nanomaterials of iron compounds made from leaf extracts of *Moringa oleifera* and pomegranate and then tested its adsorption ability for sodium diclofenac. The zeta potential, XRD, BET technique, TEM, and SEM coupled to X-ray and EDX methods were used to describe the adsorbent material. The effect

of pH, kinetics, adsorption isotherm, and thermal characteristics on the adsorption efficiency of sodium diclofenac was investigated. The impregnated adsorbents were effective in the adsorption of sodium diclofenac. The pseudo-second-order system ideally fit the equilibrium data, and the Langmuir model was the equilibrium model. In terms of the thermodynamic investigation, it was established that the adsorption of all adsorbents occurs spontaneously. The adsorption mechanism was endothermic and physisorption in nature. As a result, this approach is promising since, when compared to chemical methods for nanoparticle creation, it is a cleaner and non-toxic technique (Silveira *et al.* 2020).

Green iron oxide nanoparticles were synthesized by de Souza's study team using a Fe salt and *Camellia sinensis* black tea extract, and they have been tethered on NH₂-group mesoporous silica. The multicomponent composite was evaluated and used in the adsorption of methyl orange, an anionic azo dye. According to the findings, a multicomponent composite is 3-11 times more potent than other individual adsorbents. Adsorption criteria including adsorbent concentration, starting dye dose, pH, and interaction period have been tested for multicomponent composites, and the adsorption efficiency of 105 mg/ g at 298 K has been obtained at equilibrium. When the multicomponent composite is positively charged, zeta potential measurements revealed a preferred electrostatic attraction with the oppositely charged dye at acidic pH. Kinetic studies revealed that reaction-controlled adsorption is characterized by a pseudo-first-order model. Several adsorption isotherms models were used to fit the data at equilibrium. Freundlich and Temkin's isotherms were found to be the most appropriate for describing the prepared material, implying adsorption occurred at heterogeneous surfaces with several locations of adsorbent, including protonated –NH₂ moiety and iron oxide nanoparticles, in addition to the tea polyphenols, which furnished multiple spots of adsorption via pi-pi stacking among the benzene rings of the dyes (de Souza *et al.* 2021).

With the destruction of the water sources and the depletion of drinkable water reserves, wastewater recycling and reuse have become essential. The wastewater released into the natural surroundings contains a wide range of pollutants that have detrimental long-term impacts, particularly in poor countries. Pesticides are one of the major pollutants found in high concentrations of influents and effluents from urban, industrial, and farm drainage waters. Traditional treatment procedures are accompanied by extreme expenses, increased energy use, and the development of harmful byproducts. It is required to develop and implement new pesticide removal processes that are less expensive, quicker, more tolerable, and safer for the environment. When compared to traditional technologies, wastewater treatment utilizing nanotechnology offers numerous advantages, including reduced energy and material use. Gacem *et al.* demonstrated various techniques for pesticide adsorption and degradation using nanomaterials. The limit of detection and degradation mechanisms were also explored (Gacem *et al.* 2021).

The Hazrin research group investigated the adsorption and kinetic behaviors of 2,4-dichlorophenoxyacetic acid over activated carbon derived from oil palm. The impacts of solution pH, time, temperature, and initial adsorbate concentration were studied using batch adsorption. The maximum elimination efficacy has been observed at pH 2 owing to the lack of electrostatic repulsive interaction among the adsorbate and adsorbent, as evidenced by the adsorbate's zero point charge of 4.8 and a pKa of 2.7. The non-linear form of Langmuir isotherm fits with equilibrium data at 25-45 °C and acidic pH (2) better than the non-linear Freundlich isotherm. The maximal adsorption capacity of a Langmuir monolayer was determined to be 420 mg/g, which is amongst the greatest values described for other activated carbon adsorbents employed for 2,4-dichlorophenoxyacetic acid removal. Adsorption kinetic data have been better correlated with pseudo-second-order than pseudo-first-order kinetics, representing that chemical adsorption is most prevalent than physical adsorption. The adsorbent's high adsorption absorption of 2,4-

dichlorophenoxyacetic acid underlines its capability as a credible substitute to commercial activated carbon, predominantly for standard to bulk-scale 2,4-dichlorophenoxyacetic acid detoxification of wastewater (Hazrin *et al.* 2021).

Pesticide use has escalated even more than twice during the last four decades. As a result, insecticides and their breakdown products are rapidly contaminating waterways. Organochlorine pesticides, for example, are considered the most dangerous due to their extended half-lives in the ecosystem (up to 15 years) and bioaccumulation. This is a severe health issue that necessitates sophisticated water cleaning methods such as adsorption using activated carbon, but present approaches are limited by the expense, low recyclability, and discharge of current adsorbents. Ponnuchamy and his colleagues evaluated the adsorbents constructed from various materials for pesticide adsorption. Because of their high carbon content, biochars generated from plant sources have maximum adsorption capabilities of up to 900 mg/g. Field application strategies and post-treatment of used adsorbents are described (Ponnuchamy *et al.* 2021).

The harmful effects of insecticide residues in the aquatic environment are a major concern that must be addressed. Kodali *et al.* demonstrated the adsorption mediated removal of an organophosphorus insecticide monocrotophos using an activated coconut charcoal adsorbent. With a significant Langmuir adsorption capability of 104 mg/g, this adsorbent demonstrated good authority to eliminate monocrotophos from water at neutral pH. By LC-MS spectrometry, the residual pesticide amounts in an aqueous solution after adsorption were determined. This super adsorbent demonstrated its ability to eliminate monocrotophos in farm water samples from agricultural soils (Kodali *et al.* 2021).

Green synthetic methodologies for nanomaterials have attracted a lot of attention in recent times because they address the sustainability challenges that conventional synthesis methods face. Recent works of literature, however, do not give a clear view of bioactive

nanomaterials. Ahmed's research team, on the other hand, clarified the previous discrepancies by giving insight into the stability and toxic effects of nanomaterials, vitally evaluating the diverse biological entities and solvents needed for fabrication, shedding light on the effects that influence biosynthesis, and highlighting the applicability of nanoparticles across diverse fields. Despite the benefits of eco-friendly synthesis, existing practices face challenges such as proper and secure solvent choice, process variables that impact the method for the synthesis, nanomaterial toxicity, large-scale production, nanoparticle morphology control, time-consuming maintenance, and knowledge deficits. As a result, the green production of nanomaterials is mostly confined to the laboratory. Nonetheless, the resulting nanoparticles' biodegradability, biocompatibility, and sensitivity have broader implications in evolutionary biology, environmental cleanup, and consumer sectors. Future studies should focus on understanding the mechanics of the synthesis methods, uncovering more chemical and biochemical entities that could be used in synthesis, developing the feasibility of eco-friendly synthesis at the industrial level, and optimizing the parameters influencing the synthesis process (Ahmed *et al.* 2022).

2.4. Isolation and Identification of Pesticide Degrading Microbes

Ethion, an organophosphorus pesticide, is still posing a serious threat to both the ecosystem and public health. The aerobic decomposition of Ethion by thermophilic bacteria extracted from polluted soils near decommissioned livestock dip sites was studied by Foster *et al.* When grown in minimal salts media, two isolates are recognized as *Pseudomonas* and *Azospirillum* species, have been competent of biodegrading Ethion. Ethion, Dixon, and diethylthiosphosphate were not found as abiotic hydrolytic breakdown products. The data point to Ethion degrading quickly to encourage microbial development (Foster *et al.* 2004).

Singh and Walker have documented the various mechanisms exerted by the microbes to degrade several organophosphorus pesticides. The extraction, characterization,

usage, and modification, as well as the molecular mechanisms of degradation of the primary detoxifying enzymes, are discussed. The key successes and technological developments in the bioremediation of organophosphorus compounds, as well as the limitations of present technology and future challenges, have been reviewed (Singh and Walker, 2006).

Malghani *et al.* demonstrated the potential of a bacterial isolate as a bio-degrader towards bioremediation of profenofos-contaminated soil. In their investigation, a bacterial strain was extracted using an enrichment process from a profenofos-protracted soil, and its efficiency to break down profenofos has been assessed by gas chromatography. Based on its biochemical and physiological features, as well as an examination of its 16S rRNA gene sequence, the isolated bacterium has been confirmed as *Pseudomonas aeruginosa*. The strain thrived well at pH 5.5–7.2 at a wide range of temperatures. The remediation of profenofos-polluted soil has been investigated employing free soil and profenofos treated soil that reflected in faster degradation of profenofos over untreated soil. Within 48 h of incubation with a mineral salt medium, 87 % of the profenofos was removed. The metabolites of profenofos degradation suggested that the degradation proceeded by a hydrolysis pathway, and one of the byproducts detected was 4 Bromo-2-chlorophenol, which was mineralized by *Pseudomonas aeruginosa* (Malghani *et al.* 2009).

An enrichment culture approach was utilized to extract bacteria responsible for profenofos biodegradation in soil by Malghani and co-workers. The pure cultures, *Pseudomonas putida*, and *Burkholderia gladioli* were isolated and characterized using 16S rRNA gene sequencing and biochemical techniques. Both strains thrived effectively at acidic to neutral pH and various temperatures. The bioremediation of profenofos polluted soil has been investigated employing soil treated with 200 µg/g profenofos. The soil with bacteria inoculation showed a faster degradation rate of profenofos over control soil (Malghani *et al.* 2009).

Chanika *et al.* reported the isolation and identification of two bacteria, *Pseudomonas putida*, and *Acinetobacter rhizosphaerae* to degrade the organophosphorus and fenamiphos pesticides. The dominant nature of these isolated strains from the enrichment culture was confirmed by gel electrophoresis techniques. The strains hydrolyzed the selected pesticides and also displayed efficient bioremediation towards the oxidized forms of fenamiphos residues (Chanika *et al.* 2011).

Tang identified a new triazophos-degrading bacterium from sewage sludge in an organophosphorus pesticide wastewater treatment system. The bacterium is gram-positive, and competent in triazophos hydrolysis via cometabolism. The isolate was identified as a *Bacillus sp.* by various studies. It breakdown 99 % of triazophos in the presence of nutrients in 5 days. The enzyme responsible for TAP breakdown appears to be intracellular, according to an enzyme distribution experiment (Tang and You, 2012).

2.5. Degradation of Pesticides and Plant Growth Enhancement by Microbes

The goal of their investigation has been to study the effect of microbial inoculation on plant growth enhancement as well as chlorpyrifos breakdown and deposition in different areas of the plant. Ryegrass has been cultivated in chlorpyrifos-spiked soil and inoculated with *Bacillus pumilus*. Inoculation showed a positive impact on chlorpyrifos decomposition and plant biomass production in general, with the maximum chlorpyrifos decomposition (97 %) seen after 45 days of inoculation. Interestingly, the strain colonized the rhizosphere of the inoculated plant and increased the breakdown of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol. Inoculated plants had much less chlorpyrifos buildup in their roots and shoots than control plants. The findings demonstrate the efficacy of injected exogenous bacteria in accelerating the cleanup of chlorpyrifos-polluted locations and lowering the amounts of harmful pesticide traces in crops (Ahmad *et al.* 2012).

Salunkhe and colleagues investigated the bioremediation of profenofos, an organophosphate pesticide, by four *Bacillus subtilis* strains obtained from grapevines or grape rhizosphere in liquid culture, on grapes, and in vineyard soil. All the four *B. subtilis* isolates improved profenofos break down in all three samples. The first-order kinetics module produced the best degradation rate constants. GC-MS was used to identify the breakdown metabolite 4-Bromo-2-chlorophenol (Salunkhe *et al.* 2013).

Tamilselvan and co-workers isolated and characterized efficient pesticide degrading bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. The three strains were tested for growth in minimal salt medium (MSM) with 25 ppm pesticides. For this investigation, two pesticides, metribuzin and profenofos, were chosen. Amongst isolates, *Bacillus subtilis* used the pesticides most effectively and grew the fastest. The growth of the three isolates was evaluated at varying temperature levels (25 - 55 °C), pH levels (pH 4-8), and carbon and nitrogen sources. Bacterial growth rates were highest at 35 °C and acidic pH 6. Bacterial growth was greatest in the presence of Dextrose, while the least growth was observed in Lactose broth culture. Bacterial growth was greatest in the presence of Malt extract and beef extract had the slowest development. The bacterial isolates grew well in the profenofos medium (Tamilselvan *et al.* 2014).

The degradation of organophosphorus insecticides by the new microbial consortium and isolates was examined by Siripattanakul-Ratpukdi and the research group. The enriched consortium contained three major profenofos degrading bacteria, which were identified as *P. plecoglossicida*, *P. aeruginosa*, and *P. vlaeruginosa*, respectively. Profenofos may be used as the only carbon source for a consortium and all isolates, with profenofos being removed at a rate of more than 90 % via a hydrolysis procedure. The first-order kinetic process was followed by bacterial growth and profenofos degradation rates. Profenofos biodegradation was hampered by an additional carbon supplement. Chlorpyrifos and

dicotophos pesticides were similarly degraded by the enhanced cultures (up to 70% removal). The results showed that the consortium and isolates are effective at degrading profenofos and other organophosphorus pesticides and that they might be used to remediate profenofos (Siripattanakul-Ratpukdi *et al.* 2015).

Siripattanakul-Ratpukdi and colleagues investigated bioremediation of pesticide using 20 mg/L of profenofos. The enriched consortium had three main profenofos degrading bacteria, including *P. plecoglossicida*, *P. aeruginosa*, and *P. aeruginosa*. The bacteria and the consortium used profenofos as the only carbon source and removed 90 % of profenofos through the enzymatic hydrolysis process. The rates of bacterial growth and profenofos degradation were determined by a first-order kinetic process. Adding more carbon to the mix hampered PF biodegradation. The enhanced cultures were also capable of decomposing chlorpyrifos and dicotophos, with up to 70% pesticide elimination. (Siripattanakul-Ratpukdi *et al.* 2015).

Talwar and Ninnekar identified a bacteria capable of disintegrating profenofos from pesticide-polluted soil samples. Based on visual and biochemical parameters, as well as 16S rRNA gene sequences, the isolate was recognized as *Pseudoxanthomonas suwonensis*. HPLC and GC-MS analysis revealed that 4-Bromo-2-chlorophenol is a byproduct of profenofos breakdown. The organism hydrolyzed profenofos to produce 4-Bromo-2-chlorophenol, which has been then used as a carbon source for development. As carbon sources, the organism used some other organophosphate insecticides such as quinalphos, temephos, and chlorpyrifos. The optimal conditions for profenofos breakdown by *P. suwonensis* have been reported to be neutral pH and room temperature. The degradation rate of profenofos by free and immobilized cells of *P. suwonensis* in different conditions was examined, and immobilized cells were found to be more effective than free

suspended cells in the breakdown of organophosphate pesticide polluted water (Talwar and Ninnekar, 2015).

Jabeen's research group established a profenofos deteriorating bacterial consortium which included *Bacillus* sp., *Achromobacter xylosoxidans*, *Citrobacter koseri*, and *Pseudomonas aeruginosa*. Surprisingly, the consortium decomposed profenofos more efficiently than pure isolates. Different culture conditions for profenofos degradation were improved using a 2³ complete factorial central composite design based on response surface methodology. The ideal pH, temperature, and inoculum size have been determined to be 6.83, 35 °C, and 0.59 g/L, respectively, to achieve maximum profenofos degradation of 93 %. The analysis of the metabolic byproducts revealed that the primary metabolite of profenofos was 4-Bromo-2-chlorophenol, which was further converted to simpler compounds. Notably, the consortium was capable of decomposing a wide spectrum of organophosphate and other pesticides, making it a potential tool for soil remediation with a cocktail of pollutants (Jabeen *et al.* 2015).

Palanimanickam and Sepperumal have extracted bacteria from profenofos employed paddy production field soil and tested their ability to break down profenofos in the soil in a laboratory environment. The main bacteria were found to be *Bacillus cereus* and *Aneurinibacillus migulanus*. When compared to the control, *Aneurinibacillus migulanus* expedited the breakdown of profenofos in 36 h. In the control, *m*-xylene and 4-phenyl but-3-ene have been produced as profenofos degradation metabolites that have been not found in soil subjected to *Bacillus cereus* and *Aneurinibacillus migulanus*. However, *n*-propyl benzene has been acquired as a degradation product from the soil subjected to bacteria, which was not observed in the untreated soil. When compared to the untreated soil, isopropyl benzene has been identified in soil subjected to *Bacillus cereus* and

tetramethyl benzene has been identified in soil treated with *Aneurinibacillus migulanus* as a result of profenofos deterioration (Palanimanickam and Sepperumal, 2017).

Kumar and colleagues explored the several features of organophosphate pesticide biodegradation, as well as the biological and molecular identification of several organophosphorus pesticides degrading bacteria. Organophosphates may become accessible for microbial breakdown depending on their fate. Sorption process, P–O alkyl and aryl bond hydrolysis, photocatalytic degradation, as well as enzymatic mineralization are all the various methods that environmental bacteria can use to degrade organophosphate insecticides. Microbes have isolated enzymes such as diisopropyl fluorophosphate, esterase, phosphotriesterase, and paraoxonase to examine and comprehend the catabolic processes involved in the biotransformation of these xenobiotic substances (Kumar *et al.* 2018).

Dong and colleagues evaluated the dissipation behavior of profenofos in cotton fiber throughout the growth stage and after scouring treatment. Profenofos levels in fiber from pre-opened and post-opened bolls equally declined to undetectable levels 21 days after pesticide treatment. However, a small percentage of profenofos was transformed to 4-Bromo-2-chlorophenol, a potent irritant, and a non-negligible quantity was maintained in cotton fiber after 28 days. During the traditional cotton scouring procedure, profenofos and its decomposition product might be fully eliminated. Profenofos only took 3 min to degrade in a scouring wash, and the breakdown product was 4-Bromo-2-chlorophenol (Ma *et al.* 2019).

The biodegradation of organophosphorus insecticides, profenofos, and quinalphos in a culture medium utilizing bacteria isolated from wetland paddy rhizospheres was investigated by Dash and co-workers. The isolated strain demonstrated improved pesticide tolerance, biofilm formation efficiency, and the ability to synthesize organophosphate degrading enzymes. The isolate has the most sequence similarity to *Kosakina oryzae* based

on 16S rRNA gene sequencing. Within 48 h, a biodegradation experiment using varying doses of profenofos and quinalphos revealed maximal degradation of up to 82 and 92 %, respectively. The biodegradation rates with half-lives for profenofos and quinalphos were discovered by kinetic investigations. GC-MS was used to identify the degradation products, and in silico methods were used to propose plausible degradation routes. The removal of the pesticides from the aqueous solution was executed using the biofilm of the isolated strain developed on selected substrates using mushroom, gravel, and activated charcoal biofilm carriers. Mushrooms had a high biofilm production rate and a high biodegradation rate (Dash and Osborne, 2020).

Subsanguan *et al.* used a profenofos degrading *Pseudomonas plecoglossicida* strain to explore profenofos decontamination in aerobic as well as anaerobic circumstances. The focus was on anaerobic biodegradation in the existence of nitrate. Profenofos was degraded at 10–150 mg/L under both situations, with elimination productivities up to 55 %. The culture showed good biodegradation efficiency in an aerobic environment and resulted in the formation of 4-Bromo-2-chlorophenol and 1,1'-dimethylethylphenol as degradation metabolites. The poisonousness of the contaminated water was greatly reduced following aerobic and anoxic biodegradation, according to microbial toxicity, phytotoxicity, and to-genotoxicity studies (Subsanguan *et al.* 2020).

An enrichment strategy was used by Putra's research group to extract the bacterial culture from the soil with prolonged exposure of profenofos, and its capability to break down profenofos has been assessed by gas chromatography. The ability of isolates to break down profenofos varies, with *Pseudomonas luteola* having the highest degradation rate. The isolate is rod-shaped, motile, Gram-negative, and has an optimal pH of 7 for decomposing profenofos. Profenofos degradation was studied with a soil treated with profenofos (100

mg/L) that displayed a faster degradation over the untreated soil. Within 54 h of incubation in a mineral salt medium, elimination of profenofos was 93 % (Putra *et al.* 2021).

Isworo *et al.* have reported the isolation and identification of indigenous bacterial consortium. The isolated bacterial consortium has been characterized as *Oceanobacillus iheyensis* and *Exiguobacterium profundum* by the 16S-rRNA gene technique. Further, the ability of the isolated consortium has been examined against the profenofos and malathion degrading capability. The results showed that the consortium bacteria proved able to completely profenofos and malathion in 192 h (Isworo and Oetari, 2021).

Shalini Verma and Chatterjee explored profenofos microbial breakdown by a psychrotolerant bacterium *Rahnella sp.* The strain has been extracted out of soils collected in apple orchards from the Kufri regions of Himachal Pradesh, India. The bacterium *Rahnella sp.* was capable of destroying 100 5 profenofos within 14 days at 20 °C according to quantitative analysis using HPLC. The breakdown products such as 4-Bromo-2-chlorophenol, phosphoric acid, and 3,4-dimethyl benzoic acid were detected using GC-MS and HPLC. A possible profenofos breakdown mechanism has been hypothesized based on these findings. They concluded that the initial degradation process could be caused by intracellular organophosphorus hydrolase enzyme (Verma and Chatterjee, 2021)

The same group extracted another bacteria competent of using profenofos as the only source of carbon and energy from soil samples of apple orchards in the Shimla district of Himachal Pradesh, India for profenofos bioremediation. *Bacillus altitudinis* was characterized by biochemical, FAME, and 16S rRNA gene studies. Within 30 days of incubation at 28 °C and neutral pH, the strain was able to break down 93 % of profenofos. A linear regression study of the data set demonstrated the link between bacterial population and pesticide degradation. One of the pathway metabolites found was 4-Bromo-2-chlorophenol, which was entirely reduced to lower pathway products. A possible PF

degradation pathway that determines the way from profenofos to 4-Bromo-2-chlorophenol and eventually enters the tricarboxylic acid cycle has been postulated (Mahajan *et al.* 2021).

Farhan *et al.* investigated the biodegradation of chlorpyrifos by employing native *Bacillus cereus* bacterium extracted from cotton-growing areas. The isolated cultures have been characterized using the ribotyping method. *Bacillus cereus* was tolerant to chlorpyrifos up to 125 mg/L and efficaciously metabolized 88 % of chlorpyrifos within 8 days at basic pH. *Bacillus cereus* sustained temperatures of 40 °C, which is a favorable marker for *in situ* remediations. Green manure, farmyard manure, and husks were all evaluated, and the results showed that farmyard manure had the greatest impact on degradation. According to GC–MS analysis, chlorpyrifos is initially transformed to diethylthiophosphoric acid and 3,5,6-trichloro-2-pyridinol. Subsequently, the metabolites were degraded and mineralized, leaving no hazardous byproducts which confirmed the efficacy of *Bacillus* to bio-remediate chlorpyrifos-polluted fields (Farhan *et al.* 2021).

Mishra and Sundari set out to analyze the impact of direct inoculation of chosen consortia onto plants in the influence of the root pathogenic *Sclerotium rolfsii* and the organophosphorus insecticides malathion and methyl parathion. Two bacterial isolates, a diazotrophic bacterium, fluorescent *Pseudomonad*, and a biocontrol fungus from western Uttar Pradesh, India, were chosen for the study. Individual isolates inoculated on host plants resulted in a distributed growth development while the fungus enhanced root biomass. The fluorescent *Pseudomonad* strain enriched photosynthesis pigment composition and diazotrophic bacterium isolate increased shoot and root lengths. All of the findings support the hypothesis that the synergistic activity of microbes can increase the benefits of plant growth promotion even in the presence of pathogens and pesticides (Mishra and Sundari, 2015).

From a pesticide polluted agricultural area, Akbar, S., and Sultan extracted and characterized two bacterial cultures, *Achromobacter xylosoxidans* and *Ochrobactrum sp.*, with chlorpyrifos-degradation capacity. Within 10 days, these strains have been able to metabolize 84 and 79 % of 100 mg/ L chlorpyrifos, respectively. These isolates were capable of breaking down chlorpyrifos in both sterilized and non-sterile soils and were able to break down almost 100% of the chlorpyrifos in 42 days. These strains also exhibited significant plant growth-enhancing features in the absence and presence of chlorpyrifos. Chlorpyrifos exhibited a detrimental influence on plant development and reduced the % germination, growth of the plant, and biomass, according to a plant growth experiment. Chlorpyrifos-decomposing bacterial inoculation in the soil considerably improved the plant growth properties (Akbar and Sultan, 2016).

Bacillus amyloliquefaciens, a rhizobacterium that promotes plant development, is capable of degrading a wide spectrum of organophosphorus insecticides efficiently. Meng, and colleagues published the entire genome sequence of this bacteria with genomic size, protein-coding genes, and 46 % GC content. Numerous genes previously characterized are engaged in phosphorus solubility, organophosphates pesticide degradation, indole-3-acetic acid, and siderophores generation have been identified based on genome sequencing. Interestingly, when compared to other *B. amyloliquefaciens* species, the isolated strain possessed a bigger genomic size and the greatest number of protein-coding genes. These findings demonstrate the potential of the strain *Bacillus amyloliquefaciens* for environmental bioremediation (Meng *et al.* 2019).

Kumar *et al.* explored the plant growth-promoting activity and profenofos eradication efficacy of the bacterial isolates *Acinetobacter sp.* and *Comamonas sp.* both alone and in combination. A comparison of several plant growth-promoting activities with the bacterial strains revealed that *Acinetobacter sp.* outperformed *Comamonas sp.*

Physiological criteria including root length, shoot length, sprout height, chlorophyll a, chlorophyll b, and carotenoids were greater in pot culture investigations while using the combined culture. Similarly, the combined culture exhibited the highest profenofos disintegration in pot sand and pot soil. Soil enzyme functions, as well as root cross-sectional area, total stele area, and xylem channel area, have been also greater for the bacterial consortium in a pot soil study (Kumar *et al.* 2020).

D. M. Dash and J. W. Osborne investigated the bioremediation of monocrotophos by native bacterium extracted from cultivable sugarcane soil. The isolated strain was confirmed as *Bacillus aryabhatai* by 16S rRNA gene sequencing. The strain *Bacillus aryabhatai* demonstrated the highest monocrotophos tolerance and degradation capacity. *Bacillus aryabhatai* was confirmed to be the effective strain for pesticide breakdown and plant growth-enhancing rhizobacterial characteristics. The microbial degradation of monocrotophos was tracked using a UV and HPLC, and the metabolites were characterized using GC-MS. The kinetics of degradation showed 93 % degradation within 5 days. The addition of *Bacillus aryabhatai* to the root system of *Liriope muscari* improved plant growth and monocrotophos breakdown in the soil (Dash and Osborne, 2020).

Govarthanan *et al.* investigated the feasibility for rapid chlorpyrifos biodegradation by plant growth-enhancing psychrophilic bacteria acquired from saltwater using the enrichment culture approach. The strain was characterized as *Shewanella sp.* by biochemical testing and 16 S rDNA sequencing. *Shewanella sp.* grew at a substantial rate at varied chlorpyrifos concentrations. With or without chlorpyrifos, the isolate produced plant growth stimulating factors. Within 24 h, the isolate demonstrated up to 95 % chlorpyrifos degradation. Furthermore, the media conditions were tuned for improved pesticide removal, and 93 % removal was observed in the presence of 4 % glucose in neutral pH at 33 °C. The

results of IR and HPLC revealed that *Shewanella sp.* plays a role in the biomineralization of chlorpyrifos (Govarthanan *et al.* 2020).

Khatoon reviewed the in-depth information on the current plant growth-promoting rhizobacteria core mechanisms as well as applicability as bio-control agents, plant development stimulators, and soil rhizoremediators, with the ultimate goal of promoting more agro-ecological practices for sustainable agriculture (Khatoon *et al.* 2020).

Kour's research team highlighted various categories of microbes involved in environmental remediation, their biodiversity, and recent advances and scientific understanding on using these microbes as productive bio-resources for environmental decontamination (Kour *et al.* 2021).

Muhammad Usman Ghani's study team assessed the efficacy of bacterial strains susceptible to promoting plant growth and biodegrading profenofos. The bacterial strains were extracted from an agriculture field with a history of profenofos exposure. The isolates were characterized using biochemical and molecular techniques. After 9 days of incubation, one strain, *Enterobacter cloacae*, decomposed more than 90% of the profenofos. Plant growth was also greatly boosted in plants cultivated with profenofos-polluted soil treated with the same bacterial strain (100 mg/L). The results revealed that the inoculation of profenofos disintegrating bacteria improved the growth of plants and profenofos breakdown (Ghani *et al.* 2021).

Chapter III

Green Synthesis and Characterization of Iron Nanoparticles from Tea Waste

Abstract:

Iron nanoparticles (INPs) offer the next era of environmental remediation strategies that have the potential to deliver cost-effective solutions to severe environmental cleanup problems. Nanoscale iron particles possess greater surface area, a large number of active sites, and high surface activity. They also offer a great deal of versatility for in-field applications. INPs are particularly effective at transforming and detoxifying a wide range of typical environmental contaminants, including pesticides, heavy metal ions, dyes, and other hazardous organic and inorganic pollutants. To improve the promptness and effectiveness of clean-up, the surface of the iron nanoparticles has been modified with catalysts and other capping agents. To generate zero-valent (Fe(0)) INPs, traditional approaches such as top-down and bottom-up synthesis are employed. Each of these processes has limitations, such as being expensive, requiring unique and expensive equipments, producing flammable gases during the process, and the toxicity of the reducing agents. The majority of these procedures use toxic compounds that are harmful to all living things and the ecosystem. These drawbacks have triggered research into greener approaches involving the development of simple, cleaner, and more effective and environmentally sustainable reducing agents for the fabrication of nanomaterials, culminating in the nexus of nanotechnology, environmental clean-up, and green chemistry. Hence, in the present investigation, the INPs have been fabricated *via* a greener method using tea waste for the absorptive removal of profenofos (PF) from an aqueous solution. The as-synthesized INPs have been systematically characterized by various spectral and analytical techniques.

3.1. Introduction

Nanotechnology is a new multidisciplinary domain that brings altogether chemical, physical, biological, material, pharmaceutical, and engineering sciences (Meyer and Persson, 1998). Environmental clean-up, drug transport, telecommunications, biotechnology, catalytic industry, cosmetology, the space station, cancer therapy, and material research have all benefited from nanotechnology and nanoparticles (Bhattacharyya *et al.* 2009). Because of their distinctive structural and physicochemical features, including extremely small size, morphology (sheet, cylinder, tubes, and filament), and particle dispersion, nanomaterials have attracted enormous interest. They have magnetic, refractive, thermodynamic, and tensile strengths, etc. Organic nanoparticles, also known as carbonaceous nanostructures, and inorganic nanoparticles, which are usually formed from semiconducting materials, are the two main types of nanoparticles (Hasan, 2015)

Iron is the fourth more prevalent metal in the earth's crust, and its use in human history dates back around 3000 years, to the start of the Iron Age (Frey and Reed, 2012). Despite its long history, the twentieth century offered new diverse applications and issues for metallic iron as a result of growing nanotechnology and the development of nanosized zero-valent iron. It has blatantly different characteristics than native iron, and now it has transformed the most prominent pioneered nanoparticles, as well as the most prevalent candidates for environmental clean-up, due to its cost-effectiveness, high reactivity, and excellent sorption efficiency (Li, Ling, *et al.* 2006). Oxide, mono, di as well as tri-metallic INPs are frequently documented as promising candidates for environmental clean-up (Yan, Weile, *et al.* 2013). Multiple kinds of iron oxides nanomaterials were discovered. They have antimicrobial capabilities and have been used for the degradation of dyes (Raman and Kanmani, 2016), wastewater remediation (Pasinszki and Krebsz, 2020), elimination of metal pollutants (Bhateria and Singh, 2019), insecticides (Raychoudhury and Scheytt, 2013) and also as heterogeneous catalyst materials in ecosystem functioning (Goncalves and

Alves, 2021). The development of INPs for clean-up is attributed to their magnetic susceptibility, mildness, un-hazardousness, and reversible redox capacity when interacting with water (Huber, 2005).

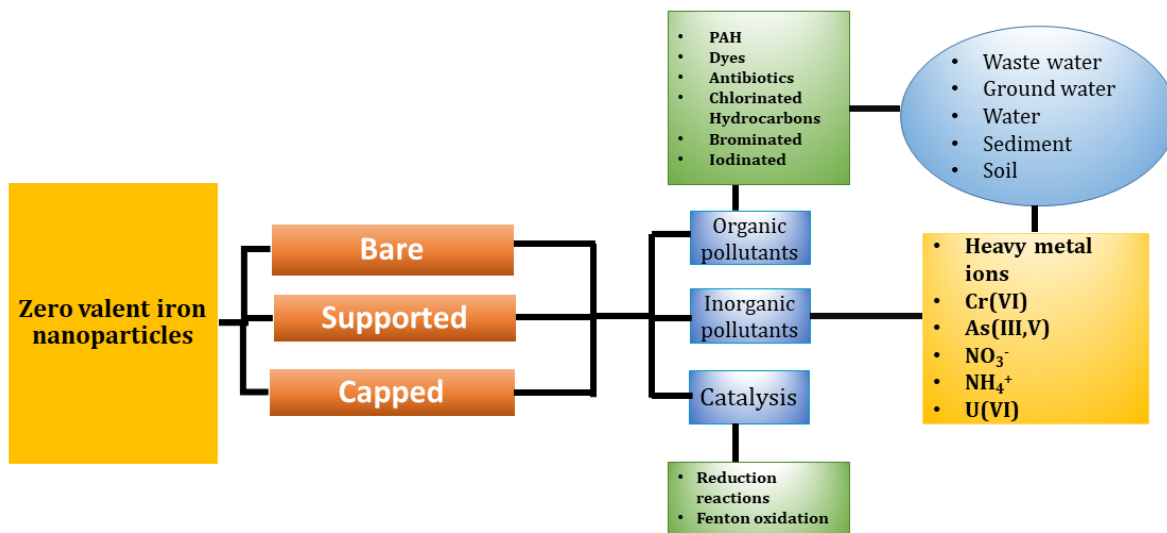


Figure 5. Various types of zero-valent INPs, and their applications

The origins of the Fe(0) nanomaterial technique could be linked back to Gillham and O'Hannesin's discovery that bulk Fe(0). It was capable of reducing a set of halogenated hydrocarbons in underground water (Gillham and O'Hannesin, 1994). The upsurge in popularity in using nano Fe(0) as a remediation strategy for polluted soil and water bodies is owing to its greater activity over bulk Fe(0), financial feasibility, and ability to treat a wide spectrum of pollutants (Figure 5). Over the last two decades, Fe(0)-based clean-up has emerged as an important area of environmental nanotechnology, with more than 50 successful trials and large-scale operations carried out globally and a substantial volume of research articles being published on the issue in recent years (Yan *et al.* 2013).

For the engineering of nanoparticles, two methodologies have been proposed: top-down and bottom-up strategies. Top-down techniques, including grinding, engraving, crushing, and tooling, entail the breakdown of bulk material, whereas bottom-up methods,

including chemical method (reduction), self as well as positional amassing which incorporate the assembly of smaller units to create a bigger framework - a process is termed as nanomaterial growth (Arole and Munde, 2014). The synthesis of nanoparticles *via* physical and chemical methods requires the use of reducing agents to react with a precursor material. The majority of these reducing agents are harmful to mankind, living organisms, and the ecosystem (Jamkhande *et al.* 2019). Due to this, exploration of biochemical techniques, including the invention of easier, cleaner, and environmentally friendly reducing compounds for the synergistic engineering of nanomaterials, has become imperative, culminating in the convergence of nanoscience, environmental clean-up, and green chemistry (Saif *et al.* 2016).

Various greener methods for the production of INPs (Figure 6) include the utilization of microorganisms like bacteria, fungi, or algae, plant-based products such as seeds, leaflets, flowers, etc have been demonstrated (Pattanayak *et al.* 2021). INPs can also be synthesized using different biological chemicals such as amino acids, vitamins, and enzymes. Implementing a greener protocol requires careful consideration of factors such as expense, energy, environmental impact, and accessibility (Fahmy *et al.* 2018). There are numerous advantages of using biologically generated nanoparticles over those produced by physical or chemical techniques (Ijaz *et al.* 2020). Biosynthesized nanoparticles frequently exhibit decreased toxic effects, aggregation, and long-term stability (Sadhasivam *et al.* 2020). As a result, biological materials, especially waste materials drawn more attention from the research community (Bolade *et al.* 2020).

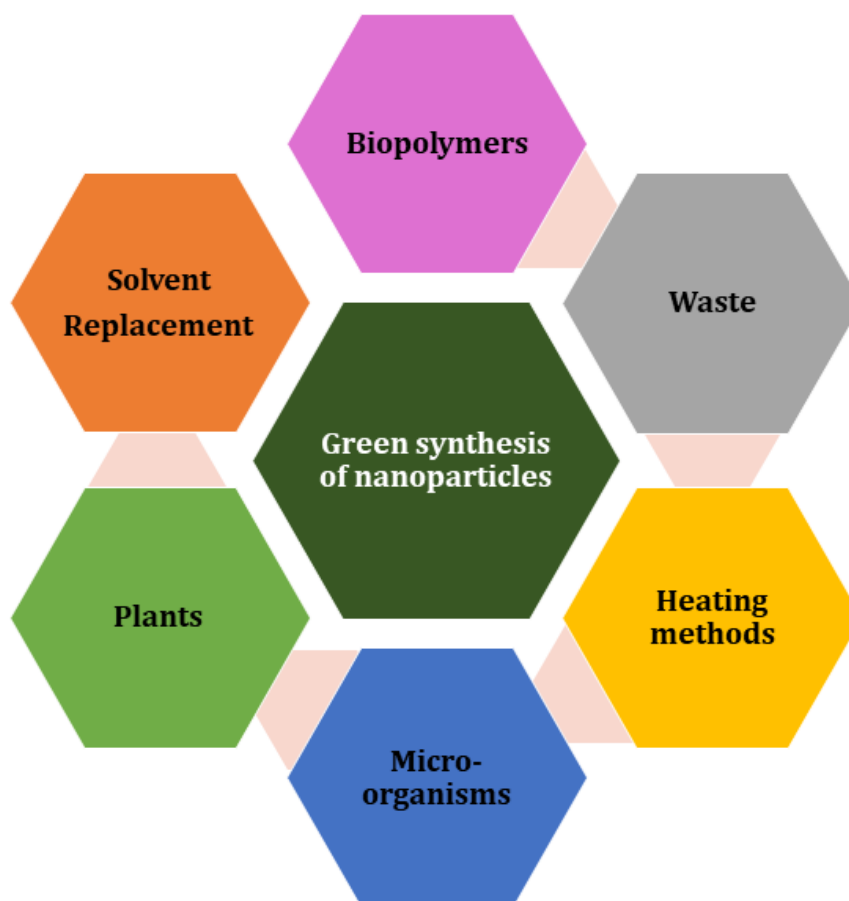


Figure 6. Various methods of green synthesis of nanoparticles

Only a limited number of reports are available on the surface characterization of zero-valent nanoparticles (Ealia and saravanakumar, 2017). To address the primary reaction pathway, reaction rate, and key intermediate or final product formations requires a thorough understanding of surface characteristics is mandatory (Samrot *et al.* 2021). The surface features also influence the mobility, diffusion, and destiny of nanomaterials in the ecosystem. Nonetheless, defining the typical iron nanomaterials is commonly impractical since Fe(0) nanoparticles synthesized using different processes might have drastically varied characteristics. INPs constitute reactive entities, with surface features that alter quickly and significantly with time, solution chemistry, and ambient circumstances. Extensive surface characterization methodologies, including morphology and surface, elemental composition,

and spatial three-dimensional distribution of functional groups, are employed to gain a deeper understanding of surface attributes (Punia *et al.* 2021). XRD, FT-IR, TEM, AFM, photoelectron spectroscopy, SEM, XPS, vibrating sample magnetometry, zeta potential, and TGA are some of the fundamental techniques used to analyses INPs.

Some researchers have synthesized INPs by using various fresh tea extracts (Huang *et al.* 2014; Xin *et al.* 2016; Ali *et al.* 2018; Lin *et al.* 2020) and also by using tea wastes (Panneerselvam *et al.* 2011; Lunge *et al.* 2014; Gautam *et al.* 2018). However, in contrast to previous research, the INPs have been fabricated by tea waste extract using a greener method for the removal of profenofos pesticide from the aqueous solution. Rising pollution of freshwater by a variety of contaminants is one of the most serious environmental issues confronting humanity on a global scale. Numerous studies have been devoted to developing more efficient, cost-effective, and robust ways for wastewater treatment that do not exacerbate environmental stress or threaten human health. Further, a huge amount of tea waste is generated every day throughout the world. Hence, we employed tea waste to fabricate iron nanoparticles by eco-friendly strategy for the adsorptive removal of profenofos pesticide from the aqueous solution. The green synthesized INPs were systematically characterized by various analytical techniques such as Ultra Violet-visible (UV-vis) spectroscopy, Fourier transforms infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), powder X-Ray diffraction (PXRD) and dynamic light scattering (DLS).

3.2. Experimental Section

3.2.1. Materials

Ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was purchased from Sigma Aldrich (India) and used without further purification. Tea waste was obtained from a local Tea stall

near Bharathidasan University, Tiruchirappalli, Tamil Nadu (India). All the experiments were performed by using distilled water.

3.2.2. Synthesis of INPs Using Tea Waste Extract

Solid tea waste was collected from a tea stall near Bharathidasan University, Tiruchirappalli, India. The collected tea waste was washed with distilled water to remove impurities and dried at ambient atmosphere. Tea extract was prepared by boiling 60.0 g of solid tea waste in one liter of distilled water at 80 °C for 30 min and allowed to cool at room temperature. The solid tea component was removed by vacuum filtration, and the filtrate solution was used to synthesize INPs. The prepared tea waste extract was added to 0.10 M of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at a volume ratio of 2:1 respectively at ambient temperature.

3.2.3. Characterization Techniques

The UV-vis spectrum of INPs was recorded using ELICO-Double beam SI-210 spectrophotometer between the range 200-800 nm. FT-IR measurements were performed using Perkin-Elmer 597 spectrophotometer in the spectral range 400-4000 cm^{-1} . The PXRD spectrum of INPs was recorded using the Rigaku Miniflex-600 instrument at Alagappa University, Karaikudi, Tamilnadu (India). The surface morphology of the INPs was investigated using SEM analysis (Carl Zeiss Sigma, Sitra, Coimbatore, India). The hydrodynamic size of INPs was established using TEM-FEI by TECNAlG2-F30 equipment functioning with 200 kV accelerated voltage. The INPs were directly mounted on the copper grids and examined under TEM and EDX (Oxford Instruments, UK) analysis to confirm the presence of elements in the prepared INPs. The zeta potential analysis of synthesized INPs was investigated using Malvern Instruments, UK.

3.3. Results and Discussion

3.3.1. Synthesis of INPs Using Tea Waste Extract

The prepared tea waste extract was added to 0.10 M of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at ambient temperature. The immediate color change appears from pale yellow to deep brown, indicating the formation of INPs (Figure 7). The formed INPs were isolated by vacuum filtration and then washed with distilled water several times, and air-dried. Afterward, the prepared INPs were used for further experiments.

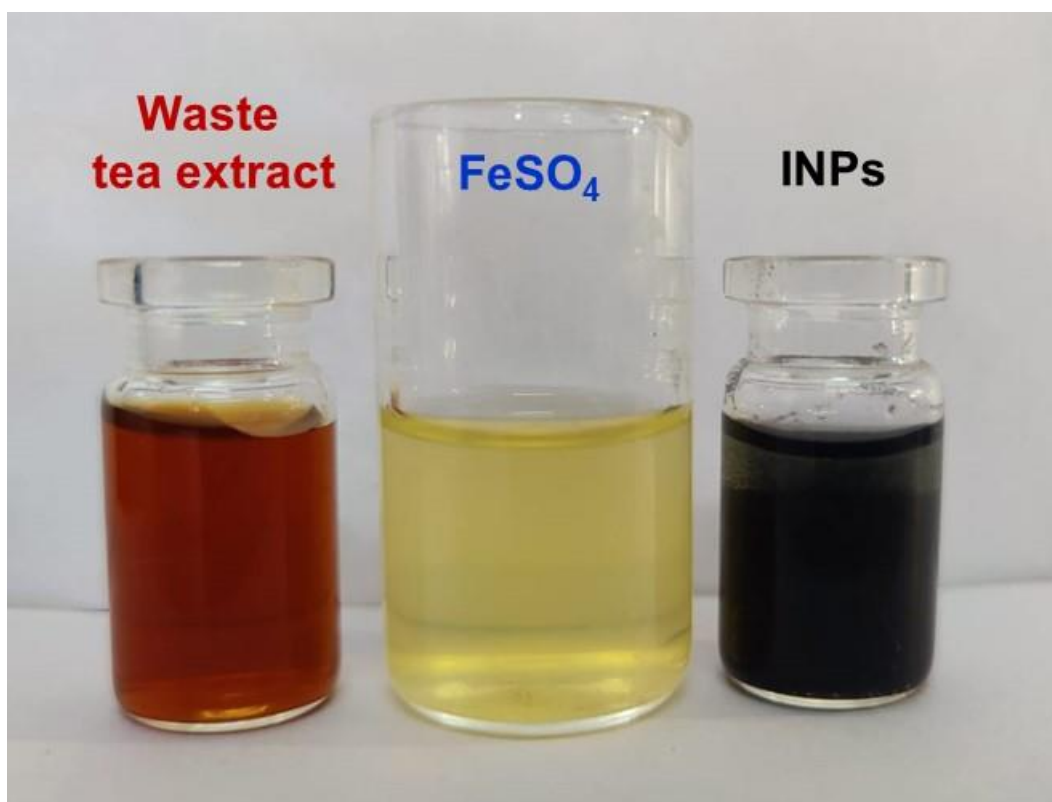


Figure 7. Photographic images of color changes during the formation of the INPs.

3.3.2. Characterization of INPs

This work describes the efficient fabrication of low-cost and green INPs as sorbents from tea waste extract for the effective removal of organophosphorus pesticide profenofos. Further, the as-synthesized INPs have been systematically characterized by various spectral and analytical techniques. The size, shape, and distribution of INPs have been studied using

TEM. The hydrodynamic diameter was calculated using DLS analysis. To measure colloid stability, zeta potential analysis was used. The elemental content of nanoparticles was determined using an EDX spectrum. The forms of INPs were determined using X-ray diffraction (XRD) examination. The presence of INPs and biomaterials on the nanoparticle's surface were identified using FT-IR. The UV-vis absorption spectrum has been recorded to examine the nature of INPs.

3.3.2.1. UV-vis Spectroscopy

One of the most essential characterization techniques for nanoparticles is UV-vis spectroscopy. The ELICO-Double beam SI-210 spectrophotometer was used to investigate the surface plasmon resonances (SPR) of fabricated INPs. SPR is the emergence of a resonance effect resulting from the interaction between the conduction band electrons of metal nanoparticles and incoming photons. The interaction is influenced by nanoparticles' size and shape, nature, and content of the distribution medium. Using the fabricated INPs in dichloromethane solution, UV-vis spectroscopic examination was performed. The visible light absorption caused by SPR excitation provides different colors to nanomaterials. The color of the solution changes as the size of the nanoparticles changes. As a result, the UV-vis absorption pattern is extremely sensitive to the production of nanoparticles. UV-vis analysis was performed on the green synthesized INPs. The UV-vis spectrum of the INPs is depicted in Figure 8. The maximal absorption of the nanoparticles is centered at 330 nm. The character of the intense band is ascribed to the particles' consistent size and shape. Further, the continuous absorption of INPs near 330 nm indicates the amorphous nature of INPs. The study's findings are likewise in good accord with those obtained by the Devatha (Devatha *et al.* 2016) and Viju Kumar (Viju Kumar and Prem, 2018) research groups.

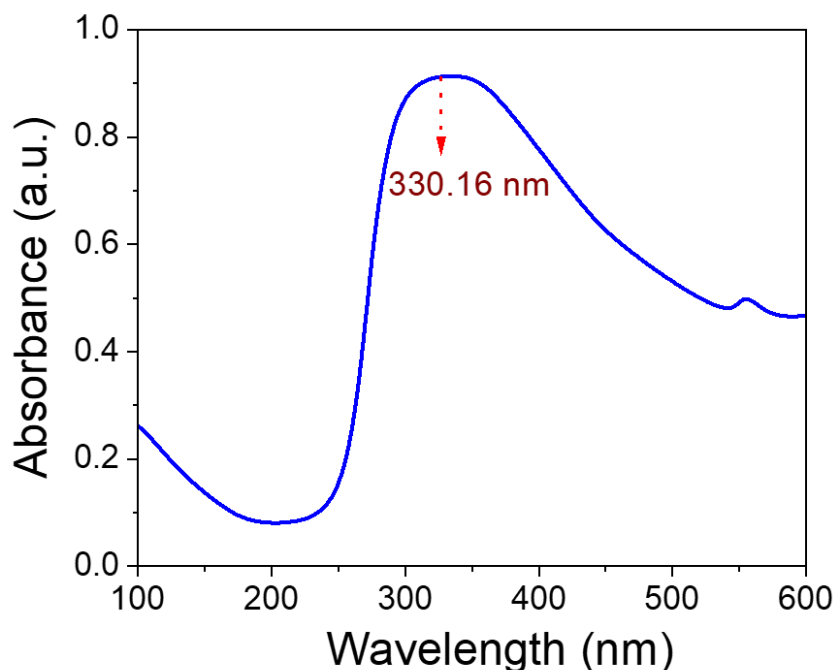


Figure 8. The UV-vis spectrum of INPs (the arrow designated the INPs UV band at 330.16 nm).

3.3.2.2. FT-IR Spectroscopy

Typically, the appearance of certain significant transmittance bands in the FT-IR spectrum of fabricated INPs indicates that the nanoparticles have been functionalized with green reducing agent molecules. It has been demonstrated that exposing INPs to tea waste extract results in encapsulation and stability of INPs. Hence, FT-IR spectroscopy was used to predict the existence of various functional groups on the surface area of INPs. It is performed to detect the prospective components of tea waste extract accountable for the reduction of iron ions and that could operate as a capping agent of the reduced INPs, hence aiding in the stabilization of INPs. The spectrum was measured using a Perkin-Elmer 597 spectrophotometer in the 400-4000 cm^{-1} range. The FT-IR spectrum of INPs is presented in Figure 9. The transmittance bands around 3220-3600 cm^{-1} may be attributed to the primary N-H or O-H stretching of the molecules from the waste tea extract or O-H stretching of surface water molecules adsorbed (Gautam *et al.* 2016). The IR band around 1600 cm^{-1} is

ascribed to aryl-substituted C=O/ C=C /C=N stretching or O-H bending vibrations of surface adsorbed water molecules. In addition, characteristic peaks corresponding to Fe-O were observed around 540-650 cm^{-1} (Mansouriieh *et al.* 2016). The thin FeO shell enabled the appearance of Fe-O absorption bands in the IR spectrum, but at a lower intensity than is typical of iron oxide nanoparticles. These findings suggested that the capping and reducing the activity of the compounds in tea waste extract shield the surface of as-fabricated iron nanoparticles against oxidation.

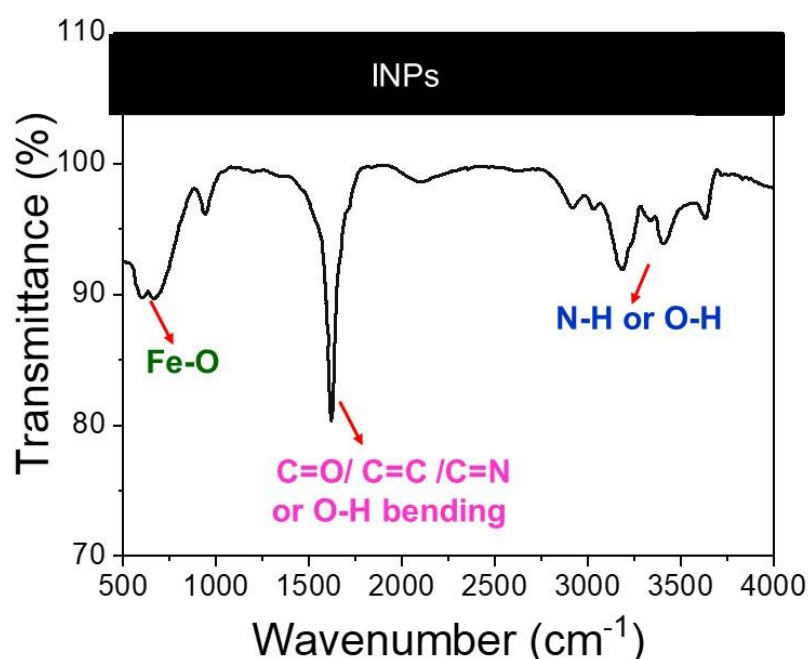


Figure 9. FT-IR spectrum of INPs fabricated from tea waste extract

3.3.2.3. Scanning Electron Microscopy (SEM)

The surface morphology of the as-synthesized INPs is studied using scanning electron microscopy. The methodology was employed to confirm the particle size and shape of the fabricated INPs. SEM examination is performed on the samples using Carl Zeiss Sigma equipment at Sitra, Coimbatore, India. The SEM image of fabricated INPs is exhibited in Figure 10. The tea extract plays a vital role in forming the nano-scale structure and size of the INPs. INPs are well-organized and round-shaped particles that were

distributed without agglomeration. The SEM analysis confirmed that the INPs have a hydrodynamic size of 60.37 ± 2.65 nm. The shape and size of the INPs were found to be similar to the results obtained by Li *et al.* (Li *et al.* 2009)

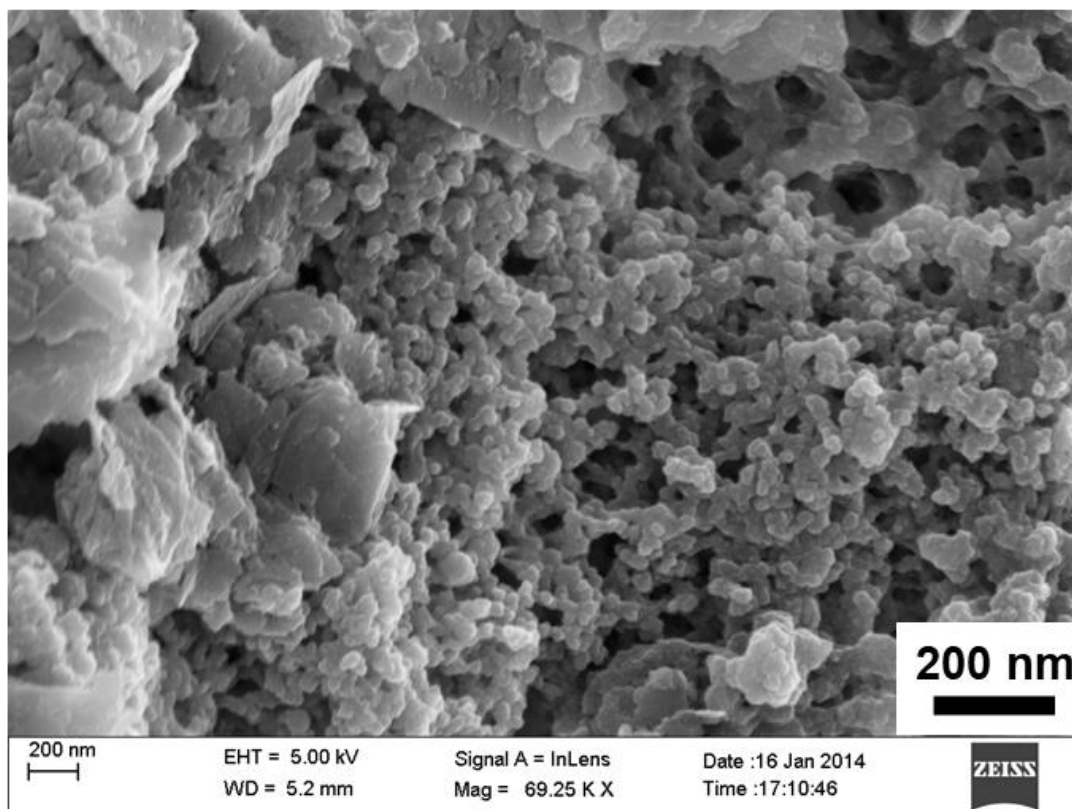


Figure 10. Morphology of INPs determined by scanning electron microscopy (SEM). Scale bar of the picture 200 nm.

3.3.2.4. Dynamic Light Scattering (DLS)

The size of nanoparticles derived from bulk materials is a crucial criterion in determining their efficiency. The average hydrodynamic size and size distribution of green-synthesized INPs were estimated by DLS methodology on TECNAIG2-F30 TEM-FEI equipment operating at 200 kV accelerated voltage. At room temperature, the intensity of the dispersed light was measured. Figure 11 depicts the particle-size distribution of green-synthesized INPs as determined by DLS analysis. DLS analysis was used to calculate the

intensity-average diameter of nanoparticles, which ranged from 0.1 to 10000 d.nm and had a mean particle size of 255.37 ± 1.06 nm. Since DLS measures the hydrodynamic size of the overall nanoparticles, including the particles of tea waste extract, the difference in the size (Dutz *et al.* 2015) of the INPs determined by SEM analysis is related to the measurement of the INPs as well as the capping materials that surround them.

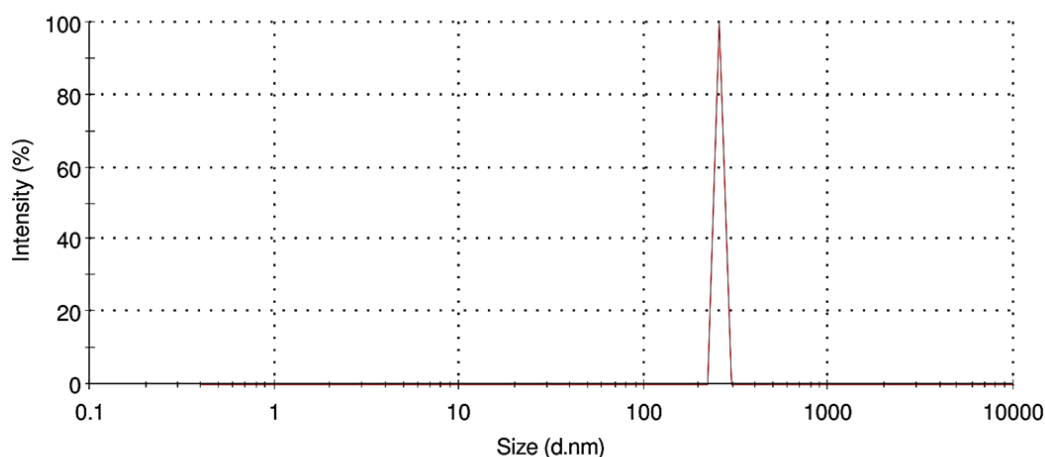


Figure 11. Hydrodynamic size parameters of INPs are determined by the dynamic light scattering (DLS) method.

3.3.2.5. Zeta Potential

The zeta (ζ) potential is stated as the electrokinetic potential in the surface of shear compared to that in distant bulk material, is frequently used to assess the surface charge and immediate surroundings of nanoparticles. The surface charge or zeta potential of nanoparticles in an electrostatic field is the most important element determining their mobility. Furthermore, the data provide an important requirement for the system's stability. The degree of the zeta potential in a dispersion reflects the extent of electrostatic repulsion between neighboring, equally charged nanoparticles.

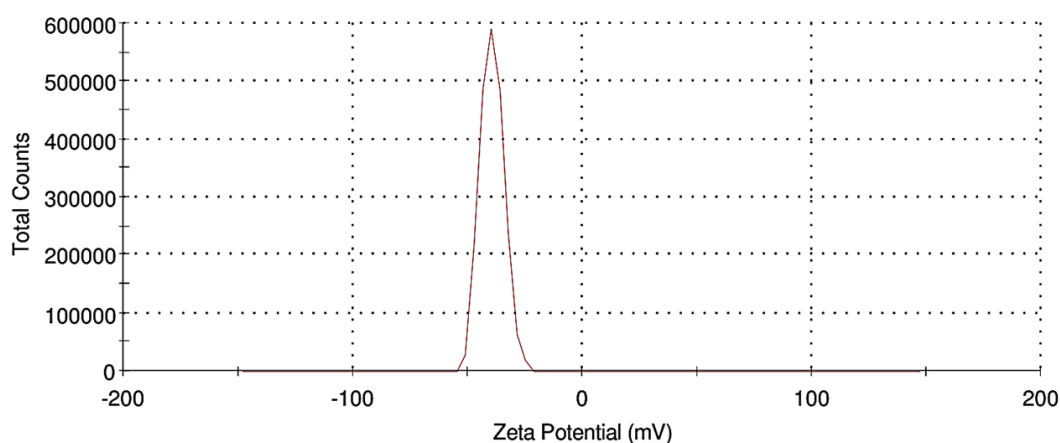


Figure 12. Zeta potential of INPs.

A high zeta potential confers stability on molecules and particles of sufficient size, where the solution or distribution will prevent agglomeration. When the potential is low, attractive forces may dominate the repulsion, causing the dispersion to disintegrate and agglomerates. Colloids with a high negative or positive zeta potential are electronically stable, whereas colloids with a low zeta potential agglomerate. Measurement of ζ potential was carried out using Malvern Instruments, UK. If the value of the potential is between ± 10 to ± 30 mV, the particles are said to have incipient stability. If the voltage oscillates between ± 30 to ± 60 mV, the particles have high to moderate stability. The zeta potential results (Figure 12) clearly showed that the prepared INPs showed anionic nature in aqueous solution because of the zeta potential value, -39.2 ± 1.25 mV (Katuwavila *et al.* 2016). It indicates the formation of stable INPs which could effectively remove the PF molecules from the aqueous phase.

3.3.2.6. Energy-Dispersive X-ray Spectroscopy (EDX) with Transmission Electron Microscopy (TEM)

EDX is the most familiar non-destructive X-ray technology for determining the elemental analysis of nanoparticles even from a small quantity of the samples. EDX is a type of electron microscopic technology that is commonly used in conjunction with SEM

and TEM to determine the link among the composition, micro-structural, and elemental spectral features. This is an important method for determining the composition, physicochemical, and chemical characteristics of biosynthesized nanoparticles. INPs were generated in this study using tea waste extract. The existence of INPs with strong and medium intense bands in the spectra was corroborated by EDX analysis. On a big scale, EDX analysis is utilized to obtain quick information on elemental composition and the existence of any impurities. The EDX spectrum of INPs with respective TEM mapping images is presented in Figure 13 and Figure 14. It revealed the elemental composition of INPs which contains iron (Fe) 71.85%, sulfur (S) 14.13%, phosphorus (P) 11.18%, and potassium (K) 1.43%. Herein, S, P, and K may be attributed to the tea extract (Gautam *et al.* 2016). The corresponding elements of prepared INPs were identified during the TEM analysis, which confirmed the successful formation of INPs from tea waste, as shown in Figure 14.

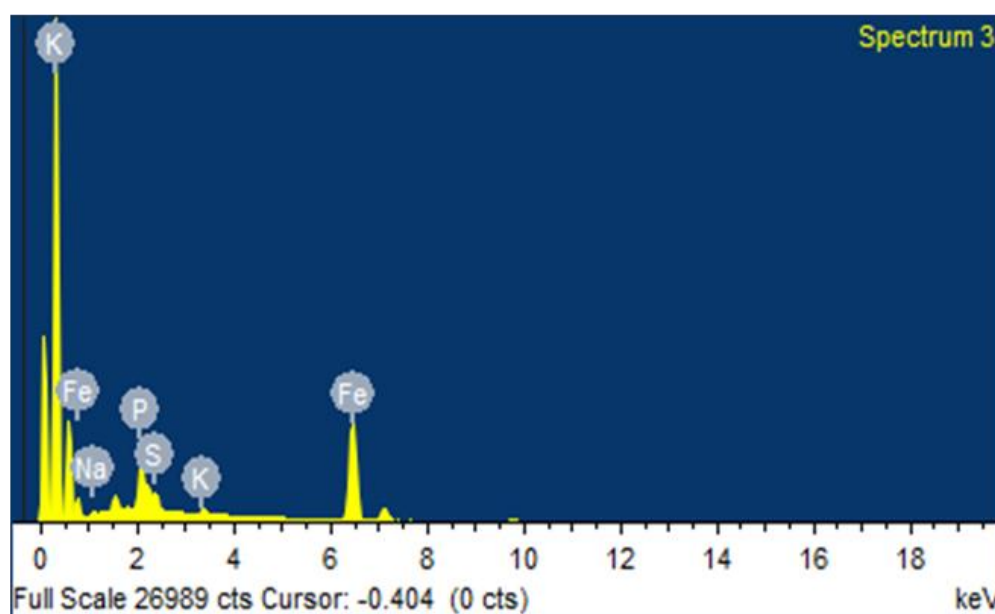


Figure 13. Elemental analysis of INPs.

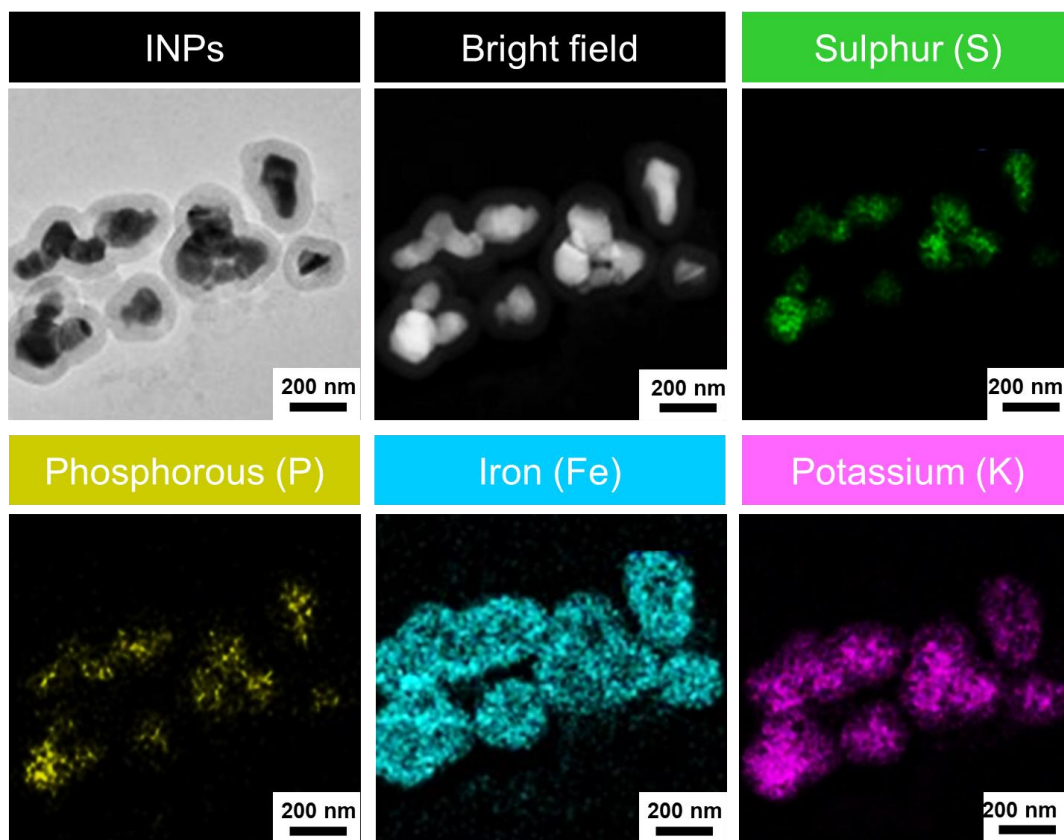


Figure 14. Elemental mapping analysis of INPs (elements were present in the nanocomposite including Iron (Fe), sulfur (S), phosphorous (P), Potassium (K)). Scale bar of the picture 200 nm.

3.3.2.7. Powder X-Ray Diffraction (PXRD)

PXRD technique is employed to measure the extent of crystallinity or the amorphous nature of nanomaterials. The size of the particles and crystallite size are two separate features of a material. Particles are made up of tiny crystallites. The size of crystallites is a basic feature of materials. The properties of nanomaterials are determined by crystal size rather than particle size. PXRD can reliably identify the size distribution of nanomaterials by analyzing millions of nanocrystals. PXRD provides various advantages, including non-destructive nature, high accuracy, consistency, and depth profiling of nanomaterials. The PXRD pattern of prepared INPs was shown in Figure 15, which is in good agreement with the JCPDS card number 87-0721 (Vinod *et al.* 2016). The

characteristic peaks of INPs at $2\theta = 44.90^\circ$, 36.52° , 33.29° , and 20.35° indicate the successful formation of zero-valent INPs from tea waste (Gautam *et al.* 2016). Moreover, the PXRD patterns suggested that the prepared INPs were amorphous, which could be more effectively interacting with PF in the aqueous phase.

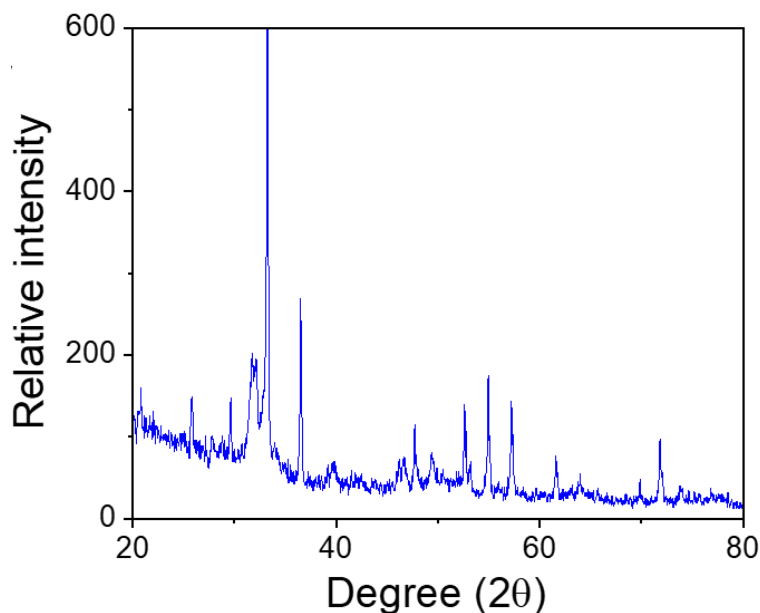


Figure 15. Powder X-ray diffraction (PXRD) of INPs.

3.3.2.8. Stability Analysis of INPs

The long-term stability of adsorbents is crucial that determines their efficacy and regulates their progress towards technological applications. Therefore, the stability of the titled INPs was examined by the SEM and DLS analysis for one week. The SEM analysis was performed on 1, 3, 5, and 7 days and the samples were incubated at room temperature. The pattern of the SEM images of INPs remains unaltered during the overall incubation time, and the results are shown in Figure 16A. The respective incubated images of INPs are shown in Figure 16B. The DLS analysis was used to examine the changes in the size, polydispersity index (PDI), and the zeta potential of the INPs. The incubation of the fabricated INPs for the particular period (Day 0-7) caused only slight changes in the size,

PDI, and zeta potential, which confirmed that the INPs are highly stable in water (Figure 17).

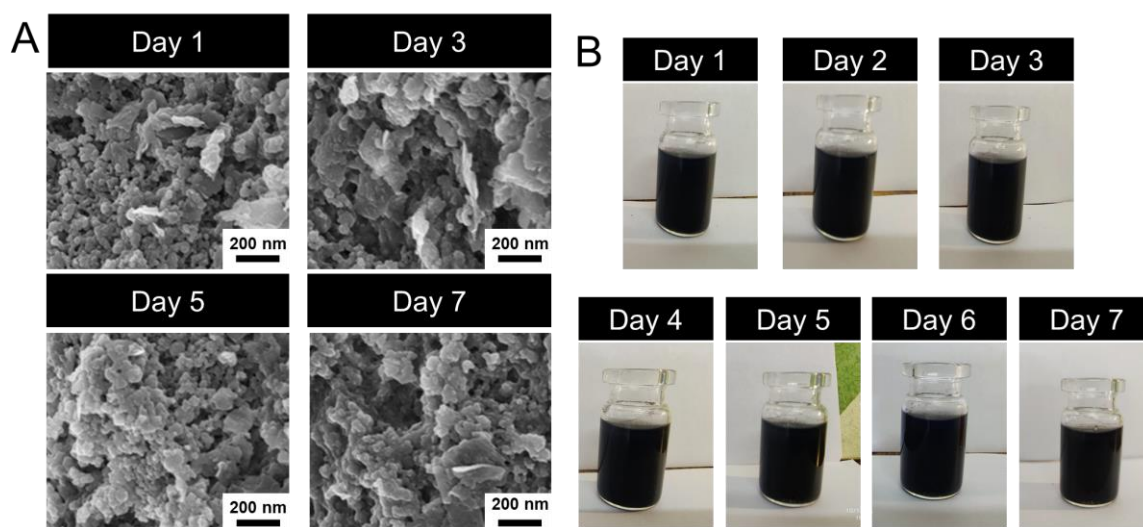


Figure 16. A) The stability of the INPs were confirmed by the SEM analysis on different incubation time (Day 1-7). B) The images of INPs at different incubation times.

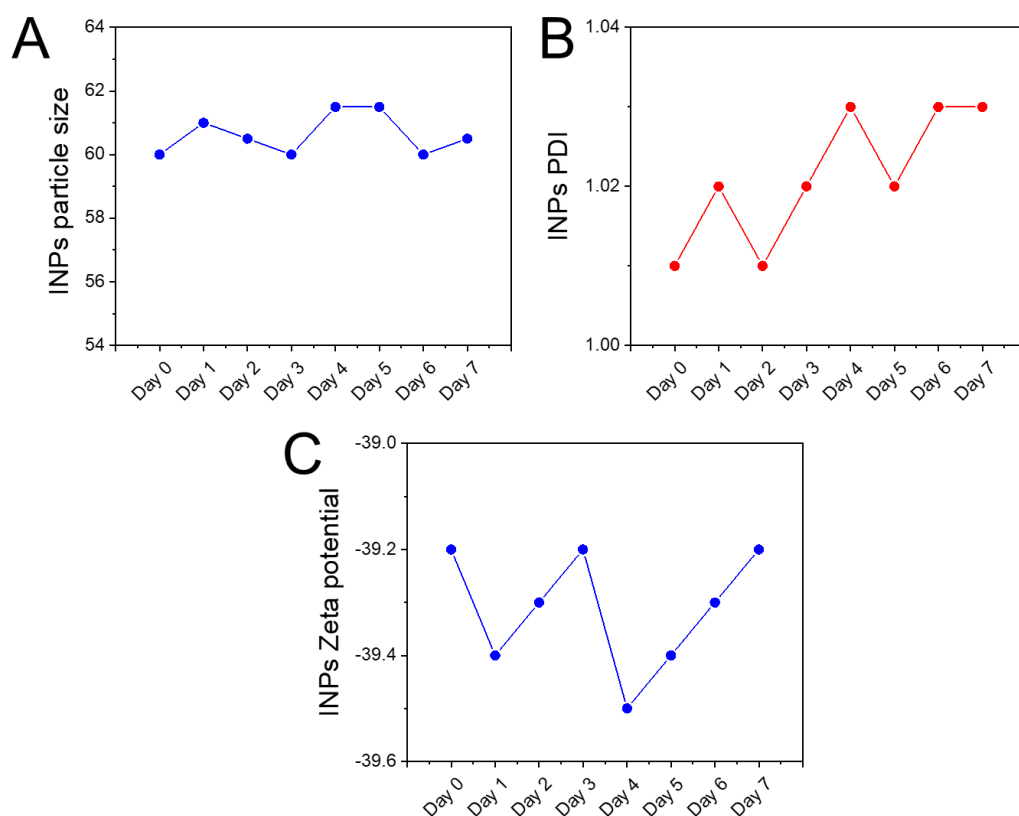


Figure 17. (A-C) The stability of the INPs was confirmed by the DLS analysis on different incubation times (Day 1-7) with particle size, PDI, and zeta potential.

3.4. Conclusion

Ecofriendly fabrication of metal nanomaterials is a strategy that reduces the formation of secondary pollutants, harsh chemicals, and noxious solvents, among other things, which have harmful effects not only on the ecosystem as well as on public health. The capability of tea waste extract to function as an alternative reducing agent for the sustainable synthesis of INPs was investigated in the present study. UV-vis, FT-IR, SEM, DLS, zeta potential, EDX with TEM elemental mapping, and PXRD techniques were used to extensively characterize the as-synthesized INPs. Green synthesized INPs were shown to have good water stability, an anionic surface nature, and an average particle size of 60.37 ± 2.65 nm. Furthermore, the existence of various elements and functional groups in the tea waste extract that are responsible for the generation of amorphous INPs was substantiated using FT-IR, EDX, TEM elemental mapping, and PXRD techniques. The findings corroborated the benefits of green synthesis and ensured the suitability of the fabricated INPs for the adsorption-mediated removal of harmful pesticides from aquatic environments.

Chapter IV

Green Synthesized Iron Nanoparticles as an Efficient Adsorbent towards the Removal of Profenofos Pesticide from the Aquatic Environment

Abstract:

Pesticide contamination has led to serious environmental issues that pose a significant threat to living organisms throughout the world. Therefore, the significant removal of pesticides from aquatic environments has attracted the attention of environmental scientists and academicians in recent decades. Hence, in the present investigation, the iron nanoparticles (INPs) from tea waste were fabricated as an efficient adsorbent towards the removal of profenofos (PF) from the aqueous solution. The as-synthesized INPs were systematically characterized by various analytical techniques. Various influencing factors such as contact time, adsorbent dosage, solution pH, and initial concentration of pesticide were systematically optimized for the maximum adsorption efficiency of PF by the prepared INPs. Based on the obtained results, the INPs showed greater adsorption efficiency towards the removal of PF. The kinetic data were analyzed using various adsorption kinetic models such as pseudo-first-order, pseudo-second-order, and intra-particle diffusion. Further, the interaction between prepared INPs and PF was evaluated using various adsorption isotherm models such as Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich (D-R) models. The monolayer Langmuir adsorption capacity of 43.67 mg/g was obtained with a minimum contact time of 15 min. According to the kinetic and isotherm data, the pseudo-second-order kinetic and Langmuir isotherm models were found to be fitted well for PF adsorption using the prepared INPs. The obtained findings of this study demonstrated that the fabricated INPs as an efficient material towards the removal of pesticide molecules from aquatic environments.

4.1. Introduction

Modern agriculture is heavily reliant on the usage of various agrochemicals, which substantially improve efficient and cost-effective crop production to fulfill the food needs of the world's rapidly expanding population (Paarlberg, 2009). Agrochemicals are widely employed in agriculture to conduit the gap between the food production and consumption required to address rising food demand (Clay, 2004). However, the unbalanced utility of agrochemicals degrades the environment and poses significant problems to food safety, environmental and human health, ecological balance, and soil biodiversity protection (Rani *et al.* 2021). Organophosphate compounds are ester derivatives of phosphoric acids and thiophosphoric acids. For over five decades, they have been used as insecticides. They are still employed as insecticides, acaricides, nematocides, helminthicides, fungicides, nerve agents, and herbicides (Mansouriieh *et al.* 2019). Despite they have been highly effective in agricultural pest management worldwide and their widespread usage has resulted in countless poisonings of off-target species, including voluminous human deaths. The significant acute mammalian toxicity concomitant with organophosphorus pesticide exposure is caused by the inactivation of the enzyme acetylcholinesterase in the nervous system by acute toxicological effects on the respiratory, myocardial, and neuromuscular systems (Milesen *et al.* 1998).

Organophosphates were introduced to replace resistant and dangerous chlorinated insecticides and quickly gained popularity in the United States and worldwide (Ma *et al.* 2019). PF [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] (Figure 18) is amongst the most routinely used organophosphate insecticides on field crops, vegetables, and fruit crops (Kumar *et al.* 2021; Kushwaha *et al.* 2016). This insecticide is moderately harmful (Toxicity Class II), and its residues have been identified in many vegetables. The primary route of exposure for humans is through dietary consumption of PF. When PF is sprayed to agricultural areas, its leftovers spread throughout the environment, including the

ambient air, surface water, and soil (Ghani *et al.* 2021). As these pesticide pollutants continue to accumulate in the ecosystem, the threat to human life, wildlife, and plants escalates inexorably in tandem. Hence, the need of the hour is to instigate efficient strategies to remove PF residues in a way that is both economically viable and environmentally friendly.

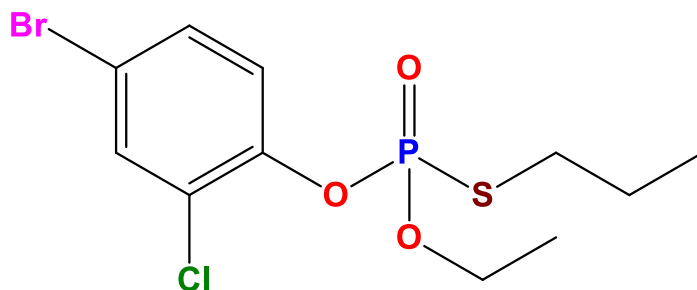


Figure 18. Chemical structure of Profenofos.

A wide range of traditional techniques such as chemical precipitation, ion exchange, membrane filtration, electrochemical methods, and bio-remediation has been used to eliminate PF molecules from aquatic bodies, and however, they have their advantages and disadvantages. As a result, there is a growing demand for safer, easier, eco-friendly, and economically viable techniques for PF removal from aqueous environments (Siripattanakul-Ratpukdi *et al.* 2017; Mansouriieh *et al.* 2016). Historically, adsorption has received more attention due to its cost-effectiveness, flexibility, high selectivity towards targeted pollutants, and greater removal efficiency, which significantly influence the capacity and selectivity towards PF species from aqueous solutions. Therefore, environmental scientists and academicians continue to focus on the development of low-cost, eco-friendly, and reproducible novel adsorbents. Since the adsorption process is reversible, the adsorbent may be reused numerous times by using the appropriate desorption techniques. Further, adsorbents used for the elimination of contaminants should meet the following requirements: 1. It must have a low impact on the environment; 2. It must have an excellent

sorption capacity and high selectivity for pollutants found in the water even at low concentrations; 3. Adsorbed contaminants must be easily removed from their surface; 4. The period necessary to achieve adsorption equilibrium should be as short as feasible; and 5. It must be recyclable. For many decades, adsorbent materials such as activated carbon, zeolites, clays, industry-based by-products, and biomaterials have been widely utilized for the elimination of various pollutants from aqueous solutions (Mojiri *et al.* 2020). However, the adsorption efficiency of these conventional materials is not satisfactory. As a result, the need for alternative adsorbents has skyrocketed (Ali and Gupta, 2006; Rashid *et al.* 2021).

Nanomaterials have proven to be able to meet most of the requirements outlined above in numerous studies conducted in the past few decades. Further, advances in nanoscience and nanotechnology have lately revealed significant possibilities for resolving environmental issues (Mahmoud *et al.* 2020; Shojaei *et al.* 2021; El-Sayed, 2020; Shojaei, 2019; Shojaei *et al.* 2021). Because of their extensive surface area, nanostructured adsorbents offer better efficiencies and quicker adsorption rates in water treatment than traditional adsorbent materials. Moreover, many effective, low-cost, and environmentally tolerable nanomaterials have been proposed to detoxify industrial effluents, groundwater, surface water, and drinking water (Shojaei *et al.* 2021; Jawad *et al.* 2020; Pourabadeh *et al.* 2020; Shojaei *et al.* 2019; Shojaei *et al.* 2021). INPs are gaining popularity as an excellent indemnification medium for a variety of pollutants and also as a promising strategy to provide cost-effective solutions to critical environmental remediation problems. INPs are conveniently available credible adsorbents that reduce pollutants more effectively by producing very little waste and ancillary pollutants (Zhao *et al.* 2016). Furthermore, INPs are increasingly used for environmental clean-up due to their small particle size, larger zone of specific surface area, a higher refractive index of reactive surface spots, and greater intrinsic reactivity (Henn and Waddill, 2006). Iron was employed to imbue the substance with magnetic characteristics and increase the removal efficiency of targeted pollutants.

Nanoscale zero-valent iron has been extensively studied in recent years to reduce the availability of metals in water and soil samples. They have yielded consistent results in limiting the availability of silver, arsenic, beryllium, cadmium, chromium, mercury, nickel, lead, uranium, zinc, and vanadium in the water samples (Galdames *et al.* 2020; Bora *et al.* 2018; Grieger *et al.* 2010). Recently, Hamadeen *et al.* synthesized effective bio-nano sorbent from *Moringa olivera* seeds waste for the removal of chlorpyrifos from wastewater (Hamadeen *et al.* 2021). Yekta demonstrated the adsorption and degradation of cyanophos by newly synthesized CaWO_4 nanoparticles (Yekta *et al.* 2016). Singhal and Lind have employed cysteine-capped silver nanoparticles to remove malathion and chlorpyrifos pesticide from drinking water (Singhal and Lind, 2018). Momić *et al.* used gold nanorods and nanospheres for the adsorption of organophosphate dimethoate pesticides (Momic *et al.* 2016). Dehaghi and co-workers effectively utilized chitosan-zinc oxide nanoparticle composite as an adsorbent to remove permethrin pesticide from water (Dehaghi *et al.* 2014). Some researchers have synthesized nanoparticles by using various fresh tea extracts. Nonetheless, those were applied for the degradation of dyes, removal of Pb, and bromothymol blue indicator (Huang *et al.* 2014; Ali *et al.* 2018; Weng *et al.* 2013; Lin *et al.* 2020; Xin *et al.* 2016). Despite some authors successfully synthesizing nanoparticles by using tea wastes, those have been employed only for the removal of metal ions and phenol red dye (Gautam *et al.* 2018; Lin *et al.* 2020; Lunge *et al.* 2014; Panneerselvam *et al.* 2011). M. Khosravi *et al.* reported the adsorption kinetics and thermodynamics investigation of PF pesticide using chemically synthesized iron/nickel bimetallic nanoparticles (Mansouriieh *et al.* 2016).

In the present study, tea waste was employed to fabricate INPs by eco-friendly strategy for the adsorptive removal of PF pesticide from the aqueous solution. The prepared INPs offer great promise for the removal of PF from the aquatic phase. The present study also investigated the impact of different influence factors such as contact time, solution pH,

the dosage of the adsorbent, and the initial concentration of PF for the maximum adsorption capacity. Further, the adsorption isotherm and kinetic properties of INPs towards the removal of PF were investigated and the underlying mechanism of profenofos adsorption has been proposed.

4.2. Experimental Section

4.2.1. Materials

PF pesticide was gained from the scientific fertilizers company, Gundur, Tiruchirappalli, Tamil Nadu (India). The fabrication of INPs has been described in the previous chapter III. All the experiments were performed by using distilled water.

4.2.2. PF Adsorption Studies Using INPs

The batch technique (Oter and Selcuk Zorer, 2021) was used to measure the PF adsorption by INPs and to evaluate the different adsorption parameters like effects of contact time (0 to 60 min), initial PF concentration (0.5 to 2.0 mg L⁻¹), pH (2 to 10), and adsorbent dosage (0.2 to 1 g L⁻¹). The adsorption experiments were conducted by mixing different amounts of INPs with 100 mL of known concentration PF solution in glass Erlenmeyer flasks. The reaction content was placed and shaken in a mechanical shaker at a speed of 150 rpm. The solution pH was adjusted by using 0.1 M HCl/NaOH. After a certain time interval, the INPs were removed from the solution by centrifugation at a speed of 8000 rpm. Then, the supernatant solution was analyzed for the residual concentration of PF after adsorption experiments. Moreover, the adsorption capacity of glass Erlenmeyer flasks was examined, no significant changes were observed without INPs. Further, the adsorption isotherms and kinetics studies were also carried out under optimized conditions such as contact time, solution pH, and dose of the INPs. The removal percentage and adsorption capacity of PF onto INPs at equilibrium was estimated using the following equations:

$$\% \text{ removal of PF} = \frac{(C_i - C_e)}{C_i} \times 100 \quad (1)$$

$$\text{Adsorbed amount of PF (q)} = \frac{(C_i - C_e)}{m} \quad (2)$$

Here, C_e and C_i are final equilibrium and initial concentrations of INPs, respectively, and 'm' is the mass of INPs in g L⁻¹.

4.3. Results and Discussion

4.3.1. Fabrication and Characterization of INPs

The efficient fabrication of low-cost and green INPs as sorbents from tea waste extract and its characterization were clearly described in the previous chapter III. The schematic diagram for the effective removal of organophosphorus pesticide, profenofos (PF) is shown in Figure 19.

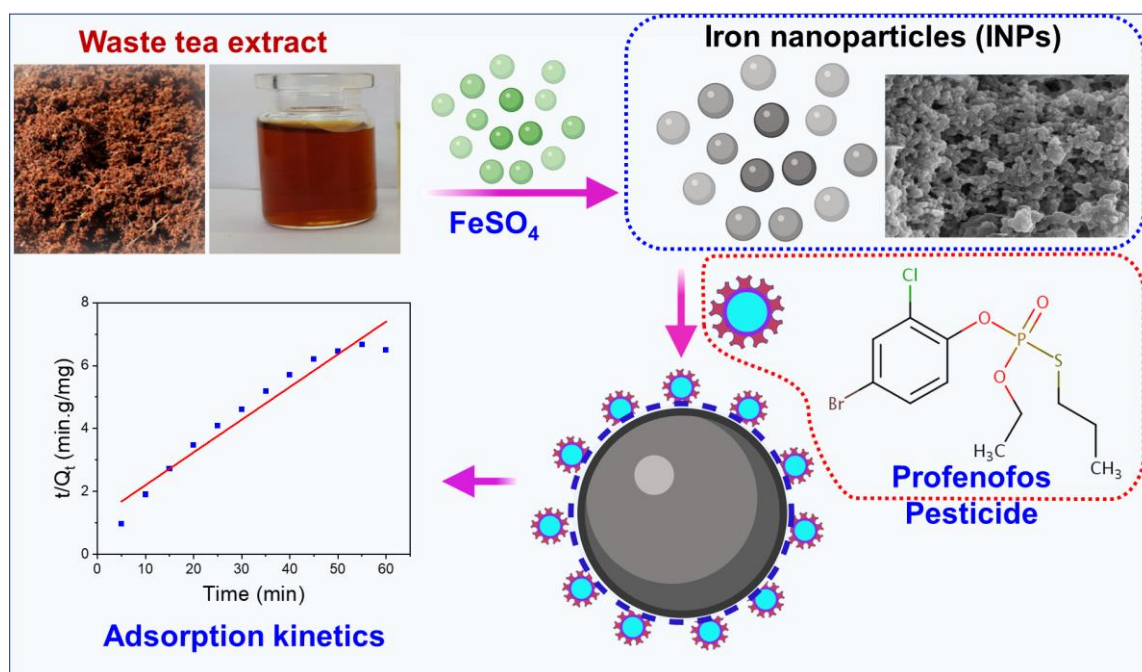


Figure 19. Schematic representation of the present investigation

4.3.2. Adsorption Parameters

4.3.2.1. Influence of Contact Time

The effect of contact time is one of the influencing parameters and plays a significant role in adsorption studies. The adsorption of PF onto the prepared INPs was investigated in batch mode under optimized conditions and obtained results are depicted in Figure 20A. About 200 mg of prepared INPs were taken in flasks containing 1 mg L⁻¹ of the initial concentration of PF solution. Then, the reaction content was shaken in a mechanical shaker with a speed of 150 rpm. Figure 20A demonstrates that PF adsorption increases with time and finally reached the equilibrium stage and beyond that, there were no significant changes in PF adsorption from the solution. The equilibrium stage was reached with a minimum contact time of 15 min. Therefore, 15 min was fixed as the optimum contact time for further studies. With increasing duration of contact time, the percentage of sorption increased from 59 to 88% and became nearly constant after 15 min. The findings indicated that PF adsorption was rapid in the earlier stages and gradually slow down near the equilibrium. This may be related to the availability of many active sites on the INPs surface in the initial stage. Afterward, additional vacant surface sites were difficult to be adsorbed due to repulsive interactions between the adsorbate molecules. These results are analogous with the other reports describing the removal of some organophosphorus pesticides by metal nanoparticles (Mansouriieh *et al.* 2016; Yang *et al.* 2017).

4.3.2.2. Influence of Initial Concentration of Profenofos

The initial PF concentration was ranged from 0.5 to 2 mg L⁻¹ for all experiments, and the content of INPs was fixed as 0.2 g L⁻¹ for this study. The initial concentration of PF plays a major role during the adsorption process because the adsorption mechanism is mainly dependent on the fixed active sites of the adsorbent and the initial concentration of

PF molecules presented in the aqueous solution. Figure 20B shows that the adsorption of PF is enhanced as the initial concentration of PF is increased. With an increase in the initial concentration of PF, there was likely a higher mass transport driving force because more adsorbate molecules were competing for binding sites on the adsorbent and facilitating adsorption. At higher concentrations of PF, this trend shifted in the other direction, which might be due to the binding site saturation retarding the adsorption process (Mansouriieh *et al.* 2016).

4.3.2.3. Influence of Initial pH

The impact of pH on adsorption efficiency is explained by the interaction between the adsorbent surface and the adsorbate molecules. The surface charge of the adsorbent plays an important role during the adsorption process. Therefore, the effect of pH is an essential factor in adsorption studies. The influence of pH on the removal of PF was examined at different pH ranging from 2 to 10 using PF initial concentration of 1 mg L⁻¹ and 200 mg INPs. The obtained results are demonstrated in Figure 20C. According to the results, the adsorption capacity of INPs was increased with increasing pH from 2 to 7 and beyond that, the adsorption capacity was gradually decreased with increasing pH. The maximum adsorption capacity was obtained at pH 6.9 and therefore the pH 6.9 was the optimum pH for further studies. The zero point charge (PHzpc) of iron oxide was reported to be 6.5 in the literature (Salmani *et al.* 2021). More than pH 6.5, iron oxide becomes negative that could not interact with anionic pollutants. Below pH 6.5, iron oxide becomes positive that could effectively interact with anionic pollutants. In the same way, the prepared INPs were effectively interacting with PF molecules less than pH 7 and above pH 7 to 10, the adsorption capacity of PF molecules considerably decreased. This may be due to the charge of INPs gradually changing from positive to negative with increasing pH. Therefore, a

considerable adsorption capacity was obtained at acidic pH due to electrostatic interaction between the positive charge of the adsorbent and negatively charged PF molecules. Therefore, a strong electrostatic attraction and complexation mechanism was involved to enhance the PF adsorption onto prepared INPs at acidic conditions. At the same time, the strong repulsion force was restricted the adsorption process of PF from an aqueous solution.

4.3.2.4. Influence of Adsorbent Dosage

To investigate the impact of adsorbent dosage on PF adsorption at ambient temperature, the amount of INPs were varied from 200 to 100 mg. The initial concentration of PF was fixed as 1 mg L⁻¹ for all runs. The effects of INPs dose on adsorption of PF is shown in Figure 20D. The adsorption efficiency of prepared INPs was increased with an increasing amount of the INPs. This may be due to the increasing number of active sites and surface area for the fixed amount of PF molecules. Figure 20D shows that as the adsorbent dosage enhanced over 0.6 g L⁻¹ of INPs, the adsorption capacity of INPs nanoparticles was dropped. The increasing adsorbent dose could cause aggregation of adsorbent, and therefore the presence of active sites could be decreased during the adsorption process. Hence, the adsorption capacity of prepared INPs was decreased when the adsorbent dose was increased (Yoon *et al.* 2014).

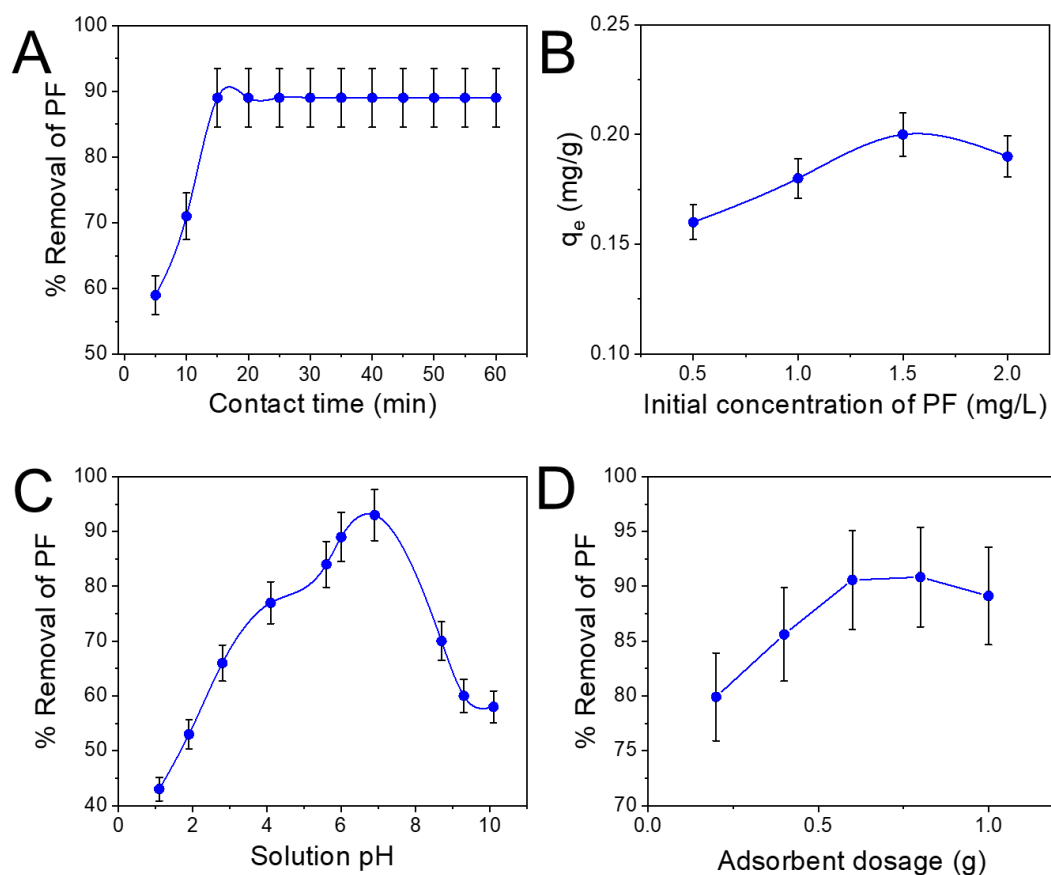


Figure 20. Factors influencing PF adsorption. A) Contact time (min). B) Initial concentration of PF (mg L^{-1}). C) Solution pH. D) INPs dosage.

4.3.3. Adsorption Isotherms

Evaluating adsorption equilibrium by fitting the equilibrium data into several isotherm models is a vital step in identifying the appropriate model for design purposes and determining the mechanism of adsorption systems (Dadfarnia *et al.* 2015; Razmi *et al.* 2019). Adsorption isotherms are essential in improving the use of adsorbents since they define how solutes interact with them and ensure the eliminated quantity of adsorbents. The Langmuir, Freundlich, Temkin, and D-R isotherm models (Yousefinia *et al.* 2021; Madhavi *et al.* 2013; Kim and Choi, 2017; Jabeen *et al.* 2013) were used to investigate the pathway behind the adsorption of PF. The correlation coefficients (R^2) values were used to assess the

suitability of the adsorption isotherm to represent the adsorption mechanism of PF molecules onto the prepared INPs.

4.3.3.1. Langmuir Isotherm

The Langmuir isotherm hypothesis is applicable for the adsorption on homogeneous surfaces. The surface is composed of identical sites that are equally available for adsorption and have equivalent adsorption energies. According to the Langmuir isotherm, the maximum adsorption relates to a saturated single-layer of solute molecules on the surface of the adsorbent with no lateral contact among the adsorbed molecules (Balouchi *et al.* 2020). The linear form of the Langmuir adsorption isotherm equation is:

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \quad (3)$$

Here, C_e is the concentration of PF in solution (mg L^{-1}) at equilibrium, q_e is the adsorbed quantity of PF, q_m (mg g^{-1}) and b (L mg^{-1}) are the maximum adsorption capacity and Langmuir constants concerned with adsorption energy, respectively. The Langmuir isotherm constant was determined from the respective slope and intercept of the straight line of C_e/q_e vs C_e .

The Langmuir adsorption isotherm for PF onto INPs is shown in Figure 21A. The Langmuir isotherm ($R^2 = 0.9989$) fits the experimental data exceptionally well, as displayed in Figure 21A. The highest adsorption capacity (q_m) of INPs towards PF was found to be 43.67 mg g^{-1} at room temperature. Because of the homogeneous distribution of active sites on the INPs surface, the Langmuir isotherm may well fit the investigational results. It is summarized that the highest adsorption is analogous to the formation of uni-layer of PF molecules on the surface of INPs and dictates no diffusion of PF in the plane of INPs surface (Baziar *et al.* 2021).

4.3.3.2. Freundlich Isotherm

Adsorption on heterogeneous surfaces is represented by the Freundlich adsorption isotherm. Both multilayer and monolayer adsorptions can be described using this isotherm model (Rahman *et al.* 2020). It is also predicated on the idea that the stronger binding sites are occupied first. The binding strength diminishes as the degree of adsorption site occupancy increases.

The non-linear form of Freundlich isotherm is designated as follows:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (4)$$

where q_e is the quantity of PF adsorbed (mg g^{-1}), C_e is the equilibrium concentration of PF in aqueous solution (mg L^{-1}), n and K_f are the Freundlich constants describing the quantity of adsorbate adsorbed onto the adsorbent for a unit equilibrium concentration. The value ($1/n$) specifies the heterogeneity of the adsorption system respectively. From the results expressed in Table 2, the K_f and n values are 1.0329 and 3.0769, respectively. It was confirmed that the PF was sufficiently adsorbed onto the surface of INPs. The R^2 value of Freundlich isotherm is 0.8760, which is too far from 1 and evidenced that Freundlich isotherm failed to forecast the experimental data (Figure 21B).

As presented in Table 2, the mean values of the regression coefficient (R^2) are 0.9989 and 0.8760 for Langmuir and Freundlich isotherm, respectively. According to the higher R^2 value, the Langmuir isotherm fits the experimental data better than the Freundlich isotherm.

4.3.3.3. Temkin Isotherm

The Temkin adsorption isotherm was derived on the assumption that due to adsorbate–adsorbate interactions, the heat of adsorption of all molecules in the layer reduces linearly with coverage. The adsorption is defined by the regular distribution of the binding

energies up to maximum binding energy (Yousefi *et al.* 2021). The linear form of Temkin's isotherm can be expressed as:

$$q_e = RT \ln K_T + \{RT/b_T\} \ln C_e \quad (5)$$

where T is absolute temperature, R is the universal gas constant, b_T is the Temkin constant related to adsorption heat (J mol^{-1}), and K_T is the Temkin isotherm constant (L mg^{-1}). The essential parameters of Temkin isotherm (Figure 21C) were determined from the linear plot of $\ln C_e$ vs q_e . As can be seen from Table 2, the Temkin isotherm also does not fits well for PF adsorption ($R^2 = 0.9598$) than the Langmuir isotherm model.

4.3.3.4. The Dubinin–Radushkevich (D-R) Isotherm

The D–R isotherm could represent adsorption on both heterogeneous and homogeneous surfaces at low concentrations. The linear form of the D–R isotherm equation is as follows:

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad (6)$$

here q_m is the D–R monolayer capability (mg g^{-1}), β sorption energy constant, and ε refers to the Polanyi potential at equilibrium concentration and equals to:

$$\varepsilon = RT \ln(1 + 1/C_e) \quad (6.1)$$

$$E = 1/(2B)^{1/2} \quad (6.2)$$

Here, E is described in free energy (kJ mol^{-1}), the value B is related to mean sorption energy.

It can be determined whether the adsorption is based on chemisorption or physisorption by the activation energy. In most cases, the D–R model's adsorption energy (E) revealed the physical nature of the elimination mechanism ($E < 8 \text{ kJ/mol}$). In nature,

physisorption occurs when the activation energy is less than 8 kJ mol^{-1} , and chemisorption occurs when the activation energy is between 8 and 16 kJ mol^{-1} . According to this assertion, our investigation discloses that the PF adsorption on INPs is based on physisorption Figure 21D (Saha *et al.* 2011).

The adsorption behavior of PF onto INPs was studied using Langmuir, Freundlich, Temkin, and D-R isotherm models. The obtained results are depicted in Figure 21. The derived isotherm parameters along with R^2 values are presented in Table 2. According to the R^2 values, the equilibrium isotherm data of PF adsorption onto INPs was fitted well into the Langmuir isotherm model than Freundlich, Temkin, and D-R isotherm models. The Langmuir adsorption capacity was found to be 43.67 mg/g for PF adsorption using prepared INPs.

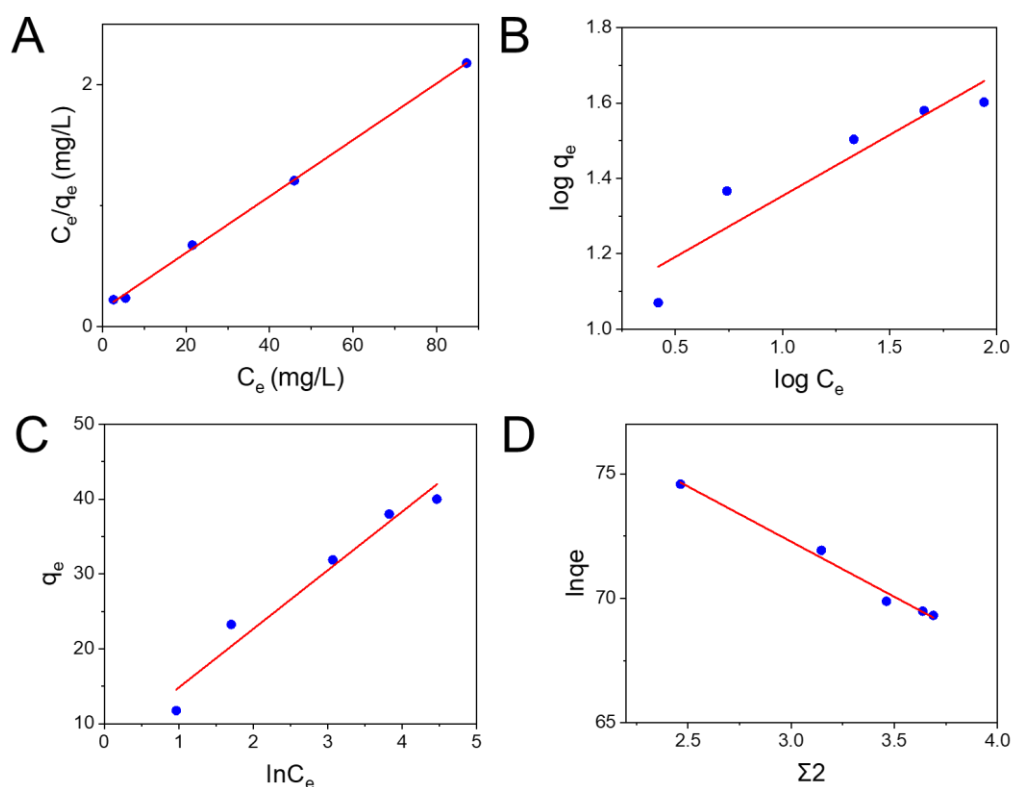


Figure 21. PF adsorption onto INPs. A) Langmuir isotherm plot. B) Freundlich isotherm plot. C) Temkin isotherm plot. D) D–R isotherm plot.

Table 2. Isotherm parameters for the adsorption PF on INPs.

Isotherm Models	Langmuir	Freundlich	Temkin	D-R
Parameters	$q_m = 43.67 \text{ mg g}^{-1}$ $b = 0.157 \text{ L mg}^{-1}$ $R^2 = 0.9989$	$n = 3.0769$ $K_F = 1.0329 \text{ L g}^{-1}$ $R^2 = 0.8760$	$\alpha = 5.4795$ $\beta = 0.1204$ $b = 207.15$ $R^2 = 0.9598$	$K_L = 4.405$ $q_m = 193.99 \text{ mg g}^{-1}$ $E = 0.3369 \text{ kJ mol}^{-1}$ $R^2 = 0.9872$

4.3.4. Adsorption Kinetics

Kinetics is one of the most significant criteria to be considered when evaluating adsorption efficiency (Namal and Kalipci, 2020). The kinetics of PF adsorption onto INPs was examined using the pseudo-first-order, pseudo-second-order, and intra-particle diffusion models (Figure 22). The calculated kinetic parameters of the pseudo-first-order, pseudo-second-order, and intra-particle diffusion models are presented in Table 3.

4.3.4.1. Pseudo-First-Order Reaction

The Lagergren first-order or pseudo-first-order rate equation is broadly employed for the solute sorption from the solution and is measured by the following equation:

$$\log(q_e - q_t) = (\log q_e) - (K_1/2.303) \times t \quad (7)$$

where q_e (mg g⁻¹) is the quantity of adsorbed PF at equilibrium, q_t (mg g⁻¹) is the quantity of adsorbed PF at the time t (min), K_1 is the rate constant for pseudo-first-order kinetics. According to the pseudo-first-order equation, the rate of adsorption site occupation is related to the number of unoccupied sites (Diagbaya *et al.* 2015; Choe *et al.* 2000). The pseudo-first-order kinetic fit for the PF adsorption onto the INPs is shown in Figure 22A and the corresponding kinetic parameters are presented in Table 3.

4.3.4.2. Pseudo-Second-Order Reaction

A pseudo-second-order equation can also be used to characterize the adsorption kinetics. The pseudo-second-order kinetic model states that the reaction rate is quick at the beginning and then slows as it reaches equilibrium; the reaction can continue at this rate for an extended period. This model is based on the adsorption ability of the adsorbent and is effective for understanding both nonphysical and nonchemical equilibrium (Dickson *et al.* 2017). It is expressed by the following equation:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (8)$$

where K_2 is the rate constant of pseudo-second-order adsorption reaction, q_t (mg g^{-1}) and q_e (mg g^{-1}) are the amounts of adsorbed solute at equilibrium at any time t (min). Figure 22B illustrates the linear form of the pseudo-second-order adsorption model, and the parameters are listed in Table 3. According to Table 3, the pseudo-second-order kinetics model along with higher R^2 values is more consistent with the investigational results than the pseudo-first-order kinetics model. The results indicate that the adsorption rate is highly dependent on adsorption sites availability over the PF concentration in the solution.

4.3.4.3. Intra-Particle Diffusion Model

It is assumed in the intra-particle diffusion model (Figure 22C) that adsorbate can be transported into the pores of the adsorbent by batch mode adsorption and is stated as follows:

$$q = K_i t^{1/2} + C_i \quad (9)$$

here C_i refers to intercept, which approximate the thickness of the boundary layer, and a significant intercept value denotes the higher boundary layer effect. K_i refers to the rate constant for intra-particle diffusion that can be calculated by the slope from the linear plot

of q against $t^{1/2}$. If the plot is linear and passes through the origin, intra-particle diffusion is the only rate-controlling step. But if the plot is linear and does not pass through the origin, it is suggested that the adsorption involves intra-particle diffusion but it is not the only rate-limiting step. The outcomes of our analysis demonstrate that intra-particle diffusion is not considered as the rate-controlling step in the PF adsorption by INPs (Sheela and Nayaka, 2012).

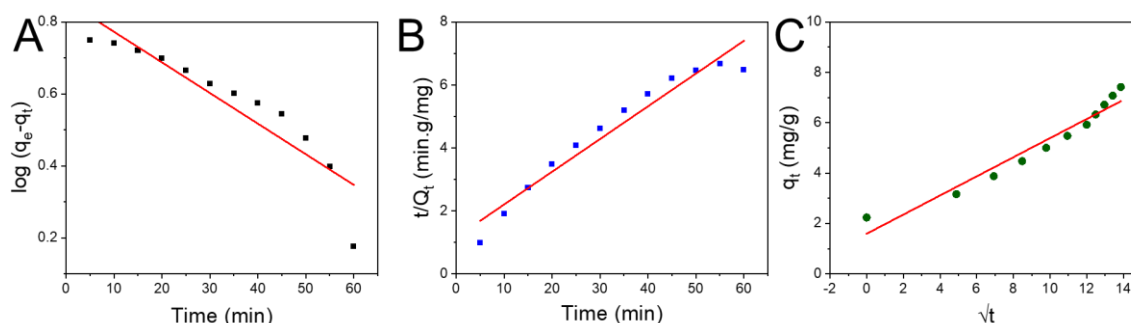


Figure 22. Kinetic adsorption models of PF. A) Pseudo-first-order kinetics. B) Pseudo-second-order kinetics. C) Kinetic plot for intra-particle diffusion at RT.

Table 3. Kinetic parameters for the adsorption of PF on INPs.

Kinetic system	System coefficients	R^2
Pseudo-first order	$q_e = 7.3468 \text{ mg g}^{-1}$ $K_1 = 1.87 \times 10^{-2} \text{ min}^{-1}$	0.9131
Pseudo-second order	$q_e = 12.722 \text{ mg g}^{-1}$ $K_2 = 7 \times 10^{-3} \text{ g mg}^{-1} \text{ min}^{-1}$	0.9792
Intra-particle diffusion	$K_{diff} = 1.7199 \text{ mg g}^{-1} \text{ min}^{1/2}$ $C = 9.0107$	0.9399

4.3.5. Adsorption Mechanism

Adsorption is a surface phenomenon that results when adsorbate molecules accumulate on the surface of the adsorbent. It is based on the mass transport of a specific or group of components of adsorbate congregate at the interface. A variety of mechanisms have

been proposed in the literature for the removal of pesticides from water. Van der Waals forces, electrostatic attraction, hydrophobic interactions, hydrogen bonding formation, π - π stacking, ion exchange, and surface complexation exist between the adsorbent and adsorbate in the aqueous medium. However, the adsorptive removal of harmful pesticides is triggered by one or more of these interactions. In general, the decontamination of pesticides by prepared adsorbent is typically a function of adsorbent properties as well as the chemical composition of the adsorbates. Several interaction forces including surface complexation, hydrogen bonding, and electrostatic attraction were governed the adsorption mechanism of PF from the aqueous phase using as-prepared INPs. Under acidic conditions, the INPs have effectively adsorbed the PF molecules from the aqueous phase because the protonated INPs were easily interacting with highly electronegative atoms of PF molecules through electrostatic attractions. Other forces such as surface complexation and hydrogen bonding were also enhanced the adsorption mechanism of PF molecules from aqueous solution. Further, the adsorptive removal of PF molecules was influenced by a variety of variables, including adsorbent concentration, incubation time, temperature, solution pH and particle size, etc. Among the above-mentioned mechanisms, the electrostatic attraction was the main force to adsorb the PF molecules onto the INPs. However, the electrostatic attraction becomes weaker in alkaline conditions because of the deprotonation of INPs. The selected pesticide profenofos is composed of the most electronegative atoms including Br, Cl, S, P, and O which may strongly involve hydrogen bonding interactions to the adsorbent INPs in acidic conditions. Further, the coordination or the chelating ability of S, P, or O atoms towards nano iron may also be responsible for the adsorption of PF molecules by INPs. Based on the obtained results, Langmuir isotherm revealed the monolayer adsorption of PF molecules on the surface of INPs. Further, the D-R isotherm unveiled that the adsorption of PF on the surface of INPs is physisorption in nature. Therefore, the underlying mechanism

is physisorption mediated monolayer adsorption of PF molecules on the surface of fabricated INPs.

4.4. Conclusion

In summary, the INPs were successfully fabricated and its further application of PF uptake was systematically investigated in this study. The prepared INPs were thoroughly characterized by using various spectral and analytical analyses. The synthesized INPs showed an excellent adsorption capacity of 43.67 mg g^{-1} for PF molecules with a minimum contact time of 15 min. Different influencing factors such as solution pH, contact time, adsorbent dose, and initial concentration were systematically investigated for the maximum uptake of PF molecules from the aqueous solution. The equilibrium data fit with the Langmuir adsorption isotherm model, demonstrating monolayer coverage of PF molecules on the INPs outer surfaces. The adsorption kinetics indicate that the adsorption system followed the pseudo-second-order kinetic model and it was confirmed by the higher R^2 (0.9792) value. Moreover, it was revealed that the rate of adsorption was more dependent on the availability of adsorption sites on INPs over PF concentration in the solution. The electrostatic interaction was mainly governed the adsorptive mechanism of PF molecules onto the INPs. The obtained findings of this study demonstrate the scope and efficacy of green INPs as effective adsorbents for the environmentally-friendly removal of pesticides from polluted water. Until now, optimization of green synthetic protocol, exact mechanisms of nanoparticle formation, regeneration, and utilization of nanomaterials are the major challenges to researchers for developing them from laboratory level to commercialization. A slight change in the parameters such as particle size, reactant concentration, reaction pH, reaction time, temperature, pressure, and environmental conditions are significantly affecting the physicochemical properties and morphologies of nanoparticles. The main drawbacks of INPs are the release of soluble iron ions and their susceptibility to surface

oxidation. Moreover, the latent toxicities of these nanomaterials are not completely addressed.

From future perspectives, we propose (i) to employ the titled INPs for the removal of other hazardous organophosphorus pesticides and other types of pollutants including dyes, heavy metals; (ii) to use the INPs as a pilot adsorbent in agricultural and industrial wastewater remediation; (iii) to estimate the practical utility, environmental and the commercial feasibility of the application. (iv) to study the toxicity effect, recovery, and reusability of INPs for their application in wastewater treatment.

Chapter V

Isolation, Screening, and Identification of Potential Profenofos Degrading Bacteria from Pesticide-Contaminated Agricultural Soil

Abstract:

The chemical structure of organophosphorus pesticides is attributed to their high toxicity and competence to irreversibly inhibit the activity of acetylcholinesterase and inflict neuronal disorder, organ failure, and eventual death among non-target species. Profenofos is one of the most widely used organophosphate pesticides on a variety of crops and its residues are being found in human blood, breast milk, and urine. Hence, there is a rising demand for the development of pesticide degrading efficacious technologies. One of the most promising bioremediation strategies has proven to be the microbial breakdown of pesticides into less harmful compounds. For the bioremediation of profenofos contaminated soil, a bacterial strain PDB1 competent of utilizing profenofos has been isolated from the agricultural soil samples of Karur district, Tamilnadu, India. The strain PDB1 is identified as *Bacillus subtilis* from the cultural, morphological, biochemical, and 16S rRNA gene studies.

5.1. Introduction

Global estimates of yield losses for main crops induced by viruses, pests, diseases, and weeds varied between 26-40%, based on crop and world territory (Willocquet *et al.* 2017). As a result, the application of chemical pesticides has been a preferred solution to boost agricultural productivity, quality and to fulfill the food requirements of the world's fast-increasing population (Salahi Moghadam *et al.* 2021). Despite its advantages their widespread and long-term usages, however, cause severe contamination of air, water, soil, and agricultural products, that jeopardizes crop production eventually being harmful to the ecosystem especially to the agriculturally beneficial microbes and aquatic animals, and consequently creates risks to human health through food chains (Rani *et al.* 2021). Massive amounts of pesticides were manually administered, but it was estimated that only a minor proportion of the compounds properly reached the target species, with the balance being settled on the soil and resulting in poisonings of off-target species (Diez, 2010). Pesticide persistence in the soil also has a severe impact on climate parameters such as rainfall intensity and schedule, as well as temperature. Climate change affects the retention of pesticides and microorganisms in the soil (Ma *et al.* 2021). Among the several pesticide categories used across the globe, organophosphates are the most frequently employed group of pesticides in contemporary usage (Milesen *et al.* 1998).

Profenofos is a common organophosphorus pesticide (Figure 23) utilized on a variety of crops. According to findings, the pesticide is an ACE inhibitor, causing an irreversible neuronal disorder in animals. Among the most serious health risks associated with profenofos residues are genotoxicity, mutagenicity, multi-organ disorder, hormone disruption, reduced overall body metabolism, and reproductive problems. Further, profenofos residues have been found in many vegetables posing a dietary risk to people and aquatic animals. Furthermore, profenofos pesticide residues have been linked to honey bee colony loss and soil microflora destruction (Kushwaha *et al.* 2016). Thus, it is imperative to

effectuate effective strategies for removing profenofos residues in a manner that is both economically viable and environmentally friendly. Traditional methods for removing and/or degrading organophosphorus pesticides, such as chemical modification, combustion, and landfills, were later shown to be problematic due to the risk of secondary exposure. Furthermore, such methods are prohibitively expensive. As a result, there is a rising demand for developing a natural solution for the efficient elimination of profenofos from the ecosystem (Jatoi *et al.* 2001).

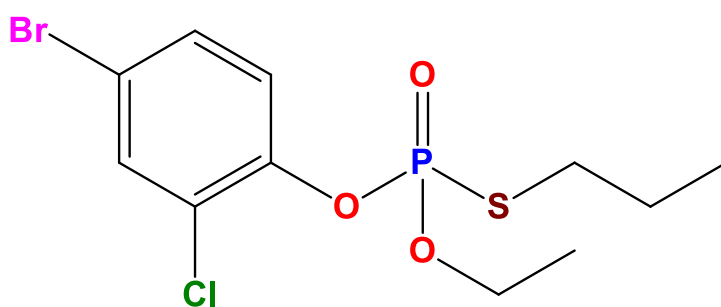


Figure 23. Molecular structure of profenofos pesticide

Biodegradation is one of the most potent, secure, and commercially sustainable strategy when compared to other contaminants removal methods. The degradation and conversion of recalcitrant pesticides can be better accomplished through bioremediation by the action of microbial enzymes that can catalyze various reactions such as hydrolysis, redox reactions, ring cleavage, etc. (Sarker *et al.* 2021). To date, there have been very few reports that describe the efficient microbial biodegradation of profenofos pesticide and their complete degradation pathways. Malghani *et al.* have reported the extraction, identification along with the characterization of bacterial strains capable of degrading profenofos pesticide (Malghani *et al.* 2009). Salunkhe *et al.* isolated *Bacillus subtilis* from grapevines and investigated the profenofos degrading ability (Salunkhe *et al.* 2013). Jabeen and co-workers have optimized the degradation capacity of bacterial consortium by surface response technique (Jabeen *et al.* 2015). Siripattanakul-Ratpukdi's research group studied the

profenofos biodegradation by a bacterial consortium (Siripattanakul-Ratpukdi *et al.* 2015). Jaoti examined the potency of rhizobacteria towards the biodegradation of profenofos and analyzed the progress of the growth of the plants (Jatoi *et al.* 2021). Recently, Verma and Chatterjee demonstrated the mechanistic pathway of profenofos biodegradation (Verma and Chatterjee, 2021).

The present investigation aims to identify novel bacterial strains from the pesticide-contaminated soil and to comprehend the profenofos degradation pathway. The strain isolated in this study can be used to bioremediate pesticide-contaminated soil in various agricultural fields.

5.2. Experimental Section

5.2.1. Sample Collection

Soil samples were gathered from various sites with ten years of pesticide exposure and taken from 10 to 15 cm depth from Karur district, Tamil Nadu, India (Latitude: 10.8154° N, Longitude: 78.5758° E) (Figure 24). Soil samples have been stored in an ambient atmosphere and preserved in plastic containers at the refrigerator. Technical grade Profenofos (99 % purity) was obtained from the Scientific Fertilizer Co Pvt. Ltd, Pesticide Division, Gundur, Tiruchirappalli, Tamil Nadu, India.

5.2.2. Physico-Chemical Characteristics of Soil

The soil samples were analyzed in the Department of Agriculture and cooperation, ICAR, Karur for demonstrating the physicochemical characteristics.

5.2.3. Medium for Isolation

Mineral salt media MSM (in g / L) (Potassium dihydrogen phosphate – 3; Sodium chloride – 0.5; Sodium sulfate – 5.8; Ammonium chloride – 1; MgSO₄.7H₂O – 0.2) and nutrient broth were purchased from HIMEDIA, India.

5.2.4. Soil Processing by Enrichment Method

By successive sub-culturing of MS-medium containing profenofos, the bacteria have been isolated using an enrichment method. 5 g of soil from the agricultural field is mixed with 100 mL MS medium and profenofos (10 mg/L). For a week, the enriched medium was stored at 30 °C under agitation (140 rpm/min). After five cycles, the enrichment culture (5 mL) was subcultured into a 100 mL new enrichment medium comprising an increasing concentration of profenofos (20, 30, 40, and 50 mg/L).

5.2.5. Isolation of Profenofos Degrading Bacteria

Pure cultures were obtained after four cycles of sub-culturing in MSM with an incremental dose of profenofos (till 50 mg/L) by conducting suitable serial dilutions of the enriched culture in MS medium and growing them in MS plates inoculated with 50 mg/L profenofos (Mu *et al.* 2018). Three pure colonies were isolated for further investigation and named PDB1, PDB2, and PDB3.

5.2.6. Screening of Potential Profenofos Degrading Bacteria

To screen the bacteria with potent profenofos pesticide tolerance, all the three isolated colonies PDB1, PDB2 and PDB3 were separately treated with various concentrations of profenofos including 40, 60, 80, and 100 mg/L in MS medium plates. Among the three treated strains, the potential bacterial strain that showed significant growth was selected for further investigation.

5.2.7. Identification of Potential Profenofos Degrading Bacteria

5.2.7.1. Cultural and Morphological Characterization

The selected strain has been cultured in nutrient broth for 24 h. The cultural and morphological characterization of the selected strain was analyzed.

5.2.7.2. Biochemical Characterization

To identify the genus of the profenofos degrading bacterium, the biochemical features such as Gram staining, citrate, gelatin hydrolysis, catalase, indole, methyl red, nitrate reduction, and pigment production were analyzed (Shirling and Gottlieb, 1996).

5.2.7.3. Molecular Characterization

The genomic DNA of the isolated strain was extracted using the usual PCI (25: 24: 1, Phenol: Chloroform: Isopropanol) extraction method. The culture was grown in LB broth at 30 °C in a rotary shaker (140 rpm/min) for 24 h. The cells were harvested by centrifugation at 4 °C for 10 min at 10000 rpm. The harvested cells were then washed thrice with sterile water. 5 mL of SET buffer was added to the cells (25 mM Tris HCl; 25 mM EDTA, pH 8; 0.3 M sucrose). After that 3 µL of lysozyme was added and agitated well on vortex mixture followed by incubation at 37°C for 45 min. After that, 600 µL of 10% SDS was added and incubated at 60 °C for 15 min followed by incubation, 3 µL of RNAase was added into the lysed mix and incubated for 15 min at 37°C. After that, 2 mL of PCI was added and centrifuged at 4 °C for 10 min at 10000 rpm. Followed by centrifugation, an aqueous layer was transferred to the fresh sterile tube and precipitated with ice-cold absolute ethyl alcohol. The solution was then kept at -80 °C for 30 min. After that, the precipitate was transferred into a fresh tube and washed with 70% ethyl alcohol, and dried at ambient atmosphere until the complete evaporation of ethyl alcohol. Finally, the pellet was dissolved in 200 µL of TE buffer for further studies (Hopwood *et al.* 1985).

5.2.7.3.1. 16S rRNA Gene Sequences

For PCR amplification of the 16S rRNA gene, the following primers were employed: 27F (5'-AGAGTTTGATCCTGGCTCAG-3'), and 1492R (5'-GGTTACCTTGTTACGACTT-3'). To create the PCR reactions, 50 µL of PCR buffer, 10 mol/µL of each primer, 5 U Taq DNA polymerase, and 10 mol/µL BSA, as well as 2 µL of DNA were used. 94°C denaturation for

three minutes, 28 amplification cycles of 20 sec each at 94°C, 58°C for 40 sec at 72 °C, and a finishing polymerization to 3 min and 30 sec was the thermocycling conditions. To check the PCR products, we used Gel Doc 2000 to visualize them on 1.0 % agarose gels. The purified PCR products of the 16S rRNA gene were sequenced (Agrigenome Labs Pvt Ltd, Kochi).

5.2.7.3.2. Phylogenetic Tree Construction

16S rRNA gene sequences analysis was performed using the BLAST (Altschul *et al.* 1997) at NCBI network services. The sequences obtained were aligned against selected sequences that are publicly available in GenBank using CLUSTAL X programs (Thompson *et al.* 1997). After verification and adjustments of the alignments, manually the phylogenetic tree has been constructed using the neighbor-joining method (Wright *et al.* 2017).

5.3. Results and discussion

5.3.1. Physico-Chemical Characteristics of the Soil Sample

Agrochemicals are regarded as a crucial component of modern agriculture, and their use is increasing at an alarming rate. Prominently, the utilization of pesticides not only increases crop production, but also induces a sequence of changes in the physical, chemical, and biological characteristics of the soil. These long-term changes have a substantial impact on the soil's quality and production capability. To check the nature, water holding capacity, and the existence of various elements, the physicochemical properties of the collected soil sample were analyzed. The physicochemical features of the analyzed soil sample are given in Table 4.

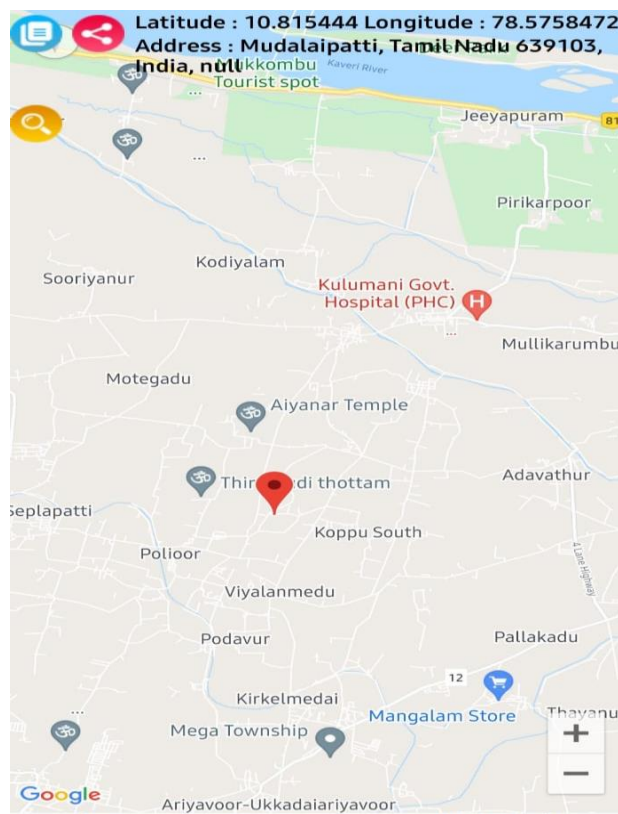


Figure 24. Location of soil samples collection

Table 4. Properties of soil sample

S.No	Parameters	Value
1.	Soil type	Red soil
2.	Density	1.47 g/mL
3.	Water holding capacity	40.39 %
4.	pH	7.2
5.	Electrical Conductivity (EC)	0.32
6.	Organic Carbon (OC)	2.30%
7.	Nitrogen (N)	254.2 kg/ha
8.	Potassium (K)	260.1 kg/ha
9.	Phosphorous (P)	20.4 kg/ha
10.	Sulfur (S)	12.1 mg/kg
11.	Zinc (Zn)	0.85 mg/kg
12.	Boron (B)	1.24 mg/kg
13.	Iron (Fe)	30 g/kg
14.	Manganese (Mn)	6.14 mg/kg
15.	Copper (Cu)	1.21 mg/kg

5.3.2. Isolation of Profenofos Degrading Bacteria

Three different bacterial strains have been isolated from the pesticides exposed agricultural soil samples by enrichment technique for further investigation and named PDB1, PDB2, and PDB3.

5.3.3. Screening of Potential Profenofos Degrading Bacteria

To screen the bacteria with potent profenofos pesticide tolerance and their growth, all the three isolated colonies were examined by the plating method. All the three isolates PDB1, PDB2, and PDB3 exhibited better growth up to 60 mg/L of profenofos. However, while the dose of profenofos was increased to 80 mg/L, the strains PDB1 and PDB2 showed better growth. Finally, only the strain PDB1 demonstrated significant growth and profenofos tolerance of 100 mg/L. Hence PDB1 has been chosen for further investigation. The pure colonies of the isolated strains were displayed in Figure 25 and the growth of the strains under the various concentrations of profenofos has been given in Table 5.

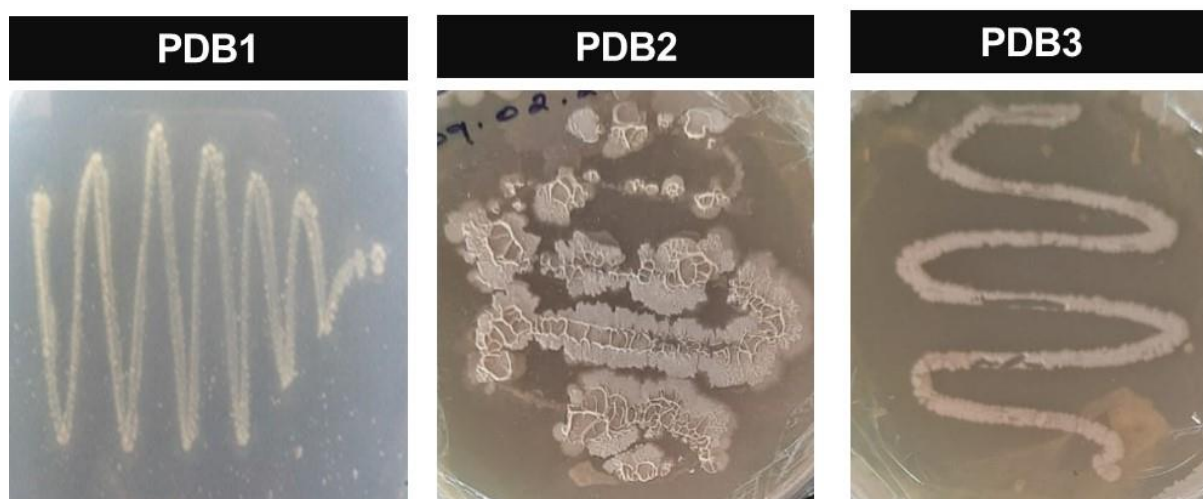


Figure 25. Pure colonies of PDB1, PDB2, and PDB3

Table 5. The growth of the strains under various profenofos concentration

	Concentration of profenofos (mg/L)			
	40	60	80	100
PDB1	+	+	+	+
PDB2	+	+	-	-
PDB3	+	+	+	-

5.3.4. Identification of Isolated Strain

5.3.4.1. Cultural and Morphological Characterization

Under the test conditions, a single bacterial isolate PDB1 was found positive at a high concentration of profenofos (100 mg/L). The strain PDB1 has been selected for profenofos degradation in soil samples collected from the Karur district of Tamil Nadu, India. At neutral pH, the PDB1 bacterium grew significantly in MS Medium added with 100 mg /L profenofos at 28°C, indicating that the bacterium was using profenofos as its sole carbon and energy source. Morphological analysis revealed that the strain PDB1 was rod-shaped and a fuzzy-white in color.

5.3.4.2. Biochemical Characterization

The biochemical properties of the PDB1 have been summarized in Table 6. The PDB1 strain exhibited positive results for Gram staining, citrate, gelatin hydrolysis, catalase, and nitrate reduction tests. For the analysis with indole, methyl red, and pigment production, the strain PDB1 showed negative responses. The biochemical analysis of the PDB1 exhibited 99 % characteristics of the *Bacillus* genus.

Table 6. Biochemical test for PDB1

Sl. No	Test	Results
1	Gram Staining	Positive
2	Citrate	Positive
3	Gelatin hydrolysis	Positive
4	Catalase	Positive
5	Indole	Negative
6	Methyl Red	Negative
7	Nitrate Reduction	Positive
8	Pigment production	Negative

5.3.5. Molecular Characterization

The genomic DNA of the PDB1 strain was isolated by the usual PCI extraction method. The isolated DNA was analyzed by gel electrophoresis technique and the corresponding DNA band size of 1500 bp for genomic DNA isolated from PDB1 strain (Figure 26).

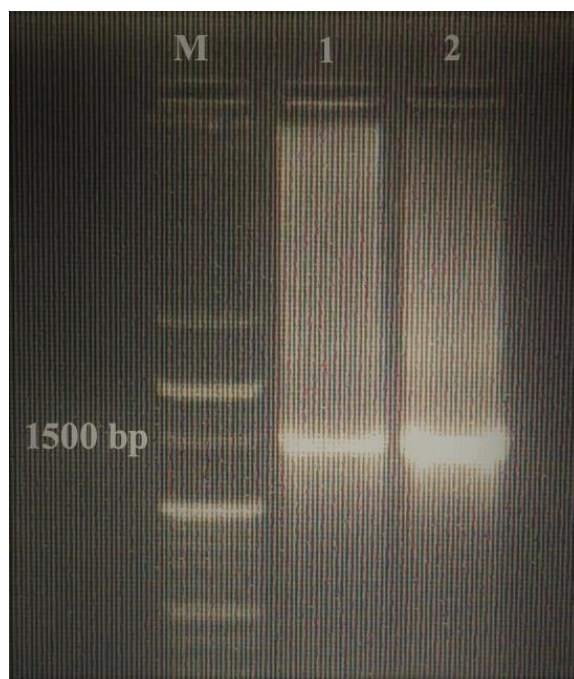


Figure 26. Genomic DNA of PDB1

5.3.5.1. 16S rRNA sequences

The PDB1 strain was further identified using 16S rRNA gene sequencing. BLAST analysis was used to compare the PDB1 nucleotide sequences to other 16S rRNA gene sequences in the NCBI database. The analysis revealed that the bacterium PDB1's 16S rRNA gene sequence exhibited a substantial degree of similarity with *subtilis* species. From this experiment, the potential strain PDB1 was identified as *Bacillus subtilis*. The 16S rRNA sequences of the *Bacillus subtilis* strain have been deposited in GenBank with accession number (SUB10926668).

5.3.5.2. Phylogenetic tree construction

The phylogenetic tree has been constructed by the neighbor-joining method (Wright *et al.* 2017). It combines sequences to outline predicted tree branches and to estimate branch lengths from trees. The phylogenetic tree of the bacterium PDB1 is shown in Figure 27 (Ankenbrand and Keller, 2016).

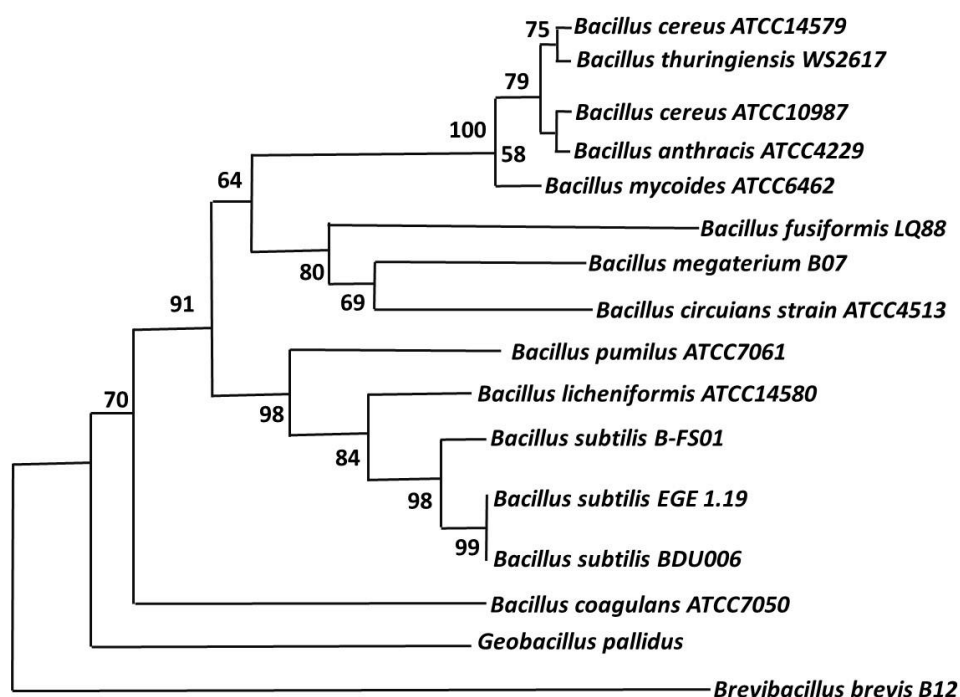


Figure 27. The phylogenetic tree of the isolated bacterium PDB1

5.4. Conclusion

Microbe-mediated biodegradation of organophosphate pesticides and the advancement of bioremediation approaches for contaminated agricultural fields by using potential microorganisms symbolize a rapidly expanding area of research worldwide. In the pristine ecosystems and non-exposed agricultural fields, naturally existing profenofos-degrading microorganisms may be scarce. As a result, the enrichment technique for isolating bacterial strains was widely employed in the investigation of pesticide biodegradation. In the present exploration, the PDB1 strain was isolated from pesticide-contaminated agricultural soil. The isolated bacterial strain was identified as *Bacillus subtilis* by various characterization techniques including cultural, morphological, biochemical, and molecular characterization. The PDB1 strain was tested for its ability to degrade the pesticide profenofos. Cultural and morphological analysis revealed that the strain PDB1 was rod-shaped and a fuzzy-white in color. The biochemical analysis of the PDB1 strain exhibited 99 % characteristics of the Gram-positive *Bacillus* genus. The molecular characterization analysis revealed that the isolated PDB1 strain is *Bacillus subtilis*.

Chapter VI

Effect of *Bacillus subtilis* on Profenofos degradation and plant growth

Abstract:

In modern agriculture, the widespread use of organophosphorus pesticides results in environmental pollution and pose severe threats to human, flora, and fauna. Hence, the efficient removal of these pesticides from polluted environments remains an enduring inspiration. Microbial degradation can be used as a proficient detoxifying strategy for the polluted ecosystem since it can break down or convert toxic pollutants into less hazardous by-products by indigenous microbes while also being cost-effective, extremely efficient, and environmentally sustainable. Further, a detailed analysis of the plant growth-promoting activity of plant-bacteria relationships could be used to promote sustainable agricultural output as well as the remediation of pesticide-polluted soil and water. Hence, a bacterial strain PDB1 was extracted from the soil sample exposed to pesticide for an extended period at Karur district, Tamilnadu, India for the bioremediation analysis. Using cultural, morphological, biochemical, and 16S rRNA gene analysis, the bacterium PDB1 was characterized as *Bacillus subtilis*. The impact of *Bacillus subtilis* on several plant-growth parameters in the pot culture method was studied in the presence and absence of the pesticide profenofos. The capacity of *Bacillus subtilis* to degrade profenofos (PF) both in liquid and soil medium was examined using gas chromatography-mass spectroscopy (GC-MS). Since this isolated bacterial strain has (PF) degrading capacity as well as other characteristics that promote plant growth, the isolate could be an attractive opportunity for the advancement of the bioremediation technique.

6.1. Introduction

Modern agriculture has relied on the constant administration of agrochemicals predominantly pesticides to increase crop productivity and food security (van Zelm *et al.* 2014). Even though pesticides play a crucial function in contemporary agriculture, their prolonged use causes severe environmental problems (Alengebawy *et al.* 2021). Continuous pesticide exposure causes immunological issues, immunodeficiency syndromes, and cancer-related problems. The excessive use of pesticides pollutes the soil and water environment. Furthermore, it has a negative impact on the genetic variation of soil microbiota, accompanying deteriorated soil fertility and plant development, which together jeopardize the long-term productivity of the agricultural field. To complicate the issue further, pesticide residues and their by-products frequently penetrate through the surface soil into groundwater, provoking widespread pollution of aquatic environments (Rani *et al.* 2021).

Organophosphates are by far the most frequently used insecticides, accounting for 34% of global pesticides consumption. They are known to inhibit cholinesterase neurotransmitters irreversibly, disrupting endocrine activities, nervous system failure, defects in childbirth, infertility, distortion of growth, development, and reproduction in fauna, birds, and mankind (Miles *et al.* 1998). Profenofos (PF) is a highly active organophosphorus pesticide that is commonly employed on crops, vegetables as well as fruits. The molecular structure of PF comprises phenolic and phosphate ester components (Figure 28). It is one of the most widely used pesticides because of its easy fabrication, cost-effectiveness, and high efficiency. PF has been categorized as a moderately dangerous pesticide of toxicity class II by the World Health Organization (WHO), and it has been shown to inhibit acetylcholinesterase. PF is genotoxic to off-target organisms, including terrestrial and aquatic creatures. PF is widely used in agriculture in many countries across the world. It has been widely used to manage and control numerous lepidopterous insects,

aphids, whiteflies, spider mites, and hoppers on a variety of crops including tomato, potato, corn, sugarcane, cotton, tobacco, and vegetables. The residual levels of PF have been measured in a variety of plants and food samples. Alarming, these organophosphate pesticide residues have been identified in human blood, breast milk, plasma, serum, and urine after exposure (Kushwaha *et al.* 2016).

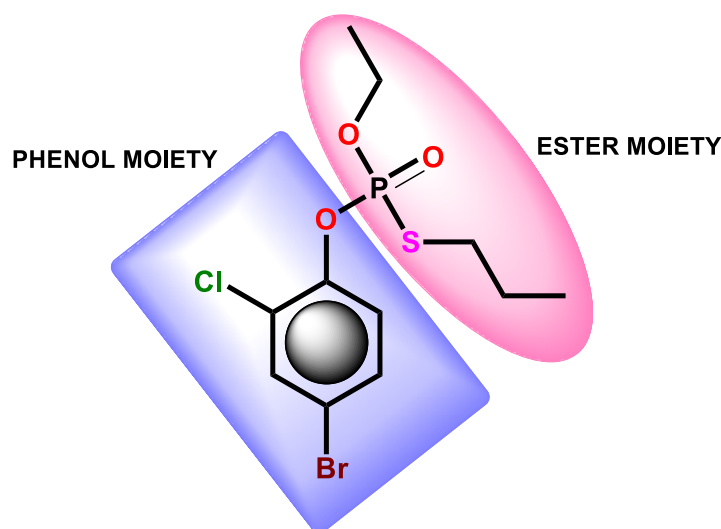


Figure 28. Chemical Structure of Profenofos

To address the ever-increasing demand and overconsumption of synthetic pesticides while taking into account their long-term negative impacts on global agro-ecosystems and living organisms, we desperately need to develop, adopt, and promote environmentally sustainable approaches (Sun *et al.* 2018). Traditional methods for removing and/or degrading organophosphorus pesticides, such as chemical treatment, combustion, and landfills, were later shown to be troublesome due to the possibility of secondary exposure. Furthermore, such procedures are prohibitively expensive. As a result, sustainable agriculture is critical in this period since it has the capabilities to fulfill our future agricultural demands (Jatoi *et al.* 2021). Hence, bio-remediation, bio-mineralization, bio-protection, bio-stimulation have been considered as safe, convenient, environmentally

friendly, and economically viable methods for the decontamination of organophosphorus pesticides (Khatoon *et al.* 2020).

Bioremediation is a technique that uses the potential of microbial degradation to provide a cost-effective and reliable way of pesticide diminution. Numerous soil and aqueous atmospheres were effectively recovered by utilizing bacteria capable of degrading the contaminants. PF is removed through hydrolysis, either chemically or through microbial activity, by transforming it to diethylthiophosphoric acid and 4-Bromo-2-chlorophenol (Kushwaha *et al.* 2016). Malghani *et al.* described the isolation and characterization of bacterial strains capable of PF decomposition (Malghani *et al.* 2009). Salunkhe *et al.* extracted *Bacillus subtilis* from grapevines and examined its ability to degrade PF (Salunkhe *et al.* 2013). Jabeen and colleagues used the surface response technique to analyze the degradation capacity of a bacterial consortium (Jabeen *et al.* 2015). The research team of Siripattanakul-Ratpukdi explored PF biodegradation by a bacterial consortium (Siripattanakul-Ratpukdi *et al.* 2015). Verma and Chatterjee briefly demonstrated a molecular mechanism of PF biodegradation (Verma and Chatterjee, 2021).

Plant-assisted bioremediation has a lot of potential for cleaning up pesticide-contaminated soil. Pesticide degrading bacteria may improve plant tolerance to pollutants by detoxifying polluted soils by direct mineralization of contaminants. Furthermore, plant exudates increase the density and efficiency of novel bacteria in the root zone. The ability of microorganisms to detoxify pollutants while also increasing plant development has previously been explored for various organophosphorus insecticides such as malathion, methyl parathion, chlorpyrifos, and monocrotophos (Pandotra *et al.* 2018). Despite, the significance of plant-bacteria relationships towards the remediation of various pollutants has been demonstrated in various studies, research on PF degradation using this strategy is currently limited.

Akbar and Sultan demonstrated that the chlorpyrifos-degrading bacteria *Achromobacter xylosoxidans* and *Ochrobactrum* sp. have the potential to become excellent options for increasing crop productivity in pesticide-contaminated soils (Akbar and Sultan, 2016). Nivedita and Sundari examined the impact of direct inoculation of specified consortia on plants in the presence of the root disease *Sclerotium rolfsii* and the organophosphate insecticides malathion and methyl parathion (Mishra and Sundari, 2015). D. M. Dash and J. W. Osborne investigated the bioremediation of monocrotophos by native isolates obtained from cultivable sugarcane soil. The inclusion of *Bacillus aryabhattai* to the rhizosphere of *Liriope muscari* improved plant growth and monocrotophos degradation in soil (Dash and Osborne, 2020). Meng Di *et al.* presented the entire genome sequence of *Bacillus amyloliquefaciens* as well as its capacity to break down a variety of organophosphorus insecticides (Meng *et al.* 2019). Fiaz Ahmad's research group portrayed the ability of inoculated external bacteria *Bacillus pumilus* to accelerate the cleanup of chlorpyrifos-contaminated soil and reduce levels of harmful pesticide residues in crops (Ahmad *et al.* 2012). Govarathanan *et al.* explored the potential for speedy degradation of chlorpyrifos and plant development enhancing psychrophilic *Shewanella* sp. cultures were obtained from saltwater using the enrichment technique (Govarathanan *et al.* 2020). Ghani *et al.* examined the efficacy of a bacterial strain, *Enterobacter cloacae*, competent in promoting plant growth and biodegrading PF (Ghani *et al.* 2021). Jaoti examined the potency of *rhizobacteria* towards the biodegradation of PF and analyzed the progress of the growth of the plants (Jatoi *et al.* 2021). Vinay Kumar *et al.* assessed the plant development and PF elimination efficacy of *Acinetobacter* and *Comamonas* sp. bacteria as separate strains and in combination and found that the consortium has greater plant growth-promoting properties than the individual bacterium (Kumar *et al.* 2020).

Inspired by the facts and results, we attempted to isolate and identify a novel bacterial strain from pesticide-contaminated soil and succeeded. Further, the metabolites

derived from PF degradation were identified by using GC-MS and a probable mechanism has been proposed. Furthermore, the isolated bacteria were tested for their ability to promote plant growth.

6.2. Experimental Section

Isolation, screening, and identification of potential profenofos degrading bacterium have been described in the previous chapter V. The collected soil from the agricultural field of Karur district, Tamil Nadu, India was sterilized for pot culture experiment.

6.2.1. Degradation of Profenofos in Liquid Culture Media

The capability of the isolated strain *Bacillus subtilis* towards the biodegradation of profenofos was investigated with a mineral salt medium comprising 100 mg/L profenofos as the only carbon source. Bacterial cells have been pre-cultured in a nutrient agar medium at 30 °C for 24 h at 140 rpm in an orbital shaker. To extract the profenofos residues, aliquots (2 mL) of the sample were withdrawn from the 100 mL liquid culture, combined with 2 mL ethyl acetate, and rapidly shaken for 30 min. The organic phase was centrifuged at 10,000 rpm for 15 min. The ethyl acetate layer was dried using anhydrous Na₂SO₄ and evaporated at room temperature. The residues have been dissolved in 100 µL of ethyl acetate.

6.2.2. GC-MS Analysis

PF degradation was determined after 3 weeks. For the GC-MS analysis, a 5 g soil sample was collected from pots B and C. 2 mL of aliquots were extracted after 3 weeks and analyzed gas chromatography-mass spectroscopy (GC-MS) analysis. To detect the metabolites of profenofos breakdown, a GC-MS system (GCMS-QP 2010 Shimadzu) provided with an auto-sampler and a mass spectrometer detector was used. For compound separation, a Phenomenex ZB 5MS column has been utilized. Helium (He) gas has been employed as a carrier gas, at a flow speed of 1 mL /min. The following temperature system was applied. The oven temperature was originally held 60 °C for one min before rising from

10 °C per min to 290 °C with a 20-minute hold time; the total run time was 45 min. The injector's temperature was kept at 250 °C. The ion trap was set to 70 eV and had a scan range of 40 to 800 m/z. In split mode, 1 L of each sample was injected (10:1). Metabolite identification was accomplished by comparing the results to standard compound mass fragmentation patterns as well as instrumental library searches (Herrmann *et al.*, 1999). (Arjunan and Muthuselvam, 2021).

6.2.3. Pot Culture Experimental Design

6.2.3.1. Analysis of Plant Growth Parameters

Pot experiments with *Vigna mungo* (L.) Hepper was performed to analyze the influence of the isolated microbial inoculation on the breakdown of profenofos and plant growth parameters. The surface of the *V. mungo* seeds have been sterilized using 0.1% mercuric chloride solution for 10 min and then cleaned by sterilized distilled water. 1.0 kg of soil samples have been spiked with profenofos to a dose of 100 mg /kg. After that, the soil was inoculated with bacterial culture to achieve the desired concentration of 1.6×10^7 cells /g. The experiment has been carried out in triplicate and a sterilized soil sample was employed as control. Sterilized *V.mungo* seeds have been sown in the soil sample and watered. Three groups of experimental pots with a minimum of six seedlings have been cultivated under the following circumstances: (A) Usual growth condition: Sterilized soil as the control without bacteria and PF; (B) under pesticide load: Sterilized soil with PF and without bacteria, (C) Under pesticide with isolated strain: Sterilized soil with isolated strain and profenofos. The pots were carefully monitored to ensure that they have been maintained at ambient environmental conditions. The seed germination progression has been monitored regularly, and plants have been developed for 3 weeks. The characteristics of plant development were documented were: percentage of germination, leaf length (cm), root length (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight.

6.2.3.2. Degradation Assay of Profenofos in Pot Culture Soil

The ability of the isolated strain *Bacillus subtilis* towards the biodegradation of profenofos in pot soil was examined with 1 kg of sterilized soil comprising 100 mg of profenofos. To extract the profenofos residues, 1 g of the pot soil samples from pot soil B (control) and pot soil C (treated) was taken. The soil samples were added with 10 mL ethyl acetate and rapidly shaken for 30 min. The organic phase was centrifuged at 10,000 rpm for 15 min. The ethyl acetate layer was dried using anhydrous Na₂SO₄ and evaporated at room temperature. The residues have been dissolved in 100 µL of ethyl acetate. The ability of *Bacillus subtilis* on biodegradation of profenofos in pot soil and to identify the degradation metabolites GC-MS analysis for extracts from pot soils B (control) and pot soil C (treated) was performed by following the same procedure mentioned in the previous section 6.2.2.

6.2.4. Statistical Analysis

All the experiments have been performed in triplicates, and statistical analysis has been used to identify distinct changes. A one-way analysis of variance (ANOVA) was employed to execute statistical analysis for percentage of germination, leaf length (cm), root length (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight involving multiple comparisons. Plant growth indicators were assessed using one-way ANOVA, exhibiting substantial changes at $p \leq 0.05$ levels with multiple comparisons.

6.3. Results and Discussion

6.3.1. Degradation of Profenofos in Liquid Culture Medium

The GC-MS methodology was utilized for the successful detection of profenofos decomposition products in the current study. After 24 - 48 h, a sample retrieved in ethyl acetate from bacterium culture with profenofos pesticide was injected into GC-MS. Figure 29 shows the GC-MS spectrum showing the degradation of profenofos. The metabolite identified as 4-Bromo-2-chlorophenol corresponded to a medium intense peak of 5.957 at 6.23 min retention time. The generation of 4-Bromo-2-chlorophenol (M.wt. 206 g/mol) demonstrates the breakdown of profenofos at the ester bond attachment in the parent compound (Palanimanickam *et al.*, 2017). Further, the degradation ability of profenofos was confirmed by the formation of diethylthiophosphoric acid (peak of 12.962 at 13 min retention time).

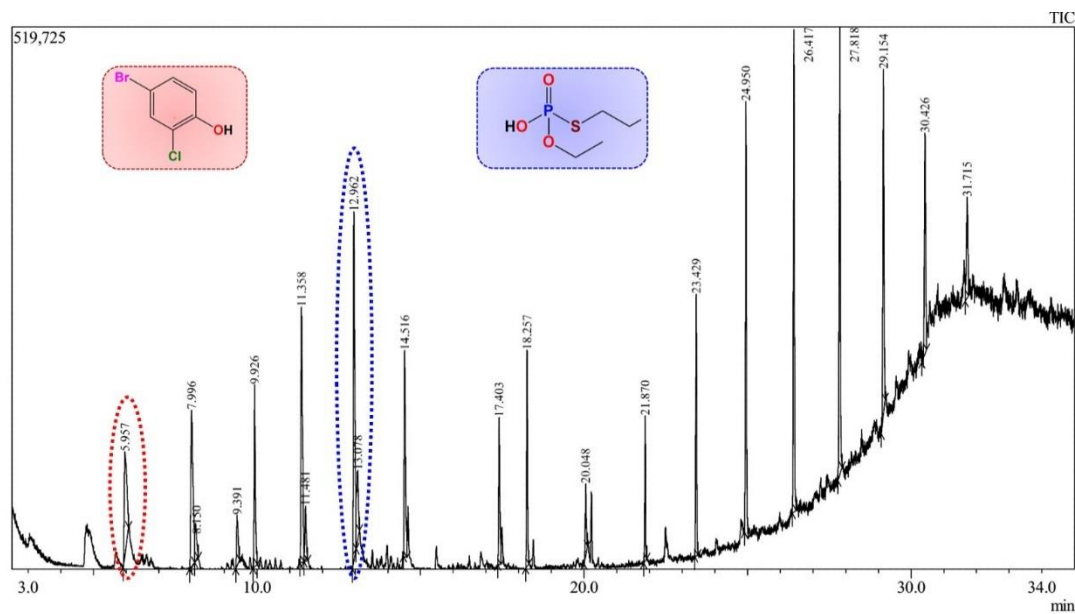


Figure 29. GC-MS spectrum showing the degradation of profenofos

6.3.2. Pot Culture Experiment

6.3.2.1. Plant Growth Parameters

Plant growth studies were used to investigate the effect of *Bacillus subtilis* activity on *Vigna mungo* (L.) Hepper development as well as profenofos breakdown. In pot soil experiments, percentage of germination, leaf length (cm), root length (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight was determined. The results are given in Table 7 and Figure 30. Profenofos application to pot soil (B) resulted in a reduction in all variables analyzed when compared with the plants grown in the absence of profenofos (A). However, when compared to pot B, the plants in pot C exhibited comparatively higher plant growth characteristics. This may be due to the presence of *Bacillus subtilis* which utilized the profenofos as the sole origin of carbon thus causing the degradation of profenofos.

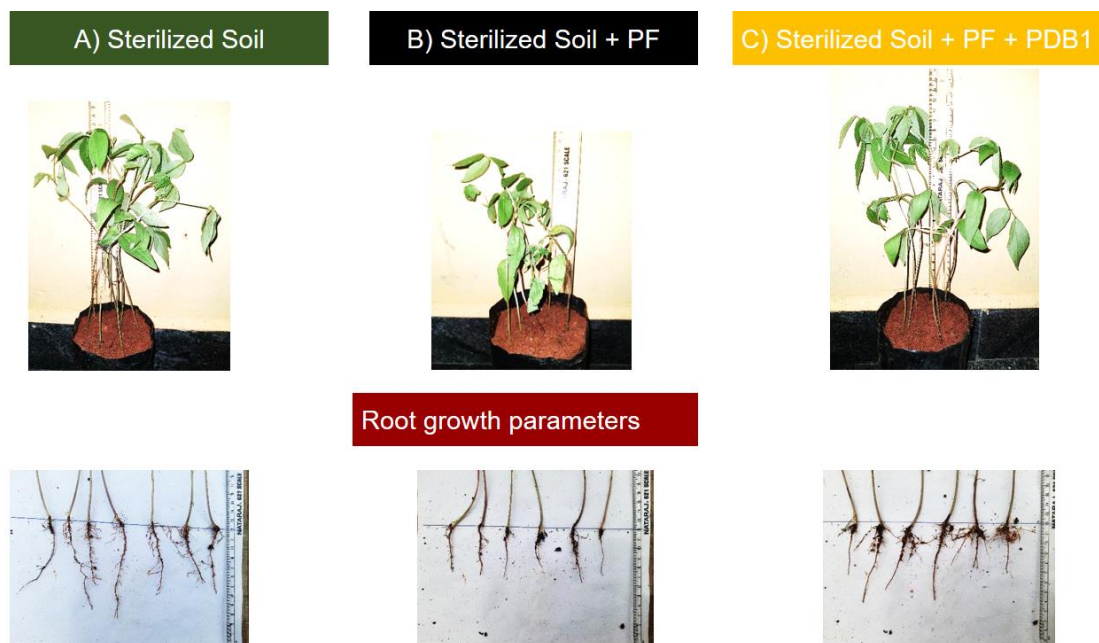


Figure 30. Growth experiment with *V. mungo* in soil supplemented with PF (100 mg /kg).

Table 7. Measurement of growth parameters of *V. mungo* in pots A, B, and C

Growth parameters	Control	PF + <i>V. mungo</i>	PF + <i>V. mungo</i> + <i>Bacillus subtilis</i>
% of germination	90.33 ± 0.88 ^a	66.33±0.66 ^a	75.00±1.15 ^a
Shoot length (cm)	4.64±0.15 ^c	3.13±0.03 ^c	4.16±0.13 ^c
Root length (cm)	7.43±0.37 ^b	5.03±0.21 ^b	6.06±0.16 ^b
Leaf length (cm)	3.63±0.12 ^c	2.53±0.17 ^c	3.11±0.10 ^c
Shoot fresh weight (g)	1.83±0.03 ^d	1.13±0.04 ^d	1.50±0.04 ^d
Root fresh weight (g)	0.80±0.03 ^{de}	0.56±0.01 ^{de}	0.63±0.02 ^e
Shoot dry weight (g)	0.62±0.02 ^e	0.25±0.01 ^e	0.57±0.01 ^e
Root dry weight (g)	0.25±0.01 ^e	0.06±0.00 ^e	0.12±0.02 ^e

The values indicate the mean ± SD of three replicates.

Different letters in the same rows indicate significantly different values.

6.3.2.2. Degradation of Profenofos in Pot Culture Soil

The ability of *Bacillus subtilis* to degrade PF in a pot soil environment was also studied using GC-MS. The GC-MS was performed for sterilized soil with PF (B) along with sterilized soil with PF and *Bacillus subtilis* (C). The results of GC-MS are given in Figure 31 and Figure 32. Figure 31 displayed a single peak of 20.005 at 19.6 min retention time. The peak is attributed to PF pesticide. Figure 32 exhibits the GC-MS result of pot C which contains sterilized soil with PF and *Bacillus subtilis*. In contrast to Figure 31, the spectrum showed multiple peaks due to the biodegradation of PF (Figure 32). Specifically, the emergence of two peaks corresponding to the metabolites of PF namely, 4-Bromo-2-chlorophenol (peak of 5.978 at 6.3 min retention time) and diethylthiophosphoric acid (peak of 13.067 at 13.2 min retention time) confirmed the *Bacillus subtilis* mediated biodegradation of PF (Kushwaha *et al.* 2016). The plausible mechanistic representation of profenofos biodegradation was portrayed in Figure 33. The results imply that the isolated bacterial strain comprised enzymes with esterase activity. Nonetheless, more research is

needed to determine the complete degradation pathway and the mineralization of intermediates.

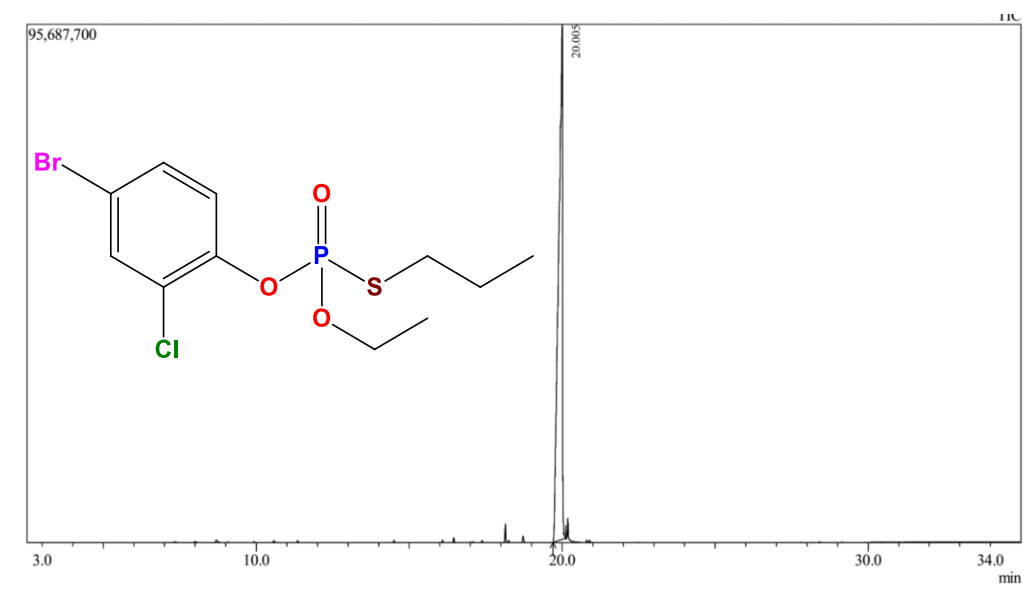


Figure 31. GC-MS result of sterilized soil with PF (control)

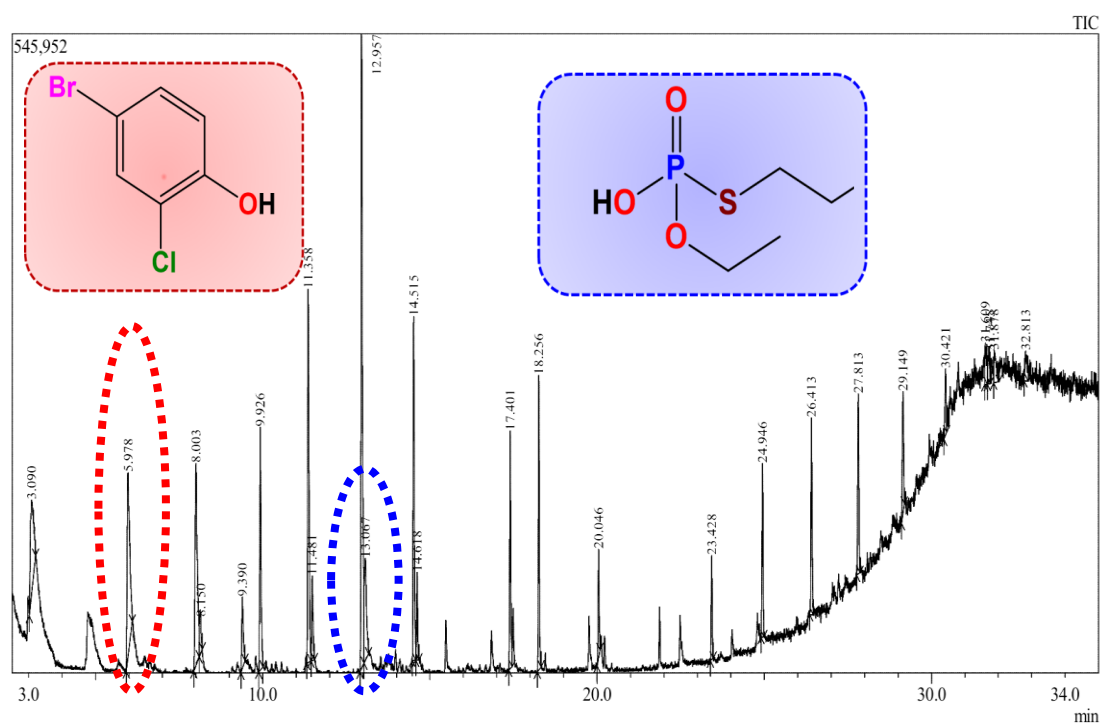


Figure 32. GC-MS result of sterilized soil with PF and *Bacillus subtilis* (treated)

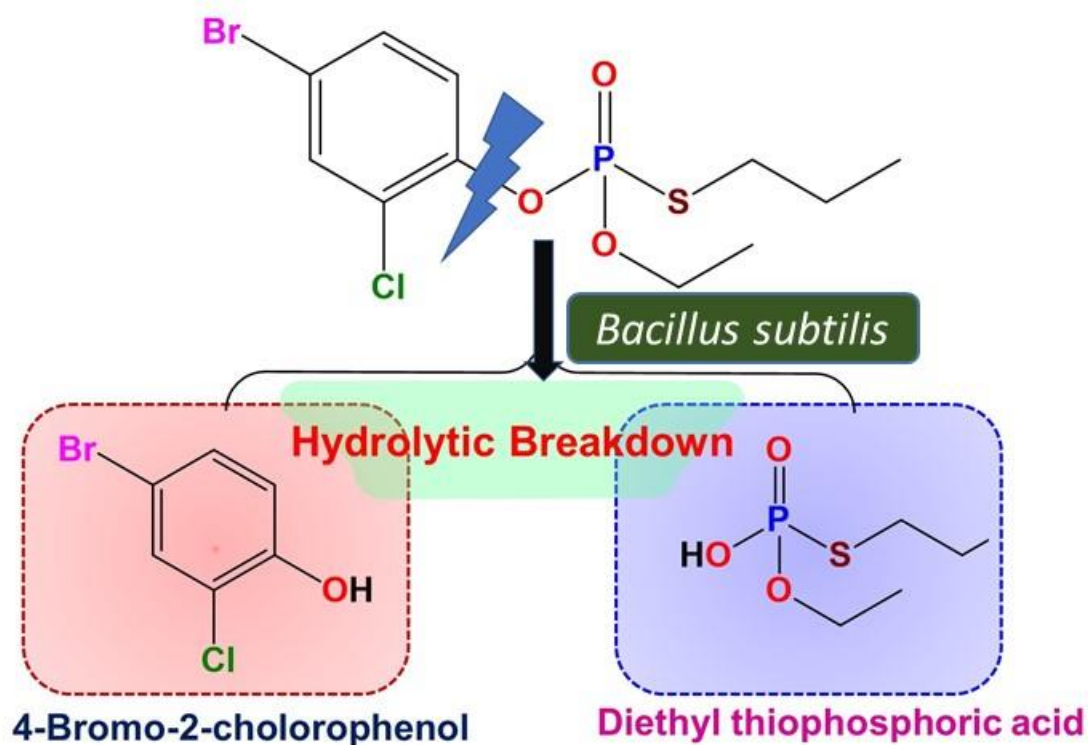


Figure 33. Plausible mechanistic representation of profenofos biodegradation

6.4. Conclusion

Plant-bacteria relationships can be used to improve pesticide-contaminated soil remediation. A bacterial strain was isolated using the enrichment methodology for the efficient remediation of PF-contaminated soil. The isolated strain was confirmed as *Bacillus subtilis* by cultural, morphological, biochemical, and 16S rRNA gene studies. *Bacillus subtilis* was tested on the plant (*V. mungo*) growth variables, including percentage of germination, leaf length (cm), root length (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight including and excluding profenofos pesticide. According to the tested criteria, the pot soil reinforced with profenofos and *Bacillus subtilis* exhibited comparatively better growth of *V. mungo* than the pot soil containing simply profenofos. The growth enhancement of *V. mungo* plant may be due to the presence of *Bacillus subtilis* which utilized the profenofos as the sole origin of carbon thus causing the degradation of profenofos. The hydrolytic breakdown of profenofos

pesticide into 4-Bromo-2-chlorophenol and diethylthiophosphoric acid metabolites by *Bacillus subtilis* was confirmed in mechanistic studies of profenofos pesticide degradation in both liquid culture and pot soil using GC-MS. This isolate, *Bacillus subtilis* has the potential to be a key contender for the advancement of the bioremediation approach due to its high biodegradation and plant growth stimulating potential. Furthermore, the findings demonstrate that *Bacillus subtilis* possesses the ability to increase agricultural productivity in pesticide-polluted agricultural soil as well as provide insights into the link between microbes and the living environment.

Summary and Conclusion

Pesticides are a vital component of today's modern agriculture. With the advancement of modern agriculture, pesticide administration to agricultural fields has become a key factor of crop production. Pesticides are extensively used regularly to meet the increasing demand for production to fulfill the food needs of the world's rapidly expanding population. Every year, roughly 2.5 million tons of pesticides are used to boost productivity and reduce crop losses brought about by pests and diseases. However, excessive pesticide uses not only pollute the surrounding soil and water environments but also accumulate pesticides and their metabolites in humans, fauna, as well as flora and causing severe problems. Organophosphorus pesticides are widely regarded as low-toxic and very efficient pesticides for crop productivity. It has been estimated that organophosphate pesticides have the greatest consumption rate of all pesticides, leaving 3 million individuals in danger by organophosphorus pesticide poisoning every year. They constitute approximately 30% of all pesticides used globally. It is estimated that only less than 0.1 % of pesticides applied to reach the targets, with a balance of 99.9 % lingering in the environment. Profenofos pesticide is one of the most widely employed organophosphorus pesticides on a variety of crops. Organophosphorus insecticides, particularly profenofos, and their metabolites are extensively spotted in crops, vegetables, birds as well as animals. Alarmingly, profenofos and its metabolites have been detected in human blood, serum, urine, and breast milk. Hence, profenofos pesticide accumulation and pollution have been classified as a major environmental health hazard. As a result, developing remedies and methodologies for removing, decontaminating, and detoxifying a profenofos-contaminated environment became a vital aspect of environmental research. Various strategies for eliminating profenofos content were developed in the past, based on

surface adsorption and bioremediation. Adsorption techniques based on iron nanoparticles are highly effective, easy to utilize, and can be used to remove pesticide contaminants. These types of nano-adsorbents appear to be quite likely to find extensive commercial applications in wastewater treatment in the near future. On the other hand, biological techniques have also been used to remediate the pesticide-contaminated environment. Several experiments have demonstrated that microbial breakdown of toxic compounds into less hazardous by-products is advantageous. Toxic pesticide bioremediation is a major, ecologically friendly, efficient, and sustainable protocol for removing leftover pesticides from farm soil areas, water sources, and other contaminated ecosystems. A diverse range of microorganisms has been isolated and identified around the world as having the potential to degrade or eliminate toxic organophosphorus pesticides into harmless materials by utilizing them as various sources of energy. The conclusions of the present investigations are summarized.

Ecofriendly fabrication of metal nanomaterials is a strategy that reduces the formation of secondary pollutants, harsh chemicals, and hazardous solvents, among other things, which have harmful effects not only on the ecosystem as well as on public health. The capability of tea waste extract to function as alternative reducing agents for the sustainable synthesis of INPs was investigated in the present study. UV-vis, FT-IR, SEM, DLS, zeta potential, EDX with TEM elemental mapping, and PXRD techniques were used to extensively characterize the as-synthesized INPs. Green synthesized INPs were shown to have good water stability, an anionic surface nature, and an average particle size of 60.37 ± 2.65 nm. Furthermore, the existence of various elements and functional groups in the tea waste extract that are responsible for the generation of amorphous INPs was substantiated using FT-IR, EDX, TEM elemental mapping, and PXRD techniques.

Hence, the green synthesized iron nanoparticles have been employed as efficient adsorbent towards the removal of profenofos from the aqueous solution. Various influencing factors such as contact time, adsorbent dosage, solution pH, and initial concentration of pesticide were systematically optimized for the maximum adsorption efficiency of profenofos by the prepared iron nanoparticles. The kinetic data were analyzed using various adsorption kinetic models such as pseudo-first-order, pseudo-second-order, and intra-particle diffusion. Further, the interaction between prepared INPs and PF was evaluated using various adsorption isotherm models such as Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich (D-R) models. The monolayer Langmuir adsorption capacity of 43.67 mg/g was obtained with a minimum contact time of 15 min. According to the kinetic and isotherm data, the pseudo-second-order kinetic and Langmuir isotherm models were found to be fitted well for PF adsorption using the prepared INPs. The electrostatic interaction was mainly governed the adsorptive mechanism of PF molecules onto the INPs.

The chemical structure of organophosphorus pesticides is attributed to their high toxicity and competence to irreversibly inhibit the activity of acetylcholinesterase and inflict neuronal disorder, organ failure, and eventual death among non-target species. Microbe-mediated biodegradation of organophosphate pesticides into less harmful compounds and the advancement of bioremediation approaches for contaminated agricultural fields by using potential microorganisms symbolize a rapidly expanding area of research worldwide. In the pristine ecosystems and non-exposed agricultural fields, naturally existing profenofos-degrading microorganisms may be scarce. As a result, the enrichment technique for isolating bacterial strains was widely employed in the investigation of pesticide biodegradation. In the present exploration, three bacterial strains were isolated from pesticide-contaminated agricultural soil and screened for their pesticide tolerance capacity. Among the strains, a

bacterium showed substantial growth against the maximum profenofos concentration. The screened bacterial strain was identified by various characterization techniques including cultural, morphological, biochemical, and molecular characterization. Cultural and morphological analysis revealed that the screened strain was rod-shaped and a fuzzy-white in color. The biochemical analysis of the screened strain exhibited 99 % characteristics of the Gram-positive *Bacillus* genus. The molecular characterization analysis revealed that the screened strain is *Bacillus subtilis*.

Bioremediation is one of the most potent, secure, and commercially sustainable strategy when compared to other contaminants removal methods. The degradation and conversion of recalcitrant pesticides can be better accomplished through bioremediation by the action of microbial enzymes. Hence, the selected bacterial strain *Bacillus subtilis* has been subjected to degrade profenofos pesticide in a liquid culture medium. Gas Chromatography-Mass Spectroscopy (GC-MS) technique evidenced the capability of *Bacillus subtilis* to break down profenofos pesticide into 4-Bromo-2-chlorophenol and diethylthiophosphoric acid metabolites. Further, plant-bacteria relationships can be used to improve pesticide-contaminated soil remediation. *Bacillus subtilis* was tested on the plant (*Vigna mungo*) growth variables, including percent germination, leaf length (cm), root length (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight including and excluding profenofos pesticide. According to the tested criteria, the pot soil reinforced with profenofos and *Bacillus subtilis* exhibited comparatively better growth of *V. mungo* than the pot soil containing simply profenofos. The growth enhancement of *V. mungo* plant may be due to the presence of *Bacillus subtilis* which utilized the profenofos as the sole origin of carbon thus causing the degradation of profenofos. The hydrolytic breakdown of profenofos pesticide into 4-Bromo-2-

chlorophenol and diethylthiophosphoric acid metabolites by *Bacillus subtilis* was confirmed in mechanistic studies of profenofos pesticide degradation in both liquid culture and pot soil using GC-MS. The findings demonstrate that *Bacillus subtilis* possesses the ability to increase agricultural productivity in pesticide-polluted agricultural soil as well as provide insights into the link between microbes and the living environment.

Overall, the findings of this study corroborated the scope and efficacy of synthesized iron nanoparticles as effective adsorbents for the environmentally-friendly removal of harmful profenofos pesticide from aquatic environment. In addition, the isolated bacterium *Bacillus subtilis* has been proved as key contender for the advancement of the bioremediation approach due to its efficient degradation of profenofos in contaminated agricultural soil environment.

References

- Abaga, N. O. Z., Alibert, P., Dousset, S., Savadogo, P. W., Savadogo, M., & Sedogo, M. (2011). Insecticide residues in cotton soils of Burkina Faso and effects of insecticides on fluctuating asymmetry in honey bees (*Apis mellifera* Linnaeus). *Chemosphere*, 83(4), 585-592.
- Abass, K., Reponen, P., Jalonen, J., & Pelkonen, O. (2007). *In vitro* metabolism and interaction of profenofos by human, mouse and rat liver preparations. *Pesticide biochemistry and physiology*, 87(3), 238-247.
- Abdullah, R. R., Ghani, S. B. A., & Sukar, N. A. (2016). Degradation of profenofos and λ -cyhalothrin using endogenous bacterial isolates and detection of the responsible genes. *J Bioremed Biodegr*, 7, 360.
- Adams, G. O., Fufeyin, P. T., Okoro, S. E., & Ehinomen, I. (2015). Bioremediation, biostimulation and bioaugmentation: a review. *International Journal of Environmental Bioremediation & Biodegradation*, 3(1), 28-39.
- Ahmad, F., Iqbal, S., Anwar, S., Afzal, M., Islam, E., Mustafa, T., & Khan, Q. M. (2012). Enhanced remediation of chlorpyrifos from soil using ryegrass (*Lolium multiflorum*) and chlorpyrifos-degrading bacterium *Bacillus pumilus* C2A1. *Journal of hazardous materials*, 237, 110-115.
- Ahmad, T., Rafatullah, M., Ghazali, A., Sulaiman, O., Hashim, R., & Ahmad, A. (2010). Removal of pesticides from water and wastewater by different adsorbents: a review. *Journal of Environmental Science and Health, Part C*, 28(4), 231-271.
- Ahmed, I., Mondol, M. M. H., Lee, H. J., & Jhung, S. H. (2021). Application of Metal-Organic Frameworks in Adsorptive Removal of Organic Contaminants from Water, Fuel and Air. *Chemistry—An Asian Journal*, 16(3), 185-196.
- Ahmed, S. F., Mofijur, M., Rafa, N., Chowdhury, A. T., Chowdhury, S., Nahrin, M., & Ong, H. C. (2022). Green approaches in synthesising nanomaterials for environmental nanobioremediation: Technological advancements, applications, benefits and challenges. *Environmental Research*, 204, 111967.
- Akbar, S., & Sultan, S. (2016). Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. *brazilian journal of microbiology*, 47, 563-570.
- 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.

- Ali, I., & Gupta, V. K. (2006). Advances in water treatment by adsorption technology. *Nature protocols*, 1(6), 2661-2667.
- Ali, I., Alharbi, O. M., Alothman, Z. A., & Alwarthan, A. (2018). Facile and eco-friendly synthesis of functionalized iron nanoparticles for cyanazine removal in water. *Colloids and Surfaces B: Biointerfaces*, 171, 606-613.
- Amer, F. I., Zakaria, I., El-Shabaka, H. A., & Ashour, I., (2007). The Effect Of An Organophosphorous Insecticide on The Hepatic, Renal And Pulmonary Tissues Of Mice Fetuses Egypt J. Med. Lab.Sci., 16(2): 99 -113.
- Andreu, V., & Pico, Y. (2004). Determination of pesticides and their degradation products in soil: critical review and comparison of methods. *TrAC Trends in Analytical Chemistry*, 23(10-11), 772-789.
- Ankenbrand, M. J., & Keller, A. (2016). bcg Tree: automatized phylogenetic tree building from bacterial core genomes. *Genome*, 59(10), 783-791.
- Arjunan, A., Muthuselvam, M., (2021). Isolation and identification of profenofos degrading bacterium for efficient bioremediation of pesticide contaminated agricultural soil. *International journal of environmental analytical chemistry* 21(2), 5415.
- Arole, V. M., & Munde, S. V. (2014). Fabrication of nanomaterials by top-down and bottom-up approaches-an overview. *J. Mater. Sci*, 1, 89-93.
- Balouchi, H., Baziar, M., Dehghan, A., Alidadi, H., & Shams, M. (2020). Combination of electrocoagulation and MOF adsorption systems for EBT removal from water. *International Journal of Environmental Analytical Chemistry*, 1-11.
- Barcelo, D. (1993). Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water. *Journal of Chromatography A*, 643(1-2), 117-143.
- Baziar, M., Zakeri, H. R., Nejad, Z. D., Shams, M., Anastopoulos, I., Giannakoudakis, D. A., & Lima, E. C. (2021). Metal-organic and Zeolitic imidazole frameworks as cationic dye adsorbents: physicochemical optimizations by parametric modeling and kinetic studies. *Journal of Molecular Liquids*, 332, 115832.
- Bhateria, R., & Singh, R. (2019). A review on nanotechnological application of magnetic iron oxides for heavy metal removal. *Journal of Water Process Engineering*, 31, 100845.

- Bhattacharyya, D., Singh, S., Satnalika, N., Khandelwal, A., & Jeon, S. H. (2009). Nanotechnology, big things from a tiny world: a review. *International Journal of u- and e-Service, Science and Technology*, 2(3), 29-38.
- Biftu, W. K., Ravindhranath, K., & Ramamoorthy, M. (2020). New research trends in the processing and applications of iron-based nanoparticles as adsorbents in water remediation methods. *Nanotechnology for Environmental Engineering*, 5, 1-12.
- Bolade, O. P., Williams, A. B., & Benson, N. U. (2020). Green synthesis of iron-based nanomaterials for environmental remediation: A review. *Environmental Nanotechnology, Monitoring & Management*, 13, 100279.
- Bora, A. J., Mohan, R., & Dutta, R. K. (2018). Simultaneous removal of arsenic, iron and manganese from groundwater by oxidation-coagulation-adsorption at optimized pH. *Water Science and Technology: Water Supply*, 18(1), 60-70
- Bose, S., Kumar, P. S., Vo, D. V. N., Rajamohan, N., & Saravanan, R. (2021). Microbial degradation of recalcitrant pesticides: a review. *Environmental Chemistry Letters*, 19, 3209-3228.
- Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environmental science & policy*, 9(7-8), 685-692.
- Chanika, E., Georgiadou, D., Soueref, E., Karas, P., Karanasios, E., Tsiropoulos, N. G., & Karpouzas, D. G. (2011). Isolation of soil bacteria able to hydrolyze both organophosphate and carbamate pesticides. *Bioresource technology*, 102(3), 3184-3192.
- Choe, S., Chang, Y. Y., Hwang, K. Y., & Khim, J. (2000). Kinetics of reductive denitrification by nanoscale zero-valent iron. *Chemosphere*, 41(8), 1307-1311.
- Clay, J. (2004). *World agriculture and the environment: a commodity-by-commodity guide to impacts and practices*. Island Press.
- Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection*, 26(9), 1337-1348.
- Cosgrove, S., Jefferson, B., & Jarvis, P. (2019). Pesticide removal from drinking water sources by adsorption: a review. *Environmental Technology Reviews*, 8(1), 1-24.
- Dadfarnia, S., Shabani, A. H., Moradi, S. E., & Emami, S. (2015). Methyl red removal from water by iron based metal-organic frameworks loaded onto iron oxide nanoparticle adsorbent. *Applied Surface Science*, 330, 85-93.

- Dalal, U., & Reddy, S. N. (2019). A novel nano zero-valent iron biomaterial for chromium (Cr 6+ to Cr 3+) reduction. *Environmental Science and Pollution Research*, 26(11), 10631-10640.
- Dash, D. M., & Osborne, J. W. (2020). Biodegradation of monocrotophos by a plant growth promoting *Bacillus aryabhatai* (VITNNDJ5) strain in artificially contaminated soil. *International Journal of Environmental Science and Technology*, 17(3), 1475-1490.
- Dash, D. M., & Osborne, W. J. (2020). Rapid biodegradation and biofilm-mediated bioremoval of organophosphorus pesticides using an indigenous *Kosakonia oryzae* strain-VITPSCQ3 in a Vertical-flow Packed Bed Biofilm Bioreactor. *Ecotoxicology and environmental safety*, 192, 110290.
- Dawson, A. H., Eddleston, M., Senarathna, L., Mohamed, F., Gawarammana, I., Bowe, S. J., & Buckley, N. A. (2010). Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. *PLoS medicine*, 7(10), 1000357.
- De Smedt, C., Ferrer, F., Leus, K., & Spanoghe, P. (2015). Removal of pesticides from aqueous solutions by adsorption on zeolites as solid adsorbents. *Adsorption Science & Technology*, 33(5), 457-485.
- de Souza, A. P. N., Licea, Y. E., Colaço, M. V., Senra, J. D., & Carvalho, N. M. (2021). Green iron oxides/amino-functionalized MCM-41 composites as adsorbent for anionic azo dye: Kinetic and isotherm studies. *Journal of Environmental Chemical Engineering*, 9(2), 105062.
- Dehaghi, S. M., Rahmanifar, B., Moradi, A. M., & Azar, P. A. (2014). Removal of permethrin pesticide from water by chitosan–zinc oxide nanoparticles composite as an adsorbent. *Journal of Saudi Chemical Society*, 18(4), 348-355.
- Dehghani, M. H., Karri, R. R., Alimohammadi, M., Nazmara, S., Zarei, A., & Saeedi, Z. (2020). Insights into endocrine-disrupting Bisphenol-A adsorption from pharmaceutical effluent by chitosan immobilized nanoscale zero-valent iron nanoparticles. *Journal of Molecular Liquids*, 311, 113317.
- Del Prado-Lu, J. L. (2015). Insecticide residues in soil, water, and eggplant fruits and farmers' health effects due to exposure to pesticides. *Environmental health and preventive medicine*, 20(1), 53-62.
- Devatha, C. P., Thalla, A. K., & Katte, S. Y. (2016). Green synthesis of iron nanoparticles using different leaf extracts for treatment of domestic waste water. *Journal of cleaner production*, 139, 1425-1435.

- Dhir, B. (2021). Nanomaterials for Remediation of Pesticides. *New Frontiers of Nanomaterials in Environmental Science*, 193-204.
- Diagbaya, P. N., Olu-Owolabi, B. I., & Adebawale, K. O. (2015). Synthesis of covalently bonded graphene oxide-iron magnetic nanoparticles and the kinetics of mercury removal. *Rsc Advances*, 5(4), 2536-2542.
- Dickson, D., Liu, G., & Cai, Y. (2017). Adsorption kinetics and isotherms of arsenite and arsenate on hematite nanoparticles and aggregates. *Journal of environmental management*, 186, 261-267.
- Diez M C (2010) Biological aspects involved in the degradation of organic pollutants. *Journal of Soil Science and Plant Nutrition*, **10(3)** 244–267.
- Digrak, M., & Kazanici, F. (2001). Effect of some organophosphorus insecticides on soil microorganisms. *Turkish Journal of Biology*, 25(1), 51-58.
- Dutz, S., Müller, R., Eberbeck, D., Hilger, I., & Zeisberger, M. (2015). Magnetic nanoparticles adapted for specific biomedical applications. *Biomedical Engineering/Biomedizinische Technik*, 60(5), 405-416.
- Ealia, S. A. M., & Saravanakumar, M. P. (2017). A review on the classification, characterisation, synthesis of nanoparticles and their application. In *IOP Conference Series: Materials Science and Engineering*, 263(3), 032019.
- Ecobichon, D. J. (2001). Pesticide use in developing countries. *Toxicology*, 160(1-3), 27-33.
- Edwards, C. A. (Ed.). (2013). *Environmental pollution by pesticides* (Vol. 3). Springer Science & Business Media.
- El-Sayed, M. E. (2020). Nanoadsorbents for water and wastewater remediation. *Science of the Total Environment*, 739, 139903.
- Fahmy, H. M., Mohamed, F. M., Marzouq, M. H., Mustafa, A. B. E. D., Alsoudi, A. M., Ali, O. A., & Mahmoud, F. A. (2018). Review of green methods of iron nanoparticles synthesis and applications. *BioNanoScience*, 8(2), 491-503.
- Fairooz, N. E., Jwad, Z. A., & Zahra, M. (2015). Adsorption isotherms and thermodynamic data for removal pesticides from aqueous solution on pomegranate peel surface. *American Journal of Applied Chemistry*, 3(4), 147-152.
- Farhan, M., Ahmad, M., Kanwal, A., Butt, Z. A., Khan, Q. F., Raza, S. A., & Wahid, A. (2021). Biodegradation of chlorpyrifos using isolates from contaminated agricultural soil, its kinetic studies. *Scientific reports*, 11(1), 1-14.

- Fazlzadeh, M., Rahmani, K., Zarei, A., Abdoallahzadeh, H., Nasiri, F., & Khosravi, R. (2017). A novel green synthesis of zero valent iron nanoparticles (NZVI) using three plant extracts and their efficient application for removal of Cr (VI) from aqueous solutions. *Advanced Powder Technology*, 28(1), 122-130.
- Frey, P. A., & Reed, G. H. (2012). The ubiquity of iron. *ACS Chem. Biol.*, 7(9), 1477–1481
- Gacem, M. A., Telli, A., & Khelil, A. O. E. H. (2021). Nanomaterials for detection, degradation, and adsorption of pesticides from water and wastewater. In *Aquananotechnology* (pp. 315-336). Elsevier.
- Galdames, A., Ruiz-Rubio, L., Orueta, M., Sánchez-Arzalluz, M., & Vilas-Vilela, J. L. (2020). Zero-Valent Iron Nanoparticles for Soil and Groundwater Remediation. *International Journal of Environmental Research and Public Health*, 17(16), 5817.
- Gautam, A., Rawat, S., Verma, L., Singh, J., Sikarwar, S., Yadav, B. C., & Kalamdhad, A. S. (2018). Green synthesis of iron nanoparticle from extract of waste tea: An application for phenol red removal from aqueous solution. *Environmental nanotechnology, monitoring & management*, 10, 377-387.
- Germany, P. A. N. (2012). Pesticides and health hazards facts and figures. *Hamburg, Germany: PAN Germany—Pestizid Aktions-Netzwerk eV.*
- Ghani, M. U., Asghar, H. N., Niaz, A., Ahmad Zahir, Z., Nawaz, M. F., & Häggblom, M. M. (2021). Efficacy of rhizobacteria for degradation of profenofos and improvement in tomato growth. *International Journal of Phytoremediation*, 1-11.
- Gillham, R. W., & O'Hannesin, S. F. (1994). Enhanced degradation of halogenated aliphatics by zero-valent iron. *Groundwater*, 32(6), 958-967.
- Gonçalves, J. R., & Alves, M. D. (2021). The Soil and Groundwater Remediation with Zero-Valent Iron Nanoparticles. In *Electrochemically Assisted Remediation of Contaminated Soils*, 315-341.
- Gotoh, M., Sakata, M., Endo, T., Hayashi, H., Seno, H., & Suzuki, O. (2001). Profenofos metabolites in human poisoning. *Forensic science international*, 116(2-3), 221-226.
- Gottimukkala, K. S. V., Harika, R. P., & Zamare, D. (2017). Green synthesis of iron nanoparticles using green tea leaves extract. *J. Nanomed. Biother. Discovery*, 7(151.10), 4172.
- Govarthanan, M., Ameen, F., Kamala-Kannan, S., Selvankumar, T., Almansob, A., Alwakeel, S. S., & Kim, W. (2020). Rapid biodegradation of chlorpyrifos by plant

- growth-promoting psychrophilic *Shewanella* sp. BT05: An eco-friendly approach to clean up pesticide-contaminated environment. *Chemosphere*, 247, 125948.
- Grieger, K. D., Fjordbøge, A., Hartmann, N. B., Eriksson, E., Bjerg, P. L., & Baun, A. (2010). Environmental benefits and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation: risk mitigation or trade-off?. *Journal of Contaminant Hydrology*, 118(3-4), 165-183.
- Habiba, R. A., Ali, H. M., & Ismail, S. M. (1992). Biochemical effects of profenofos residues in potatoes. *Journal of agricultural and food chemistry*, 40(10), 1852-1855.
- Hamadeen, H. M., Elkhatib, E. A., Badawy, M. E., & Abdelgaleil, S. A. (2021). Green low cost nanomaterial produced from *Moringa oleifera* seed waste for enhanced removal of chlorpyrifos from wastewater: Mechanism and sorption studies. *Journal of Environmental Chemical Engineering*, 9(4), 105376.
- Harnpicharnchai, K., Chaiear, N., & Charerntanyarak, L. (2013). Residues of organophosphate pesticides used in vegetable cultivation in ambient air, surface water and soil in Bueng Niam Subdistrict, Khon Kaen, Thailand. *The Southeast Asian journal of tropical medicine and public health*, 44(6), 1088-97.
- Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci*, 2277, 2502.
- Hasany, S. F., Ahmed, I., Rajan, J., & Rehman, A. (2012). Systematic review of the preparation techniques of iron oxide magnetic nanoparticles. *Nanosci. Nanotechnol*, 2(6), 148-158.
- Hassaan, M. A., & El Nemr, A. (2020). Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *The Egyptian Journal of Aquatic Research*, 46(3), 207-220
- Hazra, D. K., & Purkait, A. (2019). Role of pesticide formulations for sustainable crop protection and environment management: A review. *J. Pharmacogn. Phytochem*, 8, 686-693.
- Hazrin, H. M. M. N., Lim, A., Li, C., Chew, J. J., & Sunarso, J. (2021). Adsorption of 2, 4-dichlorophenoxyacetic acid onto oil palm trunk-derived activated carbon: Isotherm and kinetic studies at acidic, ambient condition. *Materials Today: Proceedings*.
- He, J., Fan, M., & Liu, X. (2010). Environmental behavior of profenofos under paddy field conditions. *Bulletin of environmental contamination and toxicology*, 84(6), 771-774.

- Henn, K. W., & Waddill, D. W. (2006). Utilization of nanoscale zero-valent iron for source remediation—A case study. *Remediation Journal: The Journal of Environmental Cleanup Costs, Technologies & Techniques*, 16(2), 57-77.
- Herrmann, J. M., Guillard, C., Arguello, M., Agüera, A., Tejedor, A., Piedra, L., & Fernandez-Alba, A. (1999). Photocatalytic degradation of pesticide pirimiphos-methyl: Determination of the reaction pathway and identification of intermediate products by various analytical methods. *Catalysis Today*, 54(2-3), 353-367.
- Hoag, G. E., Collins, J. B., Holcomb, J. L., Hoag, J. R., Nadagouda, M. N., & Varma, R. S. (2009). Degradation of bromothymol blue by ‘greener’ nano-scale zero-valent iron synthesized using tea polyphenols. *Journal of Materials Chemistry*, 19(45), 8671-8677.
- Huang, L., Weng, X., Chen, Z., Megharaj, M., & Naidu, R. (2014). Synthesis of iron-based nanoparticles using oolong tea extract for the degradation of malachite green. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 117, 801-804.
- Huang, L., Weng, X., Chen, Z., Megharaj, M., & Naidu, R. (2014). Green synthesis of iron nanoparticles by various tea extracts: comparative study of the reactivity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 130, 295-301.
- Huang, L., Weng, X., Chen, Z., Megharaj, M., & Naidu, R. (2014). Green synthesis of iron nanoparticles by various tea extracts: comparative study of the reactivity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 130, 295-301.
- Huber, D. L. (2005). Synthesis, properties, and applications of iron nanoparticles. *Small*, 1(5), 482-501.
- Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chemistry Letters and Reviews*, 13(3), 223-245.
- Isworo, S., & Oetari, P. S. (2021). The Chemical Compounds from Degradation of Profenofos and Malathion by Indigenous Bacterial Consortium. *Journal of Pure and Applied Microbiology*, 15(2), 897-915.
- Jabeen, H., Iqbal, S., Anwar, S., & Parales, R. E. (2015). Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. *International Biodeterioration & Biodegradation*, 100, 89-97.

- Jabeen, H., Kemp, K. C., & Chandra, V. (2013). Synthesis of nano zerovalent iron nanoparticles–graphene composite for the treatment of lead contaminated water. *Journal of environmental management*, 130, 429-435.
- Jamkhande, P. G., Ghule, N. W., Bamer, A. H., & Kalaskar, M. G. (2019). Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. *Journal of Drug Delivery Science and Technology*, 53, 101174.
- Jatoi, A. S., Hashmi, Z., Adriyani, R., Yuniarto, A., Mazari, S. A., Akhter, F., & Mubarak, N. M. (2021). Recent trends and future challenges of pesticide removal techniques–A comprehensive review. *Journal of Environmental Chemical Engineering*, 9(4), 105571.
- Jawad, A. H., Abdulhameed, A. S., Surip, S. N., & Sabar, S. (2020). Adsorptive performance of carbon modified chitosan biopolymer for cationic dye removal: kinetic, isotherm, thermodynamic, and mechanism study. *International Journal of Environmental Analytical Chemistry*, 1-15.
- Jiang, S., Wang, F., Li, Q., Sun, H., Wang, H., & Yao, Z. (2021). Environment and food safety: a novel integrative review. *Environmental Science and Pollution Research*, 28(39), 54511-54530.
- Katuwavila, N. P., Perera, A. D. L. C., Dahanayake, D., Karunaratne, V., Amaratunga, G. A., & Karunaratne, D. N. (2016). Alginate nanoparticles protect ferrous from oxidation: Potential iron delivery system. *International journal of pharmaceutics*, 513(1-2), 404-409.
- Kaur, R., Mavi, G. K., Raghav, S., & Khan, I. (2019). Pesticides classification and its impact on environment. *Int. J. Curr. Microbiol. Appl. Sci*, 8(3), 1889-1897.
- Kazemi, M., Tahmasbi, A. M., Valizadeh, R., Naserian, A. A., & Soni, A. (2012). Organophosphate pesticides: A general review. *Agricultural science research journals*, 2.
- Kazemi, M., Tahmasbi, A. M., Valizadeh, R., Naserian, A. A., Soni, A., & Moheghi, M. M. (2012). Importance and toxicological effects of organophosphorus pesticides: A comprehensive review. *Basic Research Journal of Agricultural Science and Review*, 1.
- Khan, S., Naushad, M., Al-Gheethi, A., & Iqbal, J. (2021). Engineered nanoparticles for removal of pollutants from wastewater: Current status and future prospects of nanotechnology for remediation strategies. *Journal of Environmental Chemical Engineering*, 9(5), 106160.

- Khatoon, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M. A., & Santoyo, G. (2020). Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. *Journal of Environmental Management*, 273, 111118.
- Kim, S. H., & Choi, P. P. (2017). Enhanced Congo red dye removal from aqueous solutions using iron nanoparticles: adsorption, kinetics, and equilibrium studies. *Dalton Transactions*, 46(44), 15470-15479.
- Kodali, J., Talasila, S., Arunraj, B., & Nagarathnam, R. (2021). Activated Coconut Charcoal as a super adsorbent for the removal of organophosphorous pesticide monocrotophos from water. *Case Studies in Chemical and Environmental Engineering*, 3, 100099.
- Kohler, H. R., & Triebskorn, R. (2013). Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond?. *Science*, 341(6147), 759-765.
- Kour, D., Kaur, T., Devi, R., Yadav, A., Singh, M., Joshi, D., & Saxena, A. K. (2021). Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: Present status and future challenges. *Environmental Science and Pollution Research*, 1-23.
- Kuehne, G. (2021). The human impact from Indian farmers productive but damaging use of pesticides. In *Handbook on the Human Impact of Agriculture*. Edward Elgar Publishing.
- Kumar, A., Bisht, B. S., Joshi, V. D., & Dhewa, T. (2011). Review on bioremediation of polluted environment: a management tool. *International journal of environmental sciences*, 1(6), 1079-1093.
- Kumar, A., Doan, H., Barnes, M., Chapman, J. C., & Kookana, R. S. (2010). Response and recovery of acetylcholinesterase activity in freshwater shrimp, *Paratya australiensis* (Decapoda: Atyidae) exposed to selected anti-cholinesterase insecticides. *Ecotoxicology and environmental safety*, 73(7), 1503-1510.
- Kumar, S., Kaushik, G., Dar, M. A., Nimesh, S., Lopez-Chuken, U. J., & Villarreal-Chiu, J. F. (2018). Microbial degradation of organophosphate pesticides: a review. *Pedosphere*, 28(2), 190-208.
- Kumar, V., Sharma, N., & Vangnai, A. (2021). Modeling degradation kinetics of profenofos using *Acinetobacter* sp. 33F. *Environmental Technology & Innovation*, 21, 101367.
- Kumar, V., Sharma, N., Maitra, S. S., & Lakkaboyana, S. K. (2020). *In vivo* removal of profenofos in agricultural soil and plant growth promoting activity on *Vigna radiata* by efficient bacterial formulation. *International journal of phytoremediation*, 22(6), 585-593.

- Kumari, S., Kumar, D., & Khurana, S. P. (2022). Microbial degradation of pesticides: microbial potential for degradation of pesticides. In *Development in Wastewater Treatment Research and Processes*, 41-67.
- Kushwaha, M., Verma, S., & Chatterjee, S. (2016). Profenofos, an Acetylcholinesterase-Inhibiting Organophosphorus Pesticide: A Short Review of Its Usage, Toxicity, and Biodegradation. *Journal of environmental quality*, 45(5), 1478-1489.
- Kushwaha, S., Sreelatha, G., & Padmaja, P. (2011). Evaluation of acid-treated palm shell powder for its effectiveness in the adsorption of organophosphorus pesticides: isotherm, kinetics, and thermodynamics. *Journal of Chemical & Engineering Data*, 56(5), 2407-2415.
- Leonel, A. G., Mansur, A. A., & Mansur, H. S. (2021). Advanced functional nanostructures based on magnetic iron oxide nanomaterials for water remediation: a review. *Water Research*, 190, 116693.
- Li, L., Fan, M., Brown, R. C., Van Leeuwen, J., Wang, J., Wang, W., & Zhang, P. (2006). Synthesis, properties, and environmental applications of nanoscale iron-based materials: a review. *Critical Reviews in Environmental Science and Technology*, 36(5), 405-431.
- Li, S., Yan, W., & Zhang, W. X. (2009). Solvent-free production of nanoscale zero-valent iron (nZVI) with precision milling. *Green Chemistry*, 11(10), 1618-1626.
- Li, X. Q., Elliott, D. W., & Zhang, W. X. (2006). Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects. *Critical reviews in solid state and materials sciences*, 31(4), 111-122.
- Lin, L., Liu, J., Zhang, K., & Chen, Y. (2003). An experimental study of the effects of profenofos on antioxidase in rabbits. *Wei sheng yan jiu= Journal of hygiene research*, 32(5), 434-435.
- Lin, Z., Weng, X., Owens, G., & Chen, Z. (2020). Simultaneous removal of Pb (II) and rifampicin from wastewater by iron nanoparticles synthesized by a tea extract. *Journal of Cleaner Production*, 242, 118476.
- Lopez-Sánchez, A., Luque-Badillo, A. C., Orozco-Nunnelly, D., Alencastro-Larios, N. S., Ruiz-Gómez, J. A., García-Cayuela, T., & Gradilla-Hernández, M. S. (2021). Food loss in the agricultural sector of a developing country: Transitioning to a more sustainable approach. The case of Jalisco, Mexico. *Environmental Challenges*, 5, 100327.

- Lunge, S., Singh, S., & Sinha, A. (2014). Magnetic iron oxide (Fe₃O₄) nanoparticles from tea waste for arsenic removal. *Journal of Magnetism and Magnetic Materials*, 356, 21-31.
- Ma, C. S., Zhang, W., Peng, Y., Zhao, F., Chang, X. Q., Xing, K., & Rudolf, V. H. (2021). Climate warming promotes pesticide resistance through expanding overwintering range of a global pest. *Nature communications*, 12(1), 1-10.
- Ma, M., Dong, S., Jin, W., Zhang, C., & Zhou, W. (2019). Fate of the organophosphorus pesticide profenofos in cotton fiber. *Journal of Environmental Science and Health, Part B*, 54(1), 70-75.
- Madhavi, V., Prasad, T. N. V. K. V., Reddy, A. V. B., Reddy, B. R., & Madhavi, G. (2013). Application of phytogetic zerovalent iron nanoparticles in the adsorption of hexavalent chromium. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 116, 17-25.
- Mahajan, R., Verma, S., & Chatterjee, S. (2021). Biodegradation of organophosphorus pesticide profenofos by the bacterium *Bacillus* sp. PF1 and elucidation of initial degradation pathway. *Environmental Technology*, 1-9.
- Mahajan, R., Verma, S., Chandel, S., & Chatterjee, S. (2022). Organophosphate pesticide: usage, environmental exposure, health effects, and microbial bioremediation. In *Microbial Biodegradation and Bioremediation* 473-490.
- Maharajan, A., Neelakandamoorthy, N., & Kumarasamy, P. (2012). Impact of Profenofos on oxygen consumption and gill histopathology of the Fresh water crab, *Paratelphusa jacquemontii*(Rathbun). *Res. Rev.: J. Toxicol*, 2(2), 46-55.
- Mahboob, S., Niazi, F., Sultana, S., & Ahmad, Z. (2013). Assessment of pesticide residues in water, sediments and muscles of *Cyprinus Carpio* from Head Balloki in the River Ravi. *Life Sci J*, 10, 11s.
- Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). Effects of pesticides on environment. In *Plant, soil and microbes* (pp. 253-269). Springer, Cham.
- Mahmoud, A. S., Ismail, A., Mostafa, M. K., Mahmoud, M. S., Ali, W., & Shawky, A. M. (2020). Isotherm and kinetic studies for heptachlor removal from aqueous solution using Fe/Cu nanoparticles, artificial intelligence, and regression analysis. *Separation Science and Technology*, 55(4), 684-696.
- Malghani, S., Chatterjee, N., Hu, X., & Zejiao, L. (2009). Isolation and characterization of a profenofos degrading bacterium. *Journal of Environmental Sciences*, 21(11), 1591-1597.

- Malghani, S., Chatterjee, N., Yu, H. X., & Luo, Z. (2009). Isolation and identification of profenofos degrading bacteria. *Brazilian Journal of Microbiology*, 40(4), 893-900.
- Mansouriieh, N., Sohrabi, M. R., & Khosravi, M. (2016). Adsorption kinetics and thermodynamics of organophosphorus profenofos pesticide onto Fe/Ni bimetallic nanoparticles. *International journal of environmental science and technology*, 13(5), 1393-1404.
- Mansouriieh, N., Sohrabi, M. R., & Khosravi, M. (2019). Optimization of profenofos organophosphorus pesticide degradation by zero-valent bimetallic nanoparticles using response surface methodology. *Arabian Journal of Chemistry*, 12(8), 2524-2532.
- Marican, A., & Duran-Lara, E. F. (2018). A review on pesticide removal through different processes. *Environmental Science and Pollution Research*, 25(3), 2051-2064.
- Masia, A., Vasquez, K., Campo, J., & Pico, Y. (2015). Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túrria River Basin. *Journal of Chromatography A*, 1378, 19-31.
- McDaniel, K. L., & Moser, V. C. (2004). Differential profiles of cholinesterase inhibition and neurobehavioral effects in rats exposed to fenamiphos or profenofos. *Neurotoxicology and teratology*, 26(3), 407-415.
- Meng, D., Zhai, L., Tian, Q., Guan, Z., Cai, Y., & Liao, X. (2019). Complete genome sequence of *Bacillus amyloliquefaciens* YP6, a plant growth rhizobacteria efficiently degrading a wide range of organophosphorus pesticides. *J. Integr. Agric*, 18, 2668-2672.
- Meyer, M., & Persson, O. (1998). Nanotechnology-interdisciplinarity, patterns of collaboration and differences in application. *Scientometrics*, 42(2), 195-205.
- Milesion, B. E., Chambers, J. E., Chen, W. L., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., & Wallace, K. B. (1998). Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicological sciences*, 41(1), 8-20.
- Mishra, N., & Sundari, S. K. (2015). Native PGPM consortium: a beneficial solution to support plant growth in the presence of phytopathogens and residual organophosphate pesticides. *J Bioprocess Biotech*, 5(202), 2.
- 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.

- Momić, T., Pašti, T. L., Bogdanović, U., Vodnik, V., Mraković, A., Rakočević, Z., ... & Vasić, V. (2016). Adsorption of organophosphate pesticide dimethoate on gold nanospheres and nanorods. *Journal of Nanomaterials*, 2016.
- Mondal, P., Anweshan, A., & Purkait, M. K. (2020). Green synthesis and environmental application of iron-based nanomaterials and nanocomposite: A review. *Chemosphere*, 259, 127509.
- Mu, D. S., Liang, Q. Y., Wang, X. M., Lu, D. C., Shi, M. J., Chen, G. J., & Du, Z. J. (2018). Metatranscriptomic and comparative genomic insights into resuscitation mechanisms during enrichment culturing. *Microbiome*, 6(1), 1-15.
- Mukherjee, R., Kumar, R., Sinha, A., Lama, Y., & Saha, A. K. (2016). A review on synthesis, characterization, and applications of nano zero valent iron (nZVI) for environmental remediation. *Critical reviews in environmental science and technology*, 46(5), 443-466.
- Mulla, S. I., Ameen, F., Talwar, M. P., Eqani, S. A. M. A. S., Bharagava, R. N., Saxena, G., & Ninnekar, H. Z. (2020). Organophosphate pesticides: impact on environment, toxicity, and their degradation. In *Bioremediation of industrial waste for environmental safety* (pp. 265-290). Springer, Singapore.
- Namal, O. O., & Kalipci, E. (2020). Adsorption kinetics of methylene blue removal from aqueous solutions using potassium hydroxide (KOH) modified apricot kernel shells. *International Journal of Environmental Analytical Chemistry*, 100(14), 1549-1565.
- Neuwirthova, N., Trojan, M., Svobodova, M., Vasickova, J., Simek, Z., Hofman, J., & Bielska, L. (2019). Pesticide residues remaining in soils from previous growing season (s)-Can they accumulate in non-target organisms and contaminate the food web?. *Science of the Total Environment*, 646, 1056-1062.
- Nillos, M. G., Rodriguez-Fuentes, G., Gan, J., & Schlenk, D. (2007). Enantioselective acetylcholinesterase inhibition of the organophosphorous insecticides profenofos, fonofos, and crotoxyphos. *Environmental Toxicology and Chemistry: An International Journal*, 26(9), 1949-1954.
- Omwenga, I., Zhao, S., Kanja, L., Mol, H., Rietjens, I. M., & Louisse, J. (2021). Prediction of dose-dependent *in vivo* acetylcholinesterase inhibition by profenofos in rats and humans using physiologically based kinetic (PBK) modeling-facilitated reverse dosimetry. *Archives of toxicology*, 95(4), 1287-1301.
- Oter, C., & Selcuk Zorer, O. (2021). Adsorption behaviours of Th (IV) and U (VI) using nitric acid (HNO₃) modified activated carbon: equilibrium, thermodynamic and

- kinetic studies. *International Journal of Environmental Analytical Chemistry*, 101(14), 1950-1965.
- Paarlberg, R. (2009). The ethics of modern agriculture. *Society*, 46(1), 4-8.
- Palanimanickam, A., & Sepperumal, U. (2017). Profenofos degradation potential of *Bacillus cereus* and *Aneurinibacillus migulanus* isolated from paddy crop field soil. *J. Pure Appl. Microbiol*, 11(1), 221-227.
- Pandotra, P., Raina, M., Salgotra, R. K., Ali, S., Mir, Z. A., Bhat, J. A., & Upadhahy, D. (2018). Plant-bacterial partnership: a major pollutants remediation approach. In *Modern Age Environmental Problems and their Remediation* (pp. 169-200). Springer, Cham.
- Panneerselvam, P., Morad, N., & Tan, K. A. (2011). Magnetic nanoparticle (Fe₃O₄) impregnated onto tea waste for the removal of nickel (II) from aqueous solution. *Journal of hazardous materials*, 186(1), 160-168.
- Parte, S. G., Mohekar, A. D., & Kharat, A. S. (2017). Microbial degradation of pesticide: a review. *African journal of microbiology research*, 11(24), 992-1012.
- Pasinszki, T., & Krebsz, M. (2020). Synthesis and application of zero-valent iron nanoparticles in water treatment, environmental remediation, catalysis, and their biological effects. *Nanomaterials*, 10(5), 917.
- Pattanayak, D. S., Pal, D., Thakur, C., Kumar, S., & Devnani, G. L. (2021). Bio-synthesis of iron nanoparticles for environmental remediation: Status till date. *Materials Today: Proceedings*, 44, 3150-3155.
- Pehkonen, S. O., & Zhang, Q. (2002). The degradation of organophosphorus pesticides in natural waters: a critical review. *Critical reviews in environmental science and technology*, 32(1), 17-72.
- Piwowar, A. (2021). The use of pesticides in Polish agriculture after integrated pest management (IPM) implementation. *Environmental Science and Pollution Research*, 28(21), 26628-26642.
- Ponnuchamy, M., Kapoor, A., Kumar, P. S., Vo, D. V. N., Balakrishnan, A., Jacob, M. M., & Sivaraman, P. (2021). Sustainable adsorbents for the removal of pesticides from water: a review. *Environmental Chemistry Letters*, 1-39.
- Poudel, S., Poudel, B., Acharya, B., & Poudel, P. (2020). Pesticide use and its impacts on human health and environment. *Environ. Ecosyst. Sci*, 4, 47-51.
- Pourabadeh, A., Baharinikoo, L., Shojaei, S., Mehdizadeh, B., Davoodabadi Farahani, M., & Shojaei, S. (2020). Experimental design and modelling of removal of dyes using

- nano-zero-valent iron: a simultaneous model. *International Journal of Environmental Analytical Chemistry*, 100(15), 1707-1719.
- Prabhavathy Das, G., Pasha Shaik, A., & Jamil, K. (2006). Cytotoxicity and genotoxicity induced by the pesticide profenofos on cultured human peripheral blood lymphocytes. *Drug and chemical toxicology*, 29(3), 313-322.
- Prasad, R., Kumar, V., & Prasad, K. S. (2014). Nanotechnology in sustainable agriculture: present concerns and future aspects. *African journal of Biotechnology*, 13(6), 705-713.
- Punia, P., Bharti, M. K., Chalia, S., Dhar, R., Ravelo, B., Thakur, P., & Thakur, A. (2021). Recent advances in synthesis, characterization, and applications of nanoparticles for contaminated water treatment-a review. *Ceramics International*, 47(2), 1526-1550.
- Putra, I. W. W. P., Setiyo, Y., Gunam, I. B. W., & Anggreni, A. A. M. D. (2021). Isolation and identification of profenofos pesticide degrading bacterium from soil sample of Bedugul, Indonesia. In *IOP Conference Series: Earth and Environmental Science*, 724 (1), 012037.
- Qing Li, Q., Loganath, A., Seng Chong, Y., Tan, J., & Philip Obbard, J. (2006). Persistent organic pollutants and adverse health effects in humans. *Journal of Toxicology and Environmental Health, Part A*, 69(21), 1987-2005.
- R. Foster, L. J., Kwan, B. H., & Vancov, T. (2004). Microbial degradation of the organophosphate pesticide, Ethion. *FEMS microbiology letters*, 240(1), 49-53.
- Radwan, M. A., Abu-Elamayem, M. M., Shiboob, M. H., & Abdel-Aal, A. (2005). Residual behaviour of profenofos on some field-grown vegetables and its removal using various washing solutions and household processing. *Food and Chemical Toxicology*, 43(4), 553-557.
- Rahman, M. M., Rimu, S. H., Biswas, S., Rashid, T. U., Chisty, A. H., Rahman, A., & Haque, P. (2020). Preparation of poly (acrylic acid) exfoliated clay composite by in-situ polymerisation for decolouration of methylene blue from wastewater. *International Journal of Environmental Analytical Chemistry*, 1-17.
- Raman, C. D., & Kanmani, S. (2016). Textile dye degradation using nano zero valent iron: a review. *Journal of Environmental Management*, 177, 341-355.
- Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., & Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of Cleaner Production*, 283, 124657.

- Rao, J. V., Kavitha, P., Jakka, N. M., Sridhar, V., & Usman, P. K. (2007). Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina*. *Archives of environmental contamination and toxicology*, 53(2), 227-232.
- Rao, J. V., Shilpanjali, D., Kavitha, P., & Madhavendra, S. S. (2003). Toxic effects of profenofos on tissue acetylcholinesterase and gill morphology in a euryhaline fish, *Oreochromis mossambicus*. *Archives of toxicology*, 77(4), 227-232.
- Rashid, R., Shafiq, I., Akhter, P., Iqbal, M. J., & Hussain, M. (2021). A state-of-the-art review on wastewater treatment techniques: the effectiveness of adsorption method. *Environmental Science and Pollution Research*, 28, 9050-9066.
- Rashtbari, Y., Américo-Pinheiro, J. H. P., Bahrami, S., Fazlzadeh, M., Arfaeinia, H., & Poureshgh, Y. (2020). Efficiency of Zeolite Coated with Zero-Valent Iron Nanoparticles for Removal of Humic Acid from Aqueous Solutions. *Water, Air, & Soil Pollution*, 231(10), 1-15.
- Rawtani, D., Khatri, N., Tyagi, S., & Pandey, G. (2018). Nanotechnology-based recent approaches for sensing and remediation of pesticides. *Journal of environmental management*, 206, 749-762.
- Raychoudhury, T., & Scheytt, T. (2013). Potential of zerovalent iron nanoparticles for remediation of environmental organic contaminants in water: a review. *Water Science and Technology*, 68(7), 1425-1439.
- Razmi, F. A., Ngadi, N., Wong, S., Inuwa, I. M., & Opotu, L. A. (2019). Kinetics, thermodynamics, isotherm and regeneration analysis of chitosan modified pandan adsorbent. *Journal of cleaner production*, 231, 98-109.
- Reddy, N. C., & Rao, J. V. (2008). Biological response of earthworm, *Eisenia foetida* (Savigny) to an organophosphorous pesticide, profenofos. *Ecotoxicology and Environmental Safety*, 71(2), 574-582.
- Riyaz, M., Mathew, P., Zuber, S. M., & Rather, G. A. (2022). Botanical Pesticides for an Eco-Friendly and Sustainable Agriculture: New Challenges and Prospects. In *Sustainable Agriculture* (pp. 69-96). Springer, Cham.
- Riyaz, M., Shah, R. A., & Sivasankaran, K. (2021). Pesticide Residues: Impacts on Fauna and the Environment.
- Sadhasivam, S., Vinayagam, V., & Balasubramaniyan, M. (2020). Recent advancement in biogenic synthesis of iron nanoparticles. *Journal of Molecular Structure*, 1217, 128372.

- Saha, B., Das, S., Saikia, J., & Das, G. (2011). Preferential and enhanced adsorption of different dyes on iron oxide nanoparticles: a comparative study. *The Journal of Physical Chemistry C*, 115(16), 8024-8033.
- Sahithya, K., Das, D., & Das, N. (2016). Adsorptive removal of monocrotophos from aqueous solution using biopolymer modified montmorillonite–CuO composites: equilibrium, kinetic and thermodynamic studies. *Process Safety and Environmental Protection*, 99, 43-54.
- Saif, S., Tahir, A., & Chen, Y. (2016). Green synthesis of iron nanoparticles and their environmental applications and implications. *Nanomaterials*, 6(11), 209.
- Salahi Moghadam, N., Rezaei, R., & Kavousi, A. (2021). Factors affecting farmers' excessive use of application chemical pesticides and Analyzing the self-protective behaviour about the use of chemical pesticides (Case Study: Zanjan province). *Agricultural Extension and Education Research*, 13(4), 95-112.
- Salmani, M. H., Abedi, M., Mozaffari, S. A., Mahvi, A. H., Sheibani, A., & Jalili, M. (2021). Simultaneous reduction and adsorption of arsenite anions by green synthesis of iron nanoparticles using pomegranate peel extract. *Journal of Environmental Health Science and Engineering*, 19(1), 603-612.
- Salunkhe, V. P., Sawant, I. S., Banerjee, K., Rajguru, Y. R., Wadkar, P. N., Oulkar, D. P., & Sawant, S. D. (2013). Biodegradation of profenofos by *Bacillus subtilis* isolated from grapevines (*Vitis vinifera*). *Journal of agricultural and food chemistry*, 61(30), 7195-7202.
- Samrot, A. V., Sahithya, C. S., Selvarani, J., Purayil, S. K., & Ponnaiah, P. (2021). A review on synthesis, characterization and potential biological applications of superparamagnetic iron oxide nanoparticles. *Current Research in Green and Sustainable Chemistry*, 4, 100042.
- Sarker, A., Nandi, R., Kim, J. E., & Islam, T. (2021). Remediation of chemical pesticides from contaminated sites through potential microorganisms and their functional enzymes: Prospects and challenges. *Environmental Technology & Innovation*, 23, 101777.
- Shahwan, T., Sirriah, S. A., Nairat, M., Boyacı, E., Eroğlu, A. E., Scott, T. B., & Hallam, K. R. (2011). Green synthesis of iron nanoparticles and their application as a Fenton-like catalyst for the degradation of aqueous cationic and anionic dyes. *Chemical Engineering Journal*, 172(1), 258-266.
- Sharma, G., Kumar, A., Sharma, S., Naushad, M., Dwivedi, R. P., AlOthman, Z. A., & Mola, G. T. (2019). Novel development of nanoparticles to bimetallic nanoparticles

- and their composites: a review. *Journal of King Saud University-Science*, 31(2), 257-269.
- Sheela, T., & Nayaka, Y. A. (2012). Kinetics and thermodynamics of cadmium and lead ions adsorption on NiO nanoparticles. *Chemical Engineering Journal*, 191, 123-131.
- Shojaei, S. (2019). Optimization of process variables by the application of response surface methodology for dye removal using nanoscale zero-valent iron. *International Journal of Environmental Science and Technology*, 16(8), 4601-4610.
- Shojaei, S., Nouri, A., Baharinikoo, L., Farahani, M. D., & Shojaei, S. (2021). Removal of the hazardous dyes through adsorption over nanozeolite-X: Simultaneous model, design and analysis of experiments. *Polyhedron*, 196, 114995.
- Shojaei, S., Rahmani, M., Khajeh, M., & Abbasian, A. R. (2021). Magnetic-Nanoparticle-Based Dispersive Micro-Solid Phase Extraction for the Determination of Crystal Violet in Environmental Water Samples. *ChemistrySelect*, 6(19), 4782-4790.
- Shojaei, S., Shojaei, S., & Pirkamali, M. (2019). Application of Box–Behnken design approach for removal of acid black 26 from aqueous solution using zeolite: modeling, optimization, and study of interactive variables. *Water Conservation Science and Engineering*, 4(1), 13-19.
- Shojaei, S., Shojaei, S., Band, S. S., Farizhandi, A. A. K., Ghoroghi, M., & Mosavi, A. (2021). Application of Taguchi method and response surface methodology into the removal of malachite green and auramine-O by NaX nanozeolites. *Scientific reports*, 11(1), 1-13.
- Shojaei, S., Shojaei, S., Nouri, A., & Baharinikoo, L. (2021). Application of chemometrics for modeling and optimization of ultrasound-assisted dispersive liquid–liquid microextraction for the simultaneous determination of dyes. *npj Clean Water*, 4(1), 1-8.
- Sidhu, G. K., Singh, S., Kumar, V., Dhanjal, D. S., Datta, S., & Singh, J. (2019). Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. *Critical reviews in environmental science and technology*, 49(13), 1135-1187.
- Silva, A., Martínez-Gallegos, S., Rosano-Ortega, G., Schabes-Retchkiman, P., Vega-Lebrún, C., & Albiter, V. (2017). Nanotoxicity for E. Coli and characterization of silver quantum dots produced by biosynthesis with Eichhornia crassipes. *Journal of Nanostructures*, 7(1), 1-12.
- Silveira, C., Shimabuku-Biadola, Q. L., Silva, M. F., Vieira, M. F., & Bergamasco, R. (2020). Development of an activated carbon impregnation process with iron oxide

- nanoparticles by green synthesis for diclofenac adsorption. *Environmental Science and Pollution Research*, 27(6), 6088-6102.
- Singh, B. K., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS microbiology reviews*, 30(3), 428-471.
- Singh, B. K., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS microbiology reviews*, 30(3), 428-471.
- Singh, R., Misra, V., & Singh, R. P. (2011). Synthesis, characterization and role of zero-valent iron nanoparticle in removal of hexavalent chromium from chromium-spiked soil. *Journal of Nanoparticle Research*, 13(9), 4063-4073.
- Singhal, A., & Lind, M. L. (2018). Removal of pesticide toxicity by cysteine-capped Ag nanoparticles and study of their adsorption kinetics. *International journal of nanomedicine*, 13(T-NANO 2014 Abstracts), 25.
- Singhal, A., & Lind, M. L. (2018). Removal of pesticide toxicity by cysteine-capped Ag nanoparticles and study of their adsorption kinetics. *International journal of nanomedicine*, 13, 25.
- Siripattanakul-Ratpukdi, S., Vangnai, A. S., & Patichot, W. (2017). Enhancement of Profenofos Remediation using stimulated bioaugmentation technique. *Journal of Advanced oxidation technologies*, 20(2).
- Siripattanakul-Ratpukdi, S., Vangnai, A. S., Sangthean, P., & Singkibut, S. (2015). Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. *Environmental Science and Pollution Research*, 22(1), 320-328.
- Subsanguan, T., Vangnai, A. S., & Siripattanakul-Ratpukdi, S. (2020). Aerobic and anoxic degradation and detoxification of profenofos insecticide by *Pseudomonas plecoglossicida* strain PF1. *Ecotoxicology and environmental safety*, 190, 110129.
- Sudo, M., Kunimatsu, T., & Okubo, T. (2002). Concentration and loading of pesticide residues in Lake Biwa basin (Japan). *Water Research*, 36(1), 315-329.
- Sun, S., Sidhu, V., Rong, Y., & Zheng, Y. (2018). Pesticide pollution in agricultural soils and sustainable remediation methods: a review. *Current Pollution Reports*, 4(3), 240-250.
- Talwar, M. P., & Ninnekar, H. Z. (2015). Biodegradation of pesticide profenofos by the free and immobilized cells of *Pseudoxanthomonas suwonensis* strain HNM. *Journal of basic microbiology*, 55(9), 1094-1103.

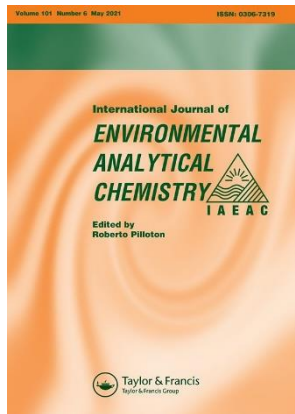
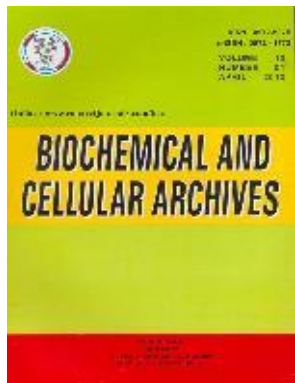
- Talwar, M. P., & Ninnekar, H. Z. (2015). Biodegradation of pesticide profenofos by the free and immobilized cells of *Pseudoxanthomonas suwonensis* strain HNM. *Journal of basic microbiology*, 55(9), 1094-1103.
- Tamilselvan, C., Joseph, S. J., Mugunthan, G., Sathish Kumar, A., & Syed Musthaq Ahamed, S. (2014). Biological degradation of metribuzin and profenofos by some efficient bacterial isolates. *International Letters of Natural Sciences*, 9.
- Tang, H., Wang, J., Zhang, S., Pang, H., Wang, X., Chen, Z., & Yu, S. (2021). Recent advances in nanoscale zero-valent iron-based materials: Characteristics, environmental remediation and challenges. *Journal of Cleaner Production*, 319, 128641.
- Tang, M., & You, M. (2012). Isolation, identification and characterization of a novel triazophos-degrading *Bacillus* sp.(TAP-1). *Microbiological research*, 167(5), 299-305.
- 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.
- Valickova, M., Derco, J., & Simovicova, K. (2013). Removal of selected pesticides by adsorption. *Acta Chimica Slovaca*, 6(1), 25-28.
- Van Dyk, J. S., & Pletschke, B. (2011). Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. *Chemosphere*, 82(3), 291-307.
- van Zelm, R., Larrey-Lassalle, P., & Roux, P. (2014). Bridging the gap between life cycle inventory and impact assessment for toxicological assessments of pesticides used in crop production. *Chemosphere*, 100, 175-181.
- Verma, J. P., Jaiswal, D. K., & Sagar, R. (2014). Pesticide relevance and their microbial degradation: a-state-of-art. *Reviews in Environmental Science and Bio/Technology*, 13(4), 429-466.
- Verma, S., & Chatterjee, S. (2021). Biodegradation of profenofos, an acetylcholine esterase inhibitor by a psychrotolerant strain *Rahnella* sp. PFF2 and degradation pathway analysis. *International Biodeterioration & Biodegradation*, 158, 105169.
- Viju Kumar, V. G., & Prem, A. A. (2018). Green synthesis and characterization of iron oxide nanoparticles using *Phyllanthus niruri* extract. *Oriental Journal of Chemistry*, 34(5), 2583.

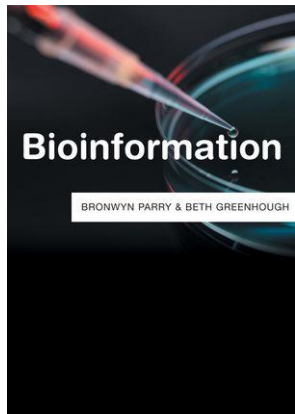
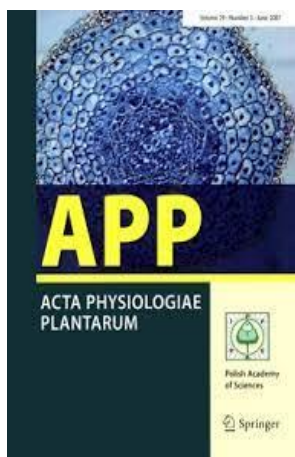
- Vinod, K. R., Saravanan, P., Sakar, M., & Balakumar, S. (2016). Insights into the nitridation of zero-valent iron nanoparticles for the facile synthesis of iron nitride nanoparticles. *RSC advances*, 6(51), 45850-45857.
- Weng, X., Huang, L., Chen, Z., Megharaj, M., & Naidu, R. (2013). Synthesis of iron-based nanoparticles by green tea extract and their degradation of malachite. *Industrial Crops and Products*, 51, 342-347.
- Willocquet, L., Nelson, N. A., Ficke, A., Savary, S., (2017). Importance of disease and pest losses on key world crops- priorities. *Synthesis and Report*, 18-19.
- World Health Organization. (2020). *The WHO recommended classification of pesticides by hazard and guidelines to classification 2019*. World Health Organization.
- Wright, M. H., Adelskov, J., & Greene, A. C. (2017). Bacterial DNA extraction using individual enzymes and phenol/chloroform separation. *Journal of microbiology & biology education*, 18(2), 18-2.
- Xin, H., Yang, X., Liu, X., Tang, X., Weng, L., & Han, Y. (2016). Biosynthesis of iron nanoparticles using tie guanyin tea extract for degradation of bromothymol blue. *Journal of Nanotechnology*, 2016.
- Yadav, I. C., & Devi, N. L. (2017). Pesticides classification and its impact on human and environment. *Environmental science and engineering*, 6, 140-158.
- Yan, W., Lien, H. L., Koel, B. E., & Zhang, W. X. (2013). Iron nanoparticles for environmental clean-up: recent developments and future outlook. *Environmental Science: Processes & Impacts*, 15(1), 63-77.
- Yang, Q., Wang, J., Zhang, W., Liu, F., Yue, X., Liu, Y., ... & Wang, J. (2017). Interface engineering of metal organic framework on graphene oxide with enhanced adsorption capacity for organophosphorus pesticide. *Chemical Engineering Journal*, 313, 19-26.
- Yekta, S., Sadeghi, M., & Babanezhad, E. (2016). Synthesis of CaWO₄ nanoparticles and its application for the adsorption-degradation of organophosphorus cyanophos. *Journal of Water Process Engineering*, 14, 19-27.
- Yoon, S. Y., Lee, C. G., Park, J. A., Kim, J. H., Kim, S. B., Lee, S. H., & Choi, J. W. (2014). Kinetic, equilibrium and thermodynamic studies for phosphate adsorption to magnetic iron oxide nanoparticles. *Chemical engineering journal*, 236, 341-347.
- Yousefi, M., Gholami, M., Oskoei, V., Mohammadi, A. A., Baziar, M., & Esrafil, A. (2021). Comparison of LSSVM and RSM in simulating the removal of ciprofloxacin from aqueous solutions using magnetization of functionalized multi-walled carbon

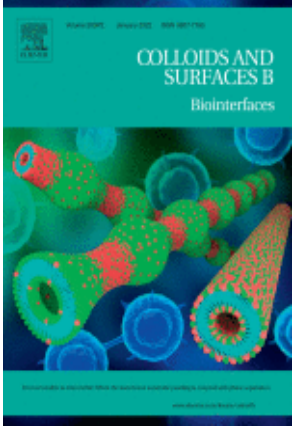
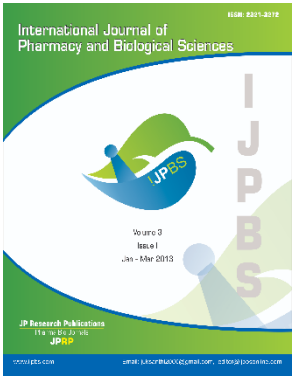
nanotubes: Process optimization using GA and RSM techniques. *Journal of Environmental Chemical Engineering*, 9(4), 105677.

- Yousefinia, S., Sohrabi, M. R., Motiee, F., & Davallo, M. (2021). The efficient removal of bisphenol A from aqueous solution using an assembled nanocomposite of zero-valent iron nanoparticles/graphene oxide/copper: Adsorption isotherms, kinetic, and thermodynamic studies. *Journal of Contaminant Hydrology*, 243, 103906.
- Zaynab, M., Fatima, M., Sharif, Y., Sughra, K., Sajid, M., Khan, K. A., & Li, S. (2021). Health and environmental effects of silent killers Organochlorine pesticides and polychlorinated biphenyl. *Journal of King Saud University-Science*, 33(6), 101511.
- Zhang, W. X. (2003). Nanoscale iron particles for environmental remediation: an overview. *Journal of nanoparticle Research*, 5(3), 323-332.
- Zhao, X., Liu, W., Cai, Z., Han, B., Qian, T., & Zhao, D. (2016). An overview of preparation and applications of stabilized zero-valent iron nanoparticles for soil and groundwater remediation. *Water research*, 100, 245-266.
- Zou, Y., Wang, X., Khan, A., Wang, P., Liu, Y., Alsaedi, A., & Wang, X. (2016). Environmental remediation and application of nanoscale zero-valent iron and its composites for the removal of heavy metal ions: a review. *Environmental science & technology*, 50(14), 7290-7304.

List of Publications

1	Fabrication of iron nanoparticles from tea waste as an efficient adsorbent towards the removal of profenofos pesticide from aquatic environment. Arjunan Annavi, Muthuselvam Manickam*. <i>International journal of environmental analytical chemistry</i>, (In press) https://doi.org/10.1080/03067319.2021.2020767.	
2	Isolation and identification of profenofos degrading bacterium for efficient bioremediation of pesticide contaminated agricultural soil. Arjunan Annavi, Muthuselvam Manickam* <i>Biochem. Cell. Arch.</i> 21, 2021, 5189-5194.	
3	An environmentally sustainable strategy for the efficient biodegradation of profenofos and plant growth enhancement by indigenous soil bacterium <i>Bacillus subtilis</i>. Arjunan Annavi, Venkatachalam Vasudevan, Manickam Muthuselvam*. <i>International Journal of Botany Studies</i>, 6, 2021, 1280-1285.	

4	Exploration of culturable Marine <i>Streptomyces</i> from Southern Coastal Region of India with antibacterial properties. Deepika Thandayuthapani, Nivetha Chinnappa, Arjunan Annavi and Muthuselvam Manickam*. <i>Journal of Pharmaceutical Research International</i>, 33, 2021, 323-334.	
5	Phylogenetic and sequence profile analysis of Non-Ribosomal Polyketide Synthase-Adenylation (NRPS) domain from Actinobacterium dagang 5. Deepika Thandayuthapani, Nivetha Chinnappa, Arjunan Annavi and Muthusevam Manickam*. <i>Bioinformation</i> 17, 2021, 809-813.	
6	Antimicrobial and antioxidant activities of <i>Streptomyces</i> sps isolated from Muthupettai mangrove soil. C. Nivetha, T. Deepika, Arjunan Annavi, P. Sivalingam, N. Revathi and Muthuselvam Manickam * <i>Journal of Pharmaceutical Research International</i>. 33, 2021, 210-234.	
7	Effect of culture conditions, cytokinins, methyl jasmonate and salicylic acid on the biomass accumulation and production of withanolides in multiple shoot culture of <i>Withania somnifera</i> (L.) Dunal using liquid culture Ganeshan Sivanandhan, Manoharan Rajesh, Muthukrishnan Arun, Murugaraj Jeyaraj, Gnanajothi Kapil Dev, Annavi Arjunan, Markandan Manickavasagam, Manickam Muthuselvam, Natesan Selvaraj, Andy Ganapathi <i>Acta Physiol Plant</i> 35, 2013, 715–728.	

8	Increased production of withanolide A, withanone, and withaferin A in hairy root cultures of <i>Withania somnifera</i> (L.) Dunal elicited with methyl jasmonate and salicylic acid. Ganeshan Sivanandhan, Gnanajothi Kapil Dev, Murugaraj Jeyaraj, Manoharan Rajesh, Annavi Arjunan, Manickam Muthuselvam, Markandan Manickavasagam, Natesan Selvaraj, Andy Ganapathi <i>Plant Cell, Tissue and Organ Culture</i> (PCTOC) 114, 2013, 121–129.	
9	Evaluation of antibacterial efficacy of phyto fabricated silver nanoparticles using <i>Mukia scabrella</i> (Musumusukkai) against drug resistance nosocomial gram negative bacterial pathogens Kandasamy Prabakar *, Periyasamy Sivalingam, Siyed Ibrahim Mohamed Rabeek, Manickam Muthuselvam, Naresh Devarajan, Annavi Arjunan, Rajamanickam Karthick, Micky Maray Suresh, John Pote Wembonyama <i>Colloids and Surfaces B: Biointerfaces</i> 104, 2013, 282-288.	
10	Assessment of fuel properties of biodiesel fuel from waste oil M. Mathiyazhagan*, S. Senthilnathan, Annavi Arjunan, M.Muthuselvam, and A. Ganapathi. <i>International Journal of Pharma and Bio Sciences</i> 3, 2012, 281 – 287.	

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Fabrication of iron nanoparticles from tea waste as an efficient adsorbent towards the removal of profenofos pesticide from aquatic environment

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ABSTRACT

Pesticide contamination has led to serious environmental issues that pose a significant threat to living organisms throughout the world. Therefore, the significant removal of pesticides from aquatic environments has attracted the attention of environmental scientists and academicians in recent decades. Hence, in the present investigation, the iron nanoparticles (INPs) from tea waste were fabricated as an efficient adsorbent towards the removal of profenofos (PF) from aqueous solution. The as-synthesised INPs were systematically characterised by various analytical techniques such as Ultra Violet-visible (UV-vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), powder X-ray diffraction (PXRD) and dynamic light scattering (DLS). Various influencing factors such as contact time, adsorbent dosage, solution pH and initial concentration of pesticide were systematically optimised for the maximum adsorption efficiency of PF by the prepared INPs. Based on the obtained results, the INPs showed greater adsorption efficiency towards the removal of PF. The kinetic data were analysed using various adsorption kinetic models such as pseudo-first-order, pseudo-second-order and intra-particle diffusion. Further, the interaction between prepared INPs and PF was evaluated using various adsorption isotherm models such as Langmuir, Freundlich, Temkin and Dubinin-Radushkevich (D-R) models. The monolayer Langmuir adsorption capacity of 43.67 mg g^{-1} was obtained with a minimum contact time of 15 min. According to the kinetic and isotherm data, the pseudo-second-order kinetic and Langmuir isotherm models were found to be well fitted for PF adsorption using the prepared INPs. The obtained findings of this study clearly demonstrated that the fabricated INPs are an efficient material towards the removal of pesticide molecules from aquatic environments.

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1. Introduction

Modern agriculture is heavily reliant on the usage of various agrochemicals, which substantially improve efficient and cost-effective crop production to fulfil the food needs of the world's rapidly expanding population [1]. Agrochemicals are widely

employed in agriculture to conduit the gap between the food production and consumption required to address rising food demand [2]. However, the unbalanced utility of agrochemicals degrades the environment and poses significant problems to food safety, environmental and human health, ecological balance and soil biodiversity protection [3]. Organophosphate compounds are ester derivatives of phosphoric acids and thiophosphoric acids. For over five decades, they have been used as insecticides. They are still employed as insecticides, acaricides, nematocides, helminthicides, fungicides, nerve agents and herbicides [4]. Although they have been highly effective in agricultural pest management worldwide, their widespread usage has resulted in countless poisonings of off-target species, including voluminous human deaths. The significant acute mammalian toxicity concomitant with organophosphorus pesticide exposure is caused by the inactivation of the enzyme acetylcholinesterase in the nervous system with acute toxicological effects on the respiratory, myocardial and neuromuscular systems [5].

Organophosphates were introduced to replace resistant and dangerous chlorinated insecticides, and quickly gained popularity in the United States and worldwide [6]. PF [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] (Figure 1) is amongst the most routinely used organophosphate insecticides on field crops, vegetables and fruit crops [7,8]. This insecticide is moderately harmful (Toxicity Class II), and its residues have been identified in many vegetables. The primary route of exposure for humans is through dietary consumption of PF. When PF is sprayed to agricultural areas, its leftovers spread throughout the environment, including the ambient air, surface water and soil [9]. As these pesticide pollutants continue to accumulate in the ecosystem, the threat to human life, wildlife and plants escalates inexorably in tandem. Hence, the need of the hour is to instigate efficient strategies to remove PF residues in a way that is both economically viable and environmentally friendly.

A wide range of traditional techniques such as chemical precipitation, ion exchange, membrane filtration, electrochemical methods and bio-remediation have been used to eliminate PF molecules from aquatic bodies and, however, they have their own advantages and disadvantages. As a result, there is a growing demand for safer, easier, eco-friendly and economically viable techniques for PF removal from aqueous environment [10,11]. Historically, adsorption has

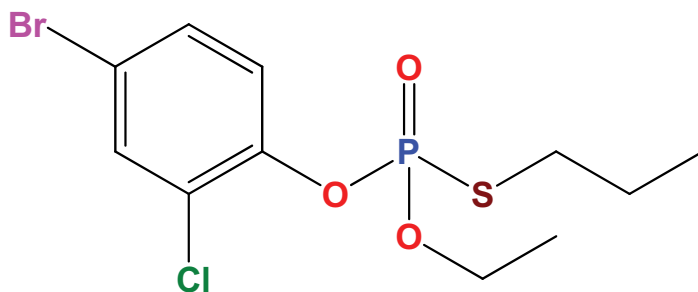


Figure 1. Chemical structure of Profenofos (PF).

received more attention due to its cost-effectiveness, flexibility, high selectivity towards targeted pollutants and greater removal efficiency, which significantly influence the capacity and selectivity towards PF species from aqueous solution. Therefore, environmental scientists and academicians continue to focus on the development of low-cost, eco-friendly and reproducible novel adsorbents. Since adsorption process is reversible, the adsorbent may be reused numerous times by using the appropriate desorption techniques. Further, adsorbents used for the elimination of contaminants should meet the following requirements: 1. It must have a low impact on the environment; 2. It must have an excellent sorption capacity and high selectivity for pollutants found in water even at low concentrations; 3. Adsorbed contaminants must be easily removed from its surface; 4. The period necessary to achieve adsorption equilibrium should be as short as feasible; and 5. It must be recyclable. For many decades, adsorbent materials such as activated carbon, zeolites and biomaterials have been widely utilised for the elimination various pollutants from aqueous solution [12]. However, the adsorption efficiency of these conventional materials is not satisfactory. As a result, the need for alternative adsorbents has skyrocketed [13,14].

Nanomaterials have proven to be able to meet most of the requirements outlined above in numerous studies conducted in the past few decades. Further, advances in nanoscience and nanotechnology have lately revealed significant possibilities for resolving environmental issues [15–19]. Because of their extensive surface area, nanostructured adsorbents offer better efficiencies and quicker adsorption rates in water treatment than traditional adsorbent materials. Moreover, many effective, low-cost and environmentally tolerable nanomaterials have been proposed to detoxify industrial effluents, groundwater, surface water and drinking water [20–24]. INPs are gaining popularity as an excellent indemnification medium for a variety of pollutants and also as a promising strategy to provide cost-effective solutions to critical environmental remediation problems. INPs are conveniently available credible adsorbents which reduce pollutants more effectively by producing very little waste and ancillary pollutants [25]. Furthermore, INPs are increasingly used for environmental clean-up due to their small particle size, larger zone of specific surface area, higher refractive index of reactive surface spots and greater intrinsic reactivity [26]. Iron was employed to imbue the substance with magnetic characteristics, increasing the removal efficiency of targeted pollutants. Nanoscale zero-valent iron has been extensively studied in recent years to reduce the availability of metals in water and soil samples. They have yielded consistent results in limiting the availability of silver, arsenic, beryllium, cadmium, chromium, mercury, nickel, lead, uranium, zinc and vanadium in the water samples [27–29]. Recently, H.M. Hamadeen *et al.* synthesised effective bio-nanosorbent from *Moringa oleifera* seeds waste for the removal of chlorpyrifos from wastewater [30]. S. Yekta demonstrated the adsorption and degradation of cyanophos by newly synthesised CaWO_4 nanoparticles [31]. A. Singhal and M.L. Lind have employed cysteine-capped silver nanoparticles to remove malathion and chlorpyrifos pesticide from drinking water [32]. T. Momić *et al.* used gold nanorods and nanospheres for the adsorption of organophosphate dimethoate pesticide [33]. S.M. Dehaghi and co-workers effectively utilised chitosan-zinc oxide nanoparticle composite as an

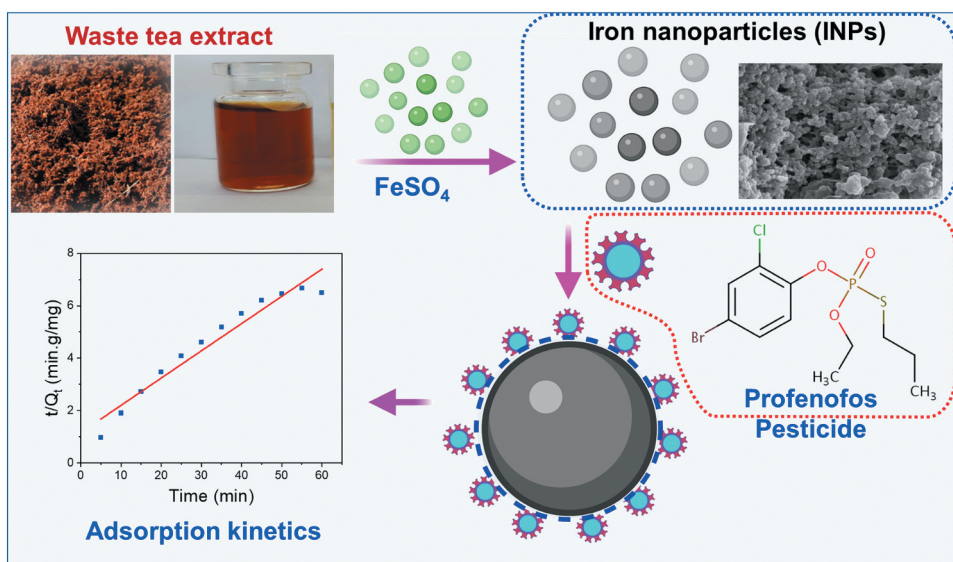


Figure 2. Schematic representation of the present investigation.

adsorbent to remove permethrin pesticide from water [34]. Some researchers have synthesised nanoparticles by using various fresh tea extracts. Nonetheless, those were applied for the degradation of dyes, removal of Pb and bromothymol blue indicator [35–39]. Despite some authors successfully synthesised nanoparticles by using tea wastes, those have been employed only for the removal of metal ions and phenol red dye [3840–42]. M. Khosravi *et al.* reported the adsorption kinetics and thermodynamics investigation of PF pesticide using chemically synthesised iron/nickel bimetallic nanoparticles [11].

However, in contrast to previous research, we have fabricated the INPs by tea waste extract using a greener method and applied it for the removal of PF from aqueous solution. Rising pollution of freshwater by a variety of contaminants is one of the most serious environmental issues confronting humanity on a global scale. Numerous studies have been devoted to develop more efficient, cost-effective and robust ways for wastewater treatment that does not exacerbate environmental stress or threaten human health. Furthermore, a huge amount of tea waste is generated every day throughout the world. Hence, we employed tea waste to fabricate INPs using an eco-friendly strategy for the adsorptive removal of PF pesticides from aqueous solution. The prepared INPs offer great promise for the removal of PF from the aquatic phase. The present study also investigated the impact of different influence factors such as contact time, solution pH, dosage of the adsorbent and initial concentration of PF on the maximum adsorption capacity. Further, the adsorption isotherm and kinetic properties of INPs towards the removal of PF were investigated, and the underlying mechanism of profenofos adsorption has been proposed (Figure 2).

2. Materials and methods

2.1. Materials

Ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was purchased from Sigma Aldrich (India) and used without further purification. PF pesticides were gained from the Scientific Fertilisers Company, Gundur, Tiruchirappalli, Tamil Nadu (India). Tea waste was obtained from a local tea stall near Bharathidasan University, Tiruchirappalli, Tamil Nadu (India). All the experiments were performed using distilled water.

2.2. Fabrication of INPs using tea waste extract

Solid tea waste was collected from a tea stall near Bharathidasan University, Tiruchirappalli, India. The collected tea waste was washed with distilled water to remove impurities and dried in the ambient atmosphere. The tea extract was prepared by boiling 60.0 g of solid tea waste in 1 L of distilled water at 80°C for 30 min and allowed to cool at room temperature. The solid tea component was removed by vacuum filtration, and the filtrate solution was used to synthesise INPs. The prepared tea waste extract was added to 0.10 M of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at a volume ratio of 2:1, respectively, at ambient temperature. An immediate colour change appears from pale yellow to deep brown, indicating the formation of INPs (Figure 3). The formed INPs were isolated by vacuum filtration and then washed with distilled water several times and air-dried. Afterwards, the prepared INPs were used for further experiments.

2.3. Characterisation of synthesised INPs

The UV-vis spectrum of the INPs was recorded using the ELICO-Double beam SI-210 spectrophotometer in the range 200–800 nm. The FT-IR measurements were performed using the Perkin-Elmer 597 spectrophotometer in the spectral range 400–4000 cm^{-1} . The PXRD spectrum of INPs was recorded using the Rigaku Miniflex-600 instrument at Alagappa University, Karaikudi, Tamilnadu (India). The surface

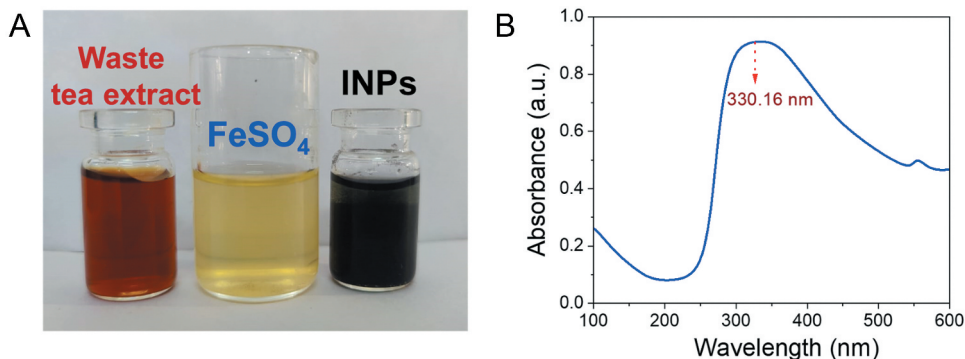


Figure 3. A) Photographic images of colour changes during the formation of the INPs. B) UV-visible spectrum of INPs (the arrow designated the INPs UV band at 330.16 nm).

morphology of the INPs was investigated using SEM analysis (Carl Zeiss Sigma, Sitra, Coimbatore, India). The hydrodynamic size of INPs was established using TEM-FEI by TECNAlG2-F30 equipment functioning with a 200 kV accelerated voltage. The INPs were directly mounted on the copper grids and examined under TEM and EDX (Oxford Instruments, UK) analysis to confirm the presence of elements in the prepared INPs. The zeta potential analysis of synthesised INPs was investigated using Malvern Instruments, UK.

2.4. PF adsorption studies using INPs

The batch technique [43] was used to measure the PF adsorption by INPs and to evaluate the different adsorption parameters like effects of contact time (0 to 60 min), initial PF concentration (0.5 to 2.0 mg L⁻¹), pH (2 to 10) and adsorbent dosage (0.2 to 1 g L⁻¹). The adsorption experiments were conducted by mixing different amounts of INPs with 100 mL of known concentration PF solution in glass Erlenmeyer flasks. The reaction content was placed and shaken in a mechanical shaker at a speed of 150 rpm. The solution pH was adjusted using 0.1 M HCl/NaOH. After a certain time interval, the INPs are removed from the solution by centrifugation at a speed of 8000 rpm. The supernatant solution was then analysed for the residual concentration of PF after adsorption experiments. Moreover, the adsorption capacity of glass Erlenmeyer flasks was examined and no significant changes were observed without INPs. Further, adsorption isotherms and kinetics studies were also carried out under optimised conditions such as contact time, solution pH and dose of the INPs. The removal percentage and adsorption capacity of PF onto INPs at equilibrium were estimated using the following equations:

$$\% \text{ removal of PF} = \frac{(C_i - C_e)}{C_i} \times 100 \quad (1)$$

$$\text{Adsorbed amount of PF (q)} = \frac{(C_i - C_e)}{m} \quad (2)$$

Here, C_e and C_i are the final equilibrium and initial concentrations of INPs, respectively, and 'm' is the mass of INPs in g L⁻¹.

3. Results and discussion

3.1. Fabrication and characterisation of INPs

This work describes the efficient fabrication of low-cost green INPs as sorbents from tea waste extract that effectively removes organophosphorus pesticide (PF) and the schematic diagram is shown in Figure 3. The UV-vis spectrum of INPs is displayed in Figure 3. The continuous absorption of INPs near 330.16 nm indicates the amorphous nature of INPs. The FT-IR spectra of INPs before and after adsorption of PF are presented in Figure 4, Figure 4). The IR absorption bands around 3220–3600 cm⁻¹ may be attributed to O-H stretching, and the band around 1600 cm⁻¹ may be related to O-H bending vibrations of surface water molecules adsorbed [44]. In addition,

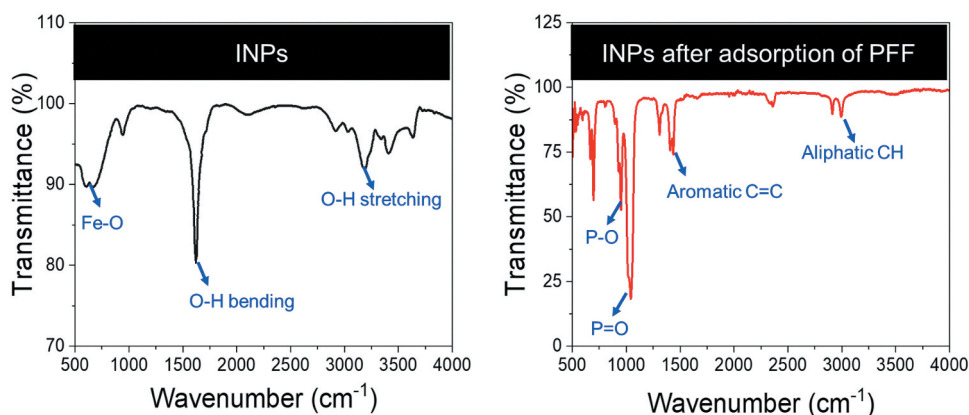


Figure 4. (A) FT-IR spectrum of INPs. (B) FT-IR spectrum of INPs after adsorption of PF.

characteristic peaks corresponding to Fe-O were observed around $540\text{--}650\text{ cm}^{-1}$ [11]. After the investigation, the FT-IR spectrum of INPs was recorded to prove the adsorption of PF (Figure 4). The spectrum exhibits weak bands around the region of $\sim 3000\text{ cm}^{-1}$, confirming the aliphatic-CH groups of PF. Further, the sharp band at $\sim 1000\text{ cm}^{-1}$ is attributed to P-OCH_3 , and the intense band at $\sim 1040\text{ cm}^{-1}$ can be assigned to P=O stretching [11]. The results of FT-IR spectra confirmed that the INPs were successfully interacting with PF molecules.

The SEM image of fabricated INPs is exhibited in Figure 5. The tea extract plays a vital role in forming the nano-scale structure and size of INPs. The INPs are well-organised round-shaped particles that are gently arranged with a hydrodynamic size of $60.37 \pm 2.65\text{ nm}$. According to the SEM image, it was observed that INPs were successfully fabricated from the tea waste. The hydrodynamic parameters of the INPs were assessed by the DLS method and demonstrate the particle size (Figure 5), which is in good accordance with the SEM analysis. Moreover, the zeta potential results clearly showed that the prepared INPs showed anionic nature in aqueous solution because of the zeta potential value, $-39.2 \pm 1.25 (\pm)\text{ mV}$ (Figure 5). This indicates the formation of stable INPs, which could effectively remove the PF molecules from the aqueous phase.

The EDX spectrum of INPs with respective mapping images are presented in Figure 6, Figure 6. It revealed the elemental composition of INPs, which contains iron (Fe) 71.85%, sulphur (S) 14.13%, phosphorus (P) 11.18% and potassium (K) 1.43%. Herein, S, P and K may be attributed to the tea extract. The corresponding elements of prepared INPs were identified during the TEM analysis, which clearly confirmed the successful formation of INPs from tea waste, as shown in Figure 6. The PXRD pattern of prepared INPs is shown in Figure 6, which is in good agreement with the JCPDS card number 87-0721. The characteristic peaks of INPs at $2\theta = 44.90^\circ$, 36.52° , 33.29° and 20.35° indicate the successful formation of zero-valent INPs from tea waste [40]. Moreover, the PXRD patterns clearly suggest that the prepared INPs are amorphous in nature, which could more effectively interact with PF in the aqueous phase.

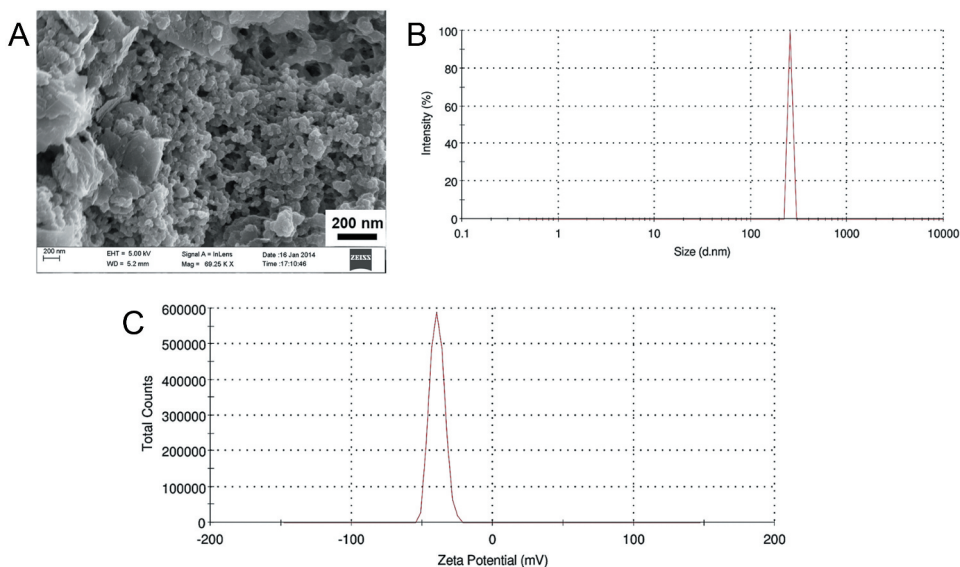


Figure 5. Morphology and hydrodynamic parameters of INPs were determined by scanning electron microscopy (SEM) and dynamic light scattering (DLS) method. A) SEM image of INPs. Scale bar of the picture 200 nm. B) Hydrodynamic size of INPs. C) Zeta potential of INPs.

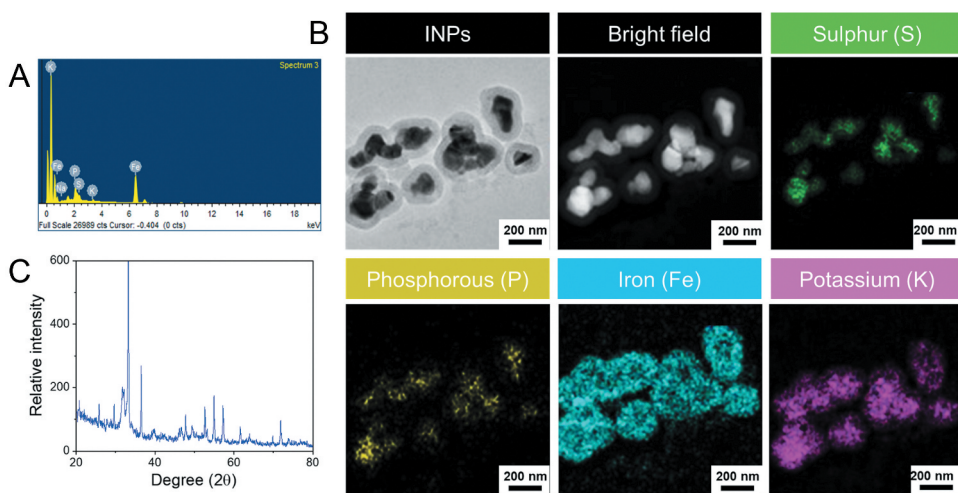


Figure 6. A) Elemental analysis of INPs. B) Elemental mapping analysis of INPs (elements were present in the nanocomposite including Iron (Fe), sulphur (S), phosphorous (P), Potassium (K)). Scale bar of the picture 200 nm. C) Powder X-ray diffraction (PXRD) of INPs.

The long-term stability of adsorbents is crucial to determine their efficacy and regulate their progress towards technological applications. Therefore, the stability of the titled INPs was examined by the SEM and DLS analysis for 1 week. The SEM analysis was performed on 1, 3, 5 and 7 days and the samples were incubated at

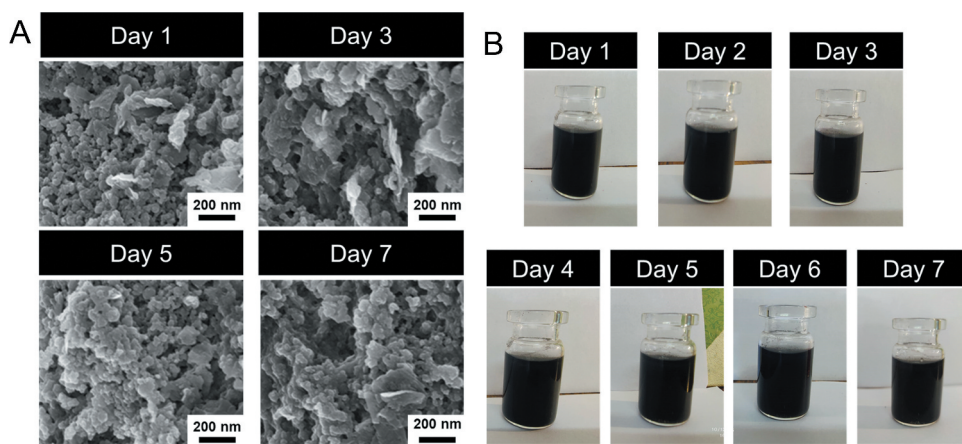


Figure 7. A) The stability of the INPs were confirmed by the SEM analysis on different incubation time (Day 1-7). B) The images of INPs at different incubation times.

room temperature. The pattern of the SEM images of INPs remains unaltered during the overall incubation time, and the results are shown in Figure 7. The respective incubated images of the INPs are shown in Figure 7. The DLS analysis was used to examine the changes in the size, polydispersity index (PDI) and the zeta potential of the INPs. The incubation of the fabricated INPs for a particular period (Days 0–7) INPs caused only slight changes in the size, PDI and zeta potential, which confirmed that the INPs are highly stable in water (Figure 8).

3.2. Adsorption parameters

3.2.1. Influence of contact time

The effect of contact time is one of the influencing parameters and plays a significant role in adsorption studies. The adsorption of PF onto the prepared INPs was investigated in batch mode under optimised conditions, and the obtained results are depicted in Figure 9. About 200 mg of prepared INPs were taken in flasks containing 1 mg L^{-1} of initial concentration of PF solution. Then, the reaction content was shaken in a mechanical shaker with a speed of 150 rpm. Figure 9 demonstrates that PF adsorption increases with time and finally reaches equilibrium stage and beyond that, there is no significant change in PF adsorption from the solution. The equilibrium stage is reached with a minimum contact time of 15 min. Therefore, 15 min was fixed as optimum contact time for further studies. With increasing duration of the contact time, the percentage of sorption increased from 59% to 88% and became nearly constant after 15 min. Our findings indicate that PF adsorption is rapid in the early stages and gradually slows down near equilibrium. This may be related to the availability of many active sites on the INPs surface in the initial stage. Afterwards, additional vacant surface sites are difficult to be adsorbed due to repulsive interactions between the adsorbate molecules. These results are analogous with the other reports describing the removal of some organophosphorus pesticides by metal nanoparticles [11,45].

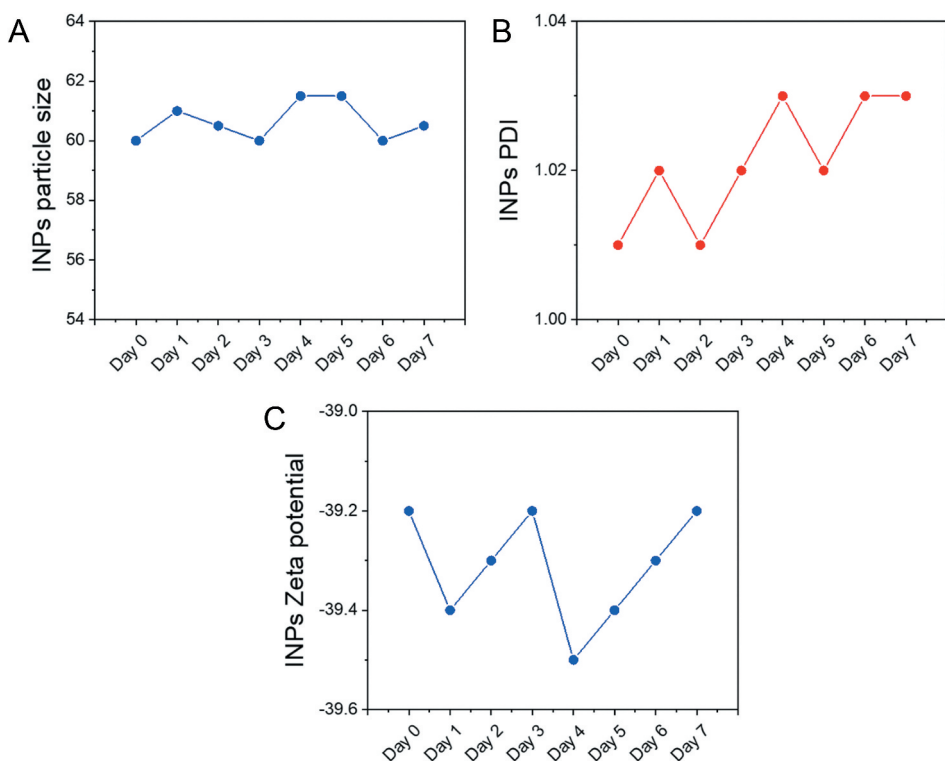


Figure 8. (A-C) The stability of the INPs were confirmed by the DLS analysis on different incubation time (Day 1-7) with particle size, PDI and zeta potential.

3.2.2. Influence of initial concentration of PF

The initial PF concentration ranged from 0.5 to 2 mg L⁻¹ for all experiments, and the content of INPs was fixed as 0.2 g L⁻¹ for this study. The initial concentration of PF plays a major role during the adsorption process because the adsorption mechanism is mainly dependent on the fixed active sites of the adsorbent and the initial concentration of PF molecules present in the aqueous solution. Figure 9 shows that the adsorption of PF is enhanced as the initial concentration of PF is increased. With an increase in the initial concentration of PF, there was likely a higher mass transport driving force because more adsorbate molecules were competing for binding sites on the adsorbent and facilitating adsorption. At higher concentrations of PF, this trend is shifted in the other direction, which might be due to the binding site saturation retarding the adsorption process [11].

3.2.3. Influence of initial pH

The impact of pH on adsorption efficiency is explained by the interaction between the adsorbent surface and the adsorbate molecules. The surface charge of the adsorbent plays an important role during the adsorption process. Therefore, the effect of pH is an essential factor in adsorption studies. The influence of pH on the removal of PF was examined at different pH ranging from 2 to 10 using PF initial concentrations of 1 mg L⁻¹

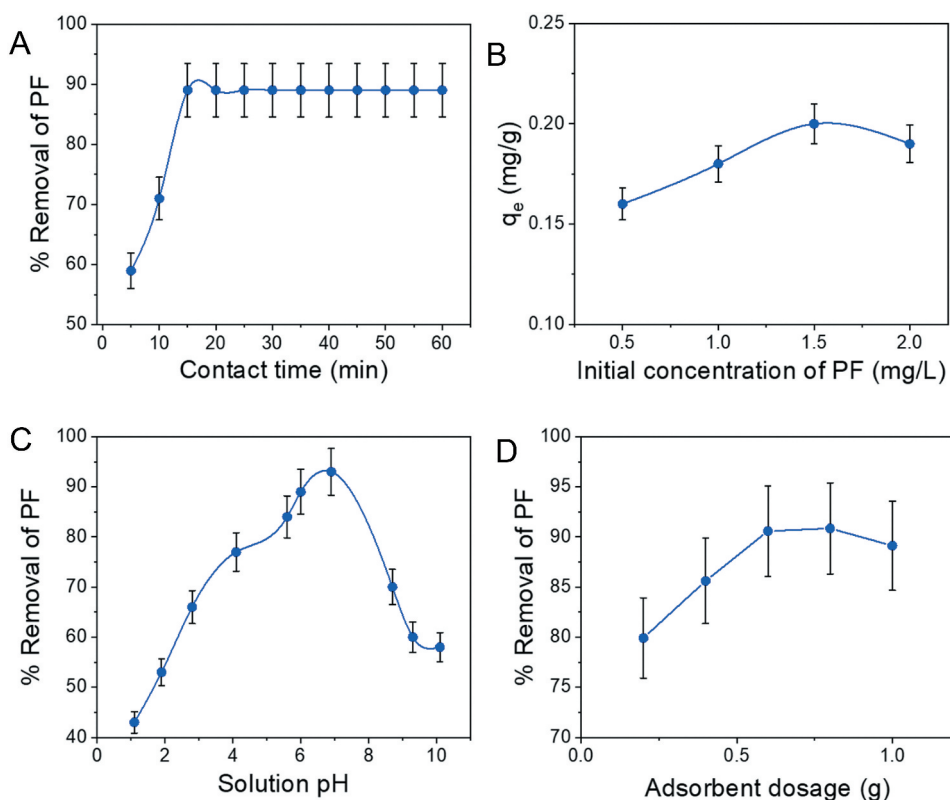


Figure 9. Factors influencing the PF adsorption. A) Contact time (min). B) Initial concentration of PF (mg L^{-1}). C) Solution pH. D) INPs dosage.

and 200 mg INPs. The obtained results are demonstrated in Figure 9. According to the results, the adsorption capacity of INPs was increased with increasing pH from 2 to 7 and beyond that, the adsorption capacity was gradually decreased with increasing pH. The maximum adsorption capacity was obtained at pH 6.9 and therefore the pH 6.9 was optimum pH for the further studies. The zero-point charge (pH_{zpc}) of iron oxide was reported to be 6.5 in the literature [46]. At more than pH 6.5, iron oxide becomes negative and could not interact with anionic pollutants. Below pH 6.5, iron oxide becomes positive and could effectively interact with anionic pollutants. In the same way, the prepared INPs were effectively interacting with PF molecules less than pH 7 and above pH 7 to 10, the adsorption capacity of PF molecules considerably decreased. This may be due to the charge of INPs gradually changing from positive to negative with increasing pH. Therefore, considerable adsorption capacity was obtained at acidic pH due to electrostatic interaction between the positive charge of the adsorbent and negatively charged PF molecules. Therefore, strong electrostatic attraction and complexation mechanisms were involved to enhance the PF adsorption onto prepared INPs at acidic conditions. At the same time, the strong repulsion force is restricted to the adsorption process of PF from aqueous solution.

3.2.4. Influence of adsorbent dosage

To investigate the impact of adsorbent dosage on PF adsorption at ambient temperature, the number of INPs was varied from 200 to 100 mg. The initial concentration of PF is fixed as 1 mg L^{-1} for all runs. The effects of INPs dose on adsorption of PF is shown in Figure 9. The adsorption efficiency of the prepared INPs increased with increasing amount of the INPs. This may be due to the increasing number of active sites and surface area for a fixed number of PF molecules. Figure 9 shows that as the adsorbent dosage was enhanced over 0.6 g L^{-1} of INPs, the adsorption capacity of INPs nanoparticles was dropped. Increasing the adsorbent dose can cause aggregation of adsorbents, and therefore the presence of active sites can be decreased during the adsorption process. Hence, the adsorption capacity of the prepared INPs decreased when the adsorbent dose was increased [47].

3.3. Adsorption isotherms

Evaluating adsorption equilibrium by fitting the equilibrium data into several isotherm models is a vital step in identifying the appropriate model for design purposes and determining the mechanism of adsorption systems [48,49]. Adsorption isotherms are essential in improving the use of adsorbents since they define how solutes interact with them and ensure the eliminated quantity of adsorbent. The Langmuir, Freundlich, Temkin and D-R isotherm models [50–53] were used to investigate the pathway behind the adsorption of PF. The correlation coefficients (R^2) values were used to assess the suitability of the adsorption isotherm to represent the adsorption mechanism of PF molecules onto the prepared INPs.

3.3.1. Langmuir isotherm

The Langmuir isotherm hypothesis is applicable for adsorption on homogeneous surfaces. The surface is composed of identical sites that are equally available for adsorption and have equivalent adsorption energies. According to the Langmuir isotherm, the maximum adsorption relates to a saturated single-layer of solute molecules on the surface of the adsorbent with no lateral contact among the adsorbed molecules [54]. The linear form of Langmuir adsorption isotherm equation is:

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \quad (3)$$

Here, C_e is the concentration of PF in solution (mg L^{-1}) at equilibrium, q_e is the adsorbed quantity of PF, q_m (mg g^{-1}) and b (L mg^{-1}) are the maximum adsorption capacity and Langmuir constants concerning the adsorption energy, respectively. The Langmuir isotherm constant was determined from the respective slope and intercept of the straight line of C_e/q_e vs C_e .

The Langmuir adsorption isotherm for PF onto INPs is shown in Figure 10. The Langmuir isotherm ($R^2 = 0.9989$) fits the experimental data exceptionally well, as displayed in Figure 10. The highest adsorption capacity (q_m) of INPs towards PF was found to be 43.67 mg g^{-1} at room temperature. Because of the homogeneous distribution of active sites on the INPs surface, the Langmuir isotherm may well fit

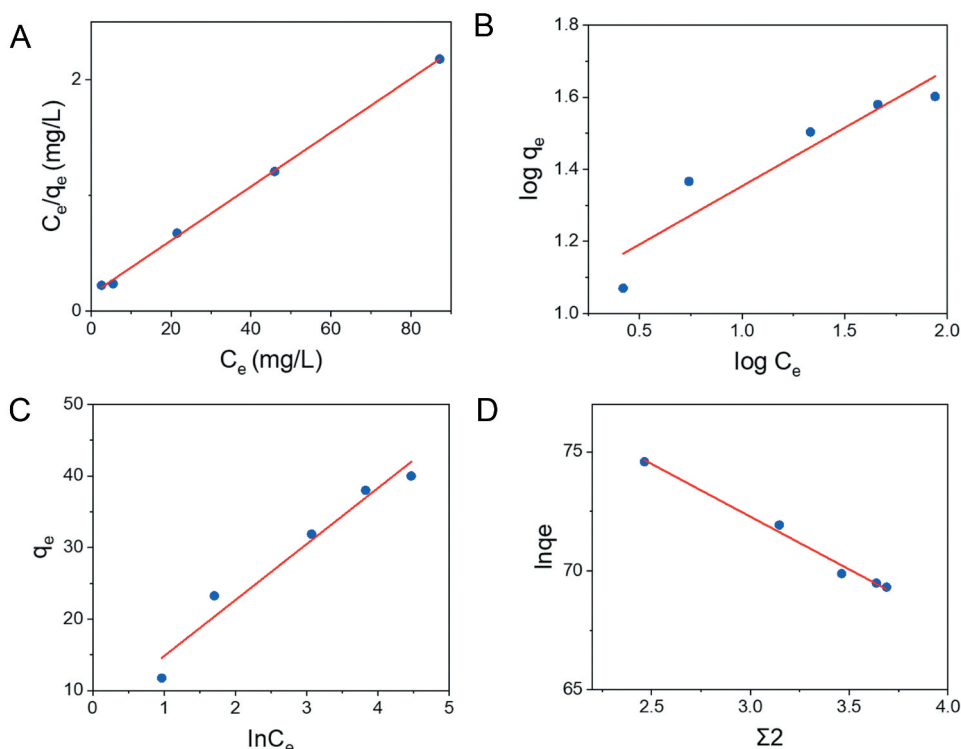


Figure 10. PF adsorption onto INPs. A) Langmuir isotherm plot. B) Freundlich isotherm plot. C) Temkin isotherm plot. D) D–R isotherm plot.

the investigation results. The highest adsorption is summarised as analogous to the formation of a uni-layer of PF molecules on the surface of INPs and dictates that there is no diffusion of PF in the plane of INPs surface [55].

3.3.2. Freundlich isotherm

Adsorption on heterogeneous surfaces is represented by the Freundlich adsorption isotherm. Both multilayer and monolayer adsorptions can be described using this isotherm model [56]. It is also predicated on the idea that stronger binding sites are occupied first. The binding strength diminishes as the degree of adsorption site occupancy increases.

The non-linear form of the Freundlich isotherm is designated as follows:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (4)$$

where q_e is the quantity of PF adsorbed (mg g^{-1}), C_e is the equilibrium concentration of PF in aqueous solution (mg L^{-1}), n and K_f are the Freundlich constants describing the quantity of adsorbate adsorbed onto the adsorbent for a unit equilibrium concentration. The value $(1/n)$ specifies the heterogeneity of the adsorption system, respectively. From the results expressed in Table 1, the K_f and n values are 1.0329 and 3.0769, respectively. It

Table 1. Isotherm parameters for the adsorption PF on INPs.

Isotherm Models	Langmuir	Freundlich	Temkin	D-R
Parameters	$q_m = 43.67 \text{ mg g}^{-1}$ $b = 0.157 \text{ L mg}^{-1}$ $R^2 = 0.9989$	$n = 3.0769$ $K_F = 1.0329 \text{ L g}^{-1}$ $R^2 = 0.8760$	$\alpha = 5.4795$ $\beta = 0.1204$ $b = 207.15$ $R^2 = 0.9598$	$K_L = 4.405$ $q_m = 193.99 \text{ mg g}^{-1}$ $E = 0.3369 \text{ kJ mol}^{-1}$ $R^2 = 0.9872$

is confirmed that the PF is sufficiently adsorbed onto the surface of the INPs. The R^2 value of the Freundlich isotherm is 0.8760, which is too far from 1 and evidenced that the Freundlich isotherm fails to forecast the experimental data (Figure 10).

As presented in Table 1, the mean values of the regression coefficient (R^2) are 0.9989 and 0.8760 for Langmuir and Freundlich isotherm, respectively. According to the higher R^2 value, Langmuir isotherm fits the experimental data better than the Freundlich isotherm.

3.3.3. Temkin Isotherm

The Temkin adsorption isotherm was derived on the assumption that due to adsorbate–adsorbate interactions, the heat of adsorption of all molecules in the layer reduces linearly with coverage. The adsorption energy is defined by the regular distribution of the binding energies up to a maximum binding energy [57]. The linear form of Temkin's isotherm can be expressed as:

$$q_e = RT \ln K_T + \{RT/b_T\} \ln C_e \quad (5)$$

where T is the absolute temperature, R is the universal gas constant, and b_T is the Temkin constant related to the adsorption heat (J mol^{-1}), K_T is the Temkin isotherm constant (L mg^{-1}). The essential parameters of the Temkin isotherm (Figure 10) were determined from the linear plot of $\ln C_e$ vs q_e . As can be seen from Table 1, the Temkin isotherm also does not fits well for PF adsorption ($R^2 = 0.9598$) than Langmuir isotherm model.

3.3.4. The Dubinin–Radushkevich (D-R) isotherm

The D–R isotherm could represent adsorption on both heterogeneous and homogeneous surfaces at low concentrations. The linear form of the D–R isotherm equation is as follows:

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad (6)$$

here q_m is the D–R monolayer capability (mg g^{-1}), β sorption energy constant and ε refers the Polanyi potential at equilibrium concentration and equals to:

$$\varepsilon = RT \ln(1 + 1/C_e) \quad (6.1)$$

$$E = 1/(2B)^{1/2} \quad (6.2)$$

Here, E is described by the free energy (kJ mol^{-1}), the value B is related to the mean sorption energy.

The activation energy can be determined by whether the adsorption is based on chemisorption or physisorption. In most cases, the D–R model's adsorption energy (E) reveals the physical nature of the elimination mechanism ($E < 8 \text{ kJ/mol}$). In nature,

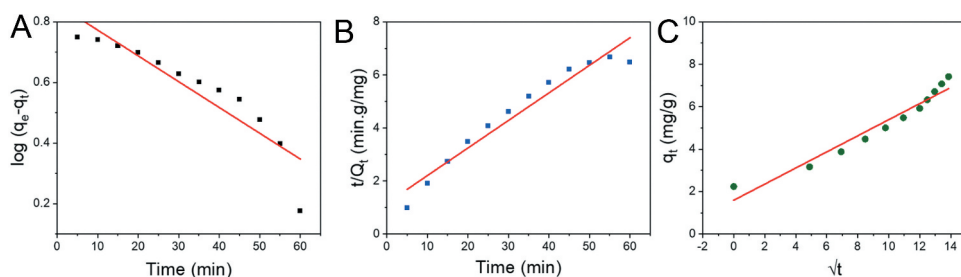


Figure 11. Kinetic adsorption models of PF. A) Pseudo-first-order kinetics. B) Pseudo-second-order kinetics. C) Kinetic plot for intra-particle diffusion at RT.

physisorption occurs when the activation energy is less than 8 kJ mol^{-1} , and chemisorption occurs when the activation energy is between 8 and 16 kJ mol^{-1} . According to this assertion, our investigation discloses that the PF adsorption on INPs is based on physisorption [58].

The adsorption behaviour of PF onto INPs was studied using Langmuir, Freundlich, Temkin and D-R isotherm models. The obtained results are depicted in Figure 10. The derived isotherm parameters along with the R^2 values are presented in Table 1. According to the R^2 values, the equilibrium isotherm data of PF adsorption onto INPs was fitted well into Langmuir isotherm model than Freundlich, Temkin and D-R isotherm models. The Langmuir adsorption capacity was found to be 43.67 mg/g for PF adsorption using prepared INPs.

3.4. Adsorption kinetics

Kinetics is one of the most significant criteria to be considered when evaluating the adsorption efficiency [59]. The kinetics of PF adsorption onto INPs was examined using the pseudo-first-order, pseudo-second-order and intra-particle diffusion models (Figure 11). The calculated kinetic parameters of the pseudo-first-order, pseudo-second-order and intra-particle diffusion models are presented in Table 2.

3.4.1. Pseudo-first-order reaction

The Lagergren first-order or pseudo-first-order rate equation are broadly employed for the solute sorption from the solution and is measured by the following equation:

$$\log(q_e - q_t) = (\log q_e) - (K_1/2.303) \times t \quad (7)$$

here $q_e \text{ (mg g}^{-1}\text{)}$ is the quantity of adsorbed PF at equilibrium, $q_t \text{ (mg g}^{-1}\text{)}$ is the quantity of adsorbed PF at time $t \text{ (min)}$ and K_1 is the rate constant for a pseudo-first-order kinetics. According to the pseudo-first-order equation, the rate of adsorption site occupation is

Table 2. Kinetic parameters for the adsorption of PF on INPs.

Kinetic system	System coefficients	R^2
Pseudo-first order	$q_e = 7.3468 \text{ mg g}^{-1}$ $K_1 = 1.87 \times 10^{-2} \text{ min}^{-1}$	0.9131
Pseudo-second order	$q_e = 12.722 \text{ mg g}^{-1}$ $K_2 = 7 \times 10^{-3} \text{ g mg}^{-1} \text{ min}^{-1}$	0.9792
Intra-particle diffusion	$K_{dif} = 1.7199 \text{ mg g}^{-1} \text{ min}^{1/2}$ $C = 9.0107$	0.9399

related to the number of unoccupied sites [60,61]. The pseudo-first-order kinetic fit for the PF adsorption onto the INPs is shown in Figure 11 and the corresponding kinetic parameters are presented in Table 2.

3.4.2. Pseudo-second-order reaction

A pseudo-second-order equation can also be used to characterise the adsorption kinetics. The pseudo-second-order kinetic model states that the reaction rate is quick at the beginning and then slows as it reaches equilibrium; the reaction can continue at this rate for an extended period of time. This model is based on the adsorption ability of the adsorbent and is effective for understanding both non-physical and nonchemical equilibrium [62]. It is expressed by the following equation:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (8)$$

where K_2 is the rate constant of the pseudo-second-order adsorption reaction, q_t (mg g^{-1}) and q_e (mg g^{-1}) are the amounts of adsorbed solute at equilibrium at any time t (min). Figure 11 illustrates the linear form of the pseudo-second-order adsorption model, and the parameters are listed in Table 2. According to Table 2, the pseudo-second-order kinetics model along with higher R^2 values is more consistent with the investigation results than the pseudo-first-order kinetics model. The results indicate that the adsorption rate is highly dependent on adsorption sites availability over the PF concentration in the solution.

3.4.3. Intra-particle diffusion model

It is assumed in the intra-particle diffusion model (Figure 11) that adsorbate can be transported into the pores of the adsorbent by batch mode adsorption and is stated as follows:

$$q = K_i t^{1/2} + C_i \quad (9)$$

here C_i refers to intercept, which approximates the thickness of the boundary layer, and a significant intercept value denotes the higher boundary layer effect. K_i refers to the rate constant for intra-particle diffusion that can be calculated by the slope from the linear plot of q against $t^{1/2}$. If the plot is linear and passes through the origin, intra-particle diffusion is the only rate-controlling step. But if the plot is linear and not passes through the origin, it is suggested that the adsorption involves intra-particle diffusion but it is not the only rate-limiting step. The outcomes of our analysis demonstrate that intra-particle diffusion is not considered as the rate-controlling step in the PF adsorption by INPs [63].

3.5. Adsorption mechanism

Adsorption is a surface phenomenon that results when adsorbate molecules accumulate on the surface of the adsorbent. It is based on the mass transport of a specific or group of components of the adsorbate congregate at the interface. A variety of mechanisms have been proposed in the literature for the removal of pesticides from water. Van der Waals forces, electrostatic attraction, hydrophobic interactions, hydrogen bonding formation, π - π stacking, ion exchange and surface complexation exist between the adsorbent and

adsorbate in an aqueous medium. However, the adsorptive removal of harmful pesticides is triggered by one or more of these interactions. In general, the decontamination of pesticides by prepared adsorbent is typically a function of adsorbent properties as well as the chemical composition of the adsorbates. Several interaction forces including surface complexation, hydrogen bonding and electrostatic attraction were governed by the adsorption mechanism of PF from the aqueous phase using as-prepared INPs. Under acidic conditions, the INPs are effectively adsorbed the PF molecules from the aqueous phase because the protonated INPs easily interacted with highly electronegative atoms of PF molecules through electrostatic attraction. Other forces such as surface complexation and hydrogen bonding also enhanced the adsorption mechanism of PF molecules from aqueous solution. Further, the adsorptive removal of PF molecules was influenced by a variety of variables, including adsorbent concentration, incubation time, temperature, solution pH and particle size, etc. Among the above mentioned mechanisms, electrostatic attraction is the main force to adsorb the PF molecules onto the INPs. However, the electrostatic attraction becomes weaker in alkaline conditions due to the deprotonation of INPs. The selected pesticide profenofos are composed of the most electronegative atoms including Br, Cl, S, P and O, which may be strongly involved in the hydrogen bonding interactions with the adsorbent INPs in acidic conditions. Further, the coordination or chelating ability of S, P or O atoms towards nano iron may also be responsible for the adsorption of PF molecules by INPs. Based on the obtained results, Langmuir isotherm revealed the monolayer adsorption of PF molecules on the surface of INPs. Further, the D-R isotherm unveiled that the adsorption of PF on the surface of INPs is physisorption in nature. Therefore, the underlying mechanism is physisorption mediated monolayer adsorption of PF molecules on the surface of fabricated INPs.

4. Conclusion

In summary, the INPs were successfully fabricated, and their further application in PF uptake was systematically investigated in this study. The prepared INPs were thoroughly characterised using UV-vis, FT-IR, SEM, EDX, PXRD, DLS, zeta potential and mapping analysis. The synthesised INPs show excellent adsorption capacity of 43.67 mg g^{-1} for PF molecules with a minimum contact time of 15 min. Various influencing factors such as solution pH, contact time, adsorbent dose and initial concentration were systematically investigated for the maximum uptake of PF molecules from aqueous solution. The equilibrium data are fit with the Langmuir adsorption isotherm model, demonstrating mono layer coverage of PF molecules on the INPs outer surfaces. The adsorption kinetics clearly indicates that the adsorption system follows a pseudo-second-order kinetic model and it is confirmed by the higher R^2 (0.9792) value. Moreover, it was revealed that the rate of adsorption was more dependent on the availability of adsorption sites on INPs over PF concentration in the solution. The electrostatic interaction is mainly governed by the adsorptive mechanism of PF molecules onto the INPs. The obtained findings of this study demonstrate the scope and efficacy of green INPs as effective adsorbent for the environmentally friendly removal of pesticides from polluted water. Until now, optimisation of green synthetic protocols, exact mechanisms of nanoparticle formation, regeneration and reutilisation of nanomaterials are major challenges for researchers to develop them from laboratory level to commercialisation. A slight change in the parameters such as particle

size, reactant concentration, reaction pH, reaction time, temperature, pressure and environmental conditions significantly affected the physicochemical properties and morphologies of nanoparticles. The main drawbacks of INPs are the release of soluble iron ions and their susceptibility to surface oxidation. Moreover, the latent toxicities of these nanomaterials are not completely addressed.

For future perspectives, we propose (i) to employ titled INPs for the removal of other hazardous organophosphorus pesticides and other types of pollutants including dyes and heavy metals; (ii) to use INPs as pilot adsorbent in agricultural and industrial wastewater remediation; (iii) to estimate the practical utility, environmental and the commercial feasibility of the application. (iv) to study the toxicity effect, recovery and reusability of INPs for their application in wastewater treatment.

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References

- [1] R. Paarlberg, *Society* **46**, 4 (2009). doi:[10.1007/s12115-008-9168-3](https://doi.org/10.1007/s12115-008-9168-3).
- [2] J. Clay, *World Agriculture and the Environment: A Commodity-by-commodity Guide to Impacts and Practices* (Washington, DC: Island Press, 2004).
- [3] L. Rani, K. Thapa, N. Kanojia, N. Sharma, S. Singh, A.S. Grewal, A.L. Srivastav and J. Kaushal, *J. Clean. Prod.* **283**, 124657 (2021). doi:[10.1016/j.jclepro.2020.124657](https://doi.org/10.1016/j.jclepro.2020.124657).
- [4] N. Mansouriieh, M.R. Sohrabi and M. Khosravi, *Arab. J. Chem.* **12**, 2524 (2019). doi:[10.1016/j.arabjc.2015.04.009](https://doi.org/10.1016/j.arabjc.2015.04.009).
- [5] B.E. Mileson, J.E. Chambers, W.L. Chen, W. Dettbarn, M. Ehrich, A.T. Eldefrawi and K.B. Wallace, *Toxicol. Sci.* **41**, 8 (1998). doi:[10.1093/toxsci/41.1.8](https://doi.org/10.1093/toxsci/41.1.8).
- [6] M. Ma, S. Dong, W. Jin, C. Zhang and W. Zhou, *J. Environ. Sci. Heal. Part B.* **54**, 70 (2019). doi:[10.1080/03601234.2018.1505036](https://doi.org/10.1080/03601234.2018.1505036).
- [7] V. Kumar, N. Sharma and A. Vangnai, *Environ. Technol. Innov.* **21**, 101367 (2021). doi:[10.1016/j.eti.2021.101367](https://doi.org/10.1016/j.eti.2021.101367).
- [8] M. Kushwaha, S. Verma and S. Chatterjee, *J. Environ. Qual.* **45**, 1478 (2016). doi:[10.2134/jeq2016.03.0100](https://doi.org/10.2134/jeq2016.03.0100).
- [9] M.U. Ghani, H.N. Asghar, A. Niaz, Z.A. Zahir, M.F. Nawaz and M.M. Häggblom, *Int. J. Phytoremed.* **11**, (2021). DOI:[10.1080/15226514.2021.1952927](https://doi.org/10.1080/15226514.2021.1952927).
- [10] S.S. Ratpukdi, A.S. Vangnai and W. Patichot, *J. Adv. Oxid. Technol.* **20**, 20170025 (2017). doi:[10.1515/jaots-2017-0025](https://doi.org/10.1515/jaots-2017-0025).
- [11] N. Mansouriieh, M.R. Sohrabi and M. Khosravi, *Int. J. Environ. Sci. Technol.* **13**, 1393 (2016). doi:[10.1007/s13762-016-0960-0](https://doi.org/10.1007/s13762-016-0960-0).

- [12] A. Mojiri, J.L. Zhou, B. Robinson, A. Ohashi, N. Ozaki, T. Kindaichi, H. Farraji and M. Vakili, *Chemosphere* **253**, 126646 (2020). doi:10.1016/j.chemosphere.2020.126646.
- [13] I. Ali and V.K. Gupta, *Nat. Protoc.* **1**, 2661 (2006). doi:10.1038/nprot.2006.370.
- [14] R. Rashid, I. Shafiq, P. Akhter, M.J. Iqbal and M. Hussain, *Environ. Sci. Pollut. Res.* **28**, 9066 (2021). doi:10.1007/s11356-021-12395-x.
- [15] A.S. Mahmoud, A. Ismail, M.K. Mostafa, M.S. Mahmoud, W. Ali and A.M. Shawky, *Sep. Sci. Technol.* **55**, 684 (2020). doi:10.1080/01496395.2019.1574832.
- [16] S. Shojaei, A. Nouri, L. Baharinikoo, M.D. Farahani and S. Shojaei, *Polyhedron* **196**, 114995 (2021). doi:10.1016/j.poly.2020.114995.
- [17] M.E. El-Sayed, *Sci. Total Environ.* **739**, 139903 (2020). doi:10.1016/j.scitotenv.2020.139903.
- [18] S. Shojaei and S. Shojaei, *Int. J. Environ. Sci. Technol.* **16**, 4601 (2019). doi:10.1007/s13762-018-1866-9.
- [19] S. Shojaei, S. Shojaei, S.S. Band, A.A.K. Farizhandi, M. Ghorogi and A. Mosavi, *Sci. Rep.* **11**, 16054 (2021). doi:10.1038/s41598-021-95649-5.
- [20] S. Shojaei, M. Rahmani, M. Khajeh and A.R. Abbasian, *Chem. Select.* **6**, 4782 (2021). doi:10.1002/slct.202100288.
- [21] A.H. Jawad, A.S. Abdulhameed, S.N. Surip and S. Sabar, *Int. J. Environ. Anal. Chem.* **2020**, 1. doi:10.1080/03067319.2020.1807966
- [22] A. Pourabadeh, L. Baharinikoo, S. Shojaei, B. Mehdizadeh, M.D. Farahani and S. Shojaei, *Int. J. Environ. Anal. Chem.* **100**, 1707 (2020). doi:10.1080/03067319.2019.1657855.
- [23] S. Shojaei, S. Shojaei and M. Pirkamali, *Water Conserv. Sci. Eng.* **4**, 13 (2019). doi:10.1007/s41101-019-00064-7.
- [24] S. Shojaei, S. Shojaei, A. Nouri and L. Baharinikoo, *Npj Clean Water.* **4**, 1 (2021). doi:10.1038/s41545-021-00113-6.
- [25] X. Zhao, W. Liu, Z. Cai, B. Han, T. Qian and D. Zhao, *Water Res.* **100**, 245 (2016). doi:10.1016/j.watres.2016.05.019.
- [26] K.W. Henn and D.W. Waddill, *Remediat. J.* **16**, 57 (2006). doi:10.1002/rem.20081.
- [27] A. Galdames, L. Ruiz-Rubio, M. Orueta, M. Sánchez-Arzalluz and J.L. Vilas-Vilela, *Int. J. Environ. Res.* **17**, 5817 (2020). doi:10.3390/ijerph17165817.
- [28] S.R. Kanel, B. Manning, L. Charlet and H. Choi, *Environ. Sci. Technol.* **39**, 1291 (2005). doi:10.1021/es048991u.
- [29] K.D. Grieger, A. Fjordbøge, N.B. Hartmann, E. Eriksson, P.L. Bjerg and A. Baun, *J. Contam. Hydrol.* **118**, 165 (2010). doi:10.1016/j.jconhyd.2010.07.011.
- [30] H.M. Hamadeen, E.A. Elkhatab, M.E.I. Badawy and S.A.M. Abdelgaleil, *J. Environ. Chem. Eng.* **9**, 105376 (2021). doi:10.1016/j.jece.2021.105376.
- [31] S. Yekta, M. Sadeghi and E. Babanezhad, *J. Water Process. Eng.* **14**, 19 (2016). doi:10.1016/j.jwpe.2016.10.004.
- [32] A. Singhal and M.L. Lind, *Int. J. Nanomed.* **13**, 25 (2018). doi:10.2147/IJN.S124700.
- [33] T. Momić, T.L. Pašti, U. Bogdanović, V. Vodnik, A. Mraković, Z. Rakočević and V. Vasić, *J. Nanomater.* **2016**, 11 (2016). doi:10.1155/2016/8910271.
- [34] S.M. Dehaghi, B. Rahmanifar, A.M. Moradi and P.A. Azar, *J. Saudi Chem. Soc.* **18**, 348 (2014). doi:10.1016/j.jscs.2014.01.004.
- [35] L. Huang, X. Weng, Z. Chen, M. Megharaj and R. Naidu, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **130**, 295 (2014). doi:10.1016/j.saa.2014.04.037.
- [36] I. Ali, O.M.L. Alharbi, Z.A. Allothman and A. Alwarthan, *Colloids Surf. B.* **171**, 606 (2018). doi:10.1016/j.colsurfb.2018.07.071.
- [37] X. Weng, L. Huang, Z. Chen, M. Megharaj and R. Naidu, *Ind. Crops Prod.* **51**, 342 (2013). doi:10.1016/j.indcrop.2013.09.024.
- [38] Z. Lin, X. Weng, G. Owens and Z. Chen, *J. Clean. Prod.* **242**, 118476 (2020). doi:10.1016/j.jclepro.2019.118476.
- [39] H. Xin, X. Yang, X. Liu, X. Tang, L. Weng and Y. Han, *J. Nanotech.* **2016**, 1 (2016). doi:10.1155/2016/4059591.
- [40] A. Gautam, S. Rawat, L. Verma, J. Singh, S. Sikarwar, B.C. Yadav and A.S. Kalamdhad, *Environ. Nanotechnol. Monit. Manag.* **10**, 377 (2018). doi:10.1016/j.enmm.2018.08.003.

- [41] S. Lunge, S. Singh and A. Sinha, *J. Magn. Magn. Mater.* **356**, 21 (2014). doi:10.1016/j.jmmm.2013.12.008.
- [42] P. Panneerselvam, N. Morad and K.A. Tan, *J. Hazard. Mater.* **186**, 160 (2011). doi:10.1016/j.jhazmat.2010.10.102.
- [43] C. Oter and O. Selçuk Zorer, *Int. J. Environ. Anal. Chem.* **101**, 1950 (2021). doi:10.1080/03067319.2019.1691184.
- [44] R. Singh, V. Misra and R.P. Singh, *J. Nanopart. Res.* **13**, 4063 (2011). doi:10.1007/s11051-011-0350-y.
- [45] Q. Yang, J. Wang, W. Zhang, F. Liu, X. Yue, Y. Liu and J. Wang, *Chem. Eng. Sci.* **313**, 19 (2017). doi:10.1016/j.ces.2016.12.041.
- [46] M.H. Salmani, M. Abedi, S.A. Mozaffari, A.H. Mahvi, A. Sheibani and M. Jalili, *J. Environ. Health Sci. Engineer.* **19**, 603 (2020). doi:10.1007/s40201-021-00631-y.
- [47] S.Y. Yoon, C.G. Lee, J.A. Park, J.H. Kim, S.B. Kim, S.H. Lee and J.W. Choi, *Chem. Eng. Technol.* **236**, 341 (2014). doi:10.1016/j.ces.2013.09.053.
- [48] S. Dadfarnia, A.M. Haji Shabani, S.E. Moradi and S. Emami, *Appl. Surf. Sci.* **330**, 85 (2015). doi:10.1016/j.apsusc.2014.12.196.
- [49] F.A. Razmi, N. Ngadi, S. Wong, I.M. Inuwa and L.A. Opotu, *J. Clean. Prod.* **231**, 98 (2019). doi:10.1016/j.jclepro.2019.05.228.
- [50] S. Yousefinia, M.R. Sohrabi, F. Motiee and M. Davallo, *J. Contam. Hydrol.* **243**, 103906 (2021). doi:10.1016/j.jconhyd.2021.103906.
- [51] V. Madhavi, T.N.V.K.V. Prasad, A.V.B. Reddy, B. Ravindra Reddy and G. Madhavi, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **116**, 17 (2013). doi:10.1016/j.saa.2013.06.045.
- [52] S.H. Kim and P.P. Choi, *Dalton Trans.* **46**, 15470 (2017). doi:10.1039/C7DT02076G.
- [53] H. Jabeen, K.C. Kemp and V. Chandra, *J. Environ. Manage.* **130**, 429 (2013). doi:10.1016/j.jenvman.2013.08.022.
- [54] H. Balouchi, M. Baziar, A. Dehghan, H. Alidadi and M. Shams, *Int. J. Environ. Anal. Chem.* **2020**, 1. doi:10.1080/03067319.2020.1737035
- [55] M. Baziar, H.R. Zakeri, S. Ghaleh Askari, Z.D. Nejad, M. Shams, I. Anastopoulos and E.C. Lima, *J. Mol. Liq.* **332**, 115832 (2021). doi:10.1016/j.molliq.2021.115832.
- [56] M.M. Rahman, S.H. Rimu, S. Biswas, T.U. Rashid, A.H. Chisty, A. Rahman and P. Haque, *Int. J. Environ. Anal. Chem.* **2020**, 1. doi:10.1080/03067319.2020.1813732
- [57] M. Yousefi, M. Gholami, V. Oskoei, A.A. Mohammadi, M. Baziar and A. Esrafil, *J. Environ. Chem. Eng.* **9**, 105677 (2021). doi:10.1016/j.jece.2021.105677.
- [58] B. Saha, S. Das, J. Saikia and G. Das, *J. Phys. Chem. C.* **115**, 8024 (2011). doi:10.1021/jp109258f.
- [59] O.O. Namal and K. Erkan, *Int. J. Environ. Anal. Chem.* **100**, 1549 (2019). doi:10.1080/03067319.2019.1656721.
- [60] P.N. Diagboya, B.I. Olu-Owolabi and K.O. Adebowale, *RSC Adv.* **5**, 2536 (2015). doi:10.1039/C4RA13126F.
- [61] S. Choe, Y.Y. Chang, K.Y. Hwang and J. Khim, *Chemosphere* **41**, 1307 (2000). doi:10.1016/S0045-6535(99)00506-8.
- [62] D. Dickson, G. Liu and Y. Cai, *J. Environ. Manage.* **186**, 261 (2017). doi:10.1016/j.jenvman.2016.07.068.
- [63] T. Sheela and Y.A. Nayaka, *Chem. Eng. J.* **191**, 123 (2012). doi:10.1016/j.ces.2012.02.080.

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ISOLATION AND IDENTIFICATION OF PROFENOFOS DEGRADING BACTERIUM FOR EFFICIENT BIOREMEDIATION OF PESTICIDE CONTAMINATED AGRICULTURAL SOIL

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ABSTRACT : The chemical structure of organophosphorus pesticides is attributed to their high toxicity and competence to irreversibly inhibit the activity of acetylcholinesterase and inflicting neuronal disorder, organ failure and eventual death among non-target species. Profenofos is one of the most widely used organophosphate pesticides on a variety of crops and its residues are being found in human blood, breast milk, and urine. Hence, there is a rising demand for the development of pesticide degrading efficacious technologies. One of most promising bioremediation strategies has proven to be microbial breakdown of pesticides into less harmful compounds. For the bioremediation of profenofos contaminated soil, a bacterial strain PDB1 competent of utilising profenofos as the only carbon and energy source, was isolated from the agricultural soil samples of Karur district, Tamilnadu, India. The strain PDB1 is identified as *Bacillus subtilis* from the morphological, biochemical, and 16S rRNA gene studies. The strain was grown in minimal salt broth with 100 ppm of profenofos as a sole carbon source. The PDB1 strain was subjected to degrade profenofos pesticide. GC–MS analysis proved the ability of PDB1 strain to degrade profenofos pesticide into 4-bromo-2-chlorophenol and diethylthiophosphoric acid metabolites.

Key words : *Bacillus subtilis*, biodegradation, GC-MS, profenofos, 4-bromo-2-chlorophenol.

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INTRODUCTION

Global estimates of yield losses for main crops induced by viruses, pests, diseases, and weeds varied between 26-40%, based on crop and world territory (Willocquet *et al*, 2017). As a result, the application of chemical pesticides has been a preferred solution to boost agricultural productivity, quality and to fulfil the food requirements of the world's fast increasing population (Moghadam *et al*, 2021). Despite of its advantages their widespread and long-term usages, however, cause severe contamination of air, water, soil and agricultural products, that jeopardizes crop production eventually being harmful to the ecosystem especially to the agriculturally beneficial microbes and aquatic animals and consequently creates risks to human health through food chains (Srivastav *et al*, 2021). Massive amounts of pesticides were manually administered, but it was estimated that only a minor proportion of the compounds properly reached the target species, with the balance being settled on the soil and

resulted in poisonings of off- target species (Diez *et al*, 2010). Pesticide persistence in soil also has a severe impact on climate parameters such as rainfall intensity and schedule, as well as temperature. Climate change affects the retention of pesticides and microorganisms in soil (Ma *et al*, 2021). Among the several pesticide categories used across the globe, organophosphates are the most frequently employed group of pesticides in contemporary usage (Milesen *et al*, 1998).

Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] (Fig. 1) is a common organophosphate insecticide used on a variety of agricultural crops. According to findings, the pesticide is an acetylcholine esterase (AChE) inhibitor, causing an irreversible neuronal disorder in animals. Among the most serious health risks associated with profenofos residues are genotoxicity, mutagenicity, multi-organ disorder, hormone disruption, reduced overall body metabolism, and reproductive problems. Further, profenofos residues have

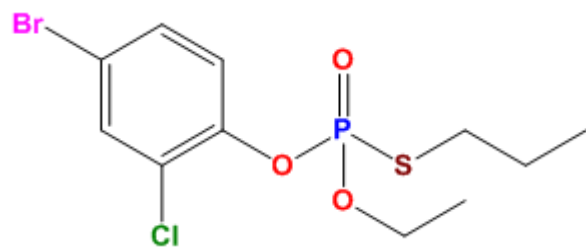


Fig. 1 : Molecular structure of profenofos pesticide.

been found in many vegetables posing a dietary risk to people and aquatic animals. Furthermore, profenofos pesticide residues have been linked to honey bee colony loss and soil microflora destruction (Chatterjee *et al*, 2016). Thus, it is imperative to effectuate effective strategies for removing profenofos residues in a manner that is both economically viable and environmentally friendly. Traditional methods for removing and/or degrading organophosphorus pesticides, such as chemical modification, combustion, and landfills, were later shown to be problematic due to the risk of secondary exposure. Furthermore, such methods are prohibitively expensive. As a result, there is a rising demand for developing a natural solution for the efficient elimination of profenofos from the eco-system (Jatoi *et al*, 1998).

Bioremediation is one of the most potent, secure, and commercially sustainable strategy when compared to other contaminants removal methods. The degradation and conversion of recalcitrant pesticides can be better accomplished through bioremediation by the action of microbial enzymes that can catalyse various reactions such as hydrolysis, redox reactions and ring cleavage etc (Islam *et al*, 2021). To date, there have been very few reports that describe the efficient microbial biodegradation of profenofos pesticide and their complete degradation pathways. Malghani *et al* (2009) have reported the extraction, identification along with the characterization of bacterial strains capable of degrading profenofos pesticide. Salunkhe *et al* (2013) isolated *Bacillus subtilis* from grapevines and investigated the profenofos degrading ability. Jabeen *et al* (2015) have optimized the degradation capacity of bacterial consortium by surface response technique. Siripattanakul-Ratpukdi's research group studied the profenofos biodegradation by bacterial consortium (Siripattanakul-Ratpukdi *et al*, 2015). Jaoti *et al* (2021) examined the potency of rhizobacteria towards the biodegradation of profenofos and analysed the progress of growth of the plants. Recently, Verma and Chatterjee (2021) demonstrated the mechanistic pathway of profenofos biodegradation.

The present investigation aims to identify novel bacterial strain from the pesticide contaminated soil and

to comprehend the profenofos degradation pathway. The strain isolated in this study can be used to bioremediate pesticide contaminated soil in various agricultural fields.

MATERIALS AND METHODS

Sample collection

Technical grade Profenofos (99 % purity) was obtained from the Scientific Fertilizer Co Pvt. Ltd, Pesticide Division, Gundur, Tiruchirappalli, Tamil Nadu, India. Profenofos-degrading microorganisms has been isolated from agricultural soils from Karur district, Tamil Nadu, India. Soil samples were gathered from various sites with ten years of pesticide uses and taken from 10 to 15 cm depth. Soil samples were air dried and stored in plastic bags at 5°C.

Soil properties

The soil samples were analysed in Department of Agriculture and cooperation, Ministry of Agriculture and farmer's welfare, Govt. of India, ICAR, Karur.

Medium for isolation

Mineral salt media MSM (in g / L) (KH_2PO_4 – 3; NaCl – 0.5; Na_2SO_4 – 5.8; NH_4Cl – 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.2) and nutrient broth were purchased from HIMEDIA, India.

Isolation of bacterium by Enrichment Method (Mu *et al*, 2018)

By successive sub-culturing of soil samples, profenofos degrading bacterium was isolated using an enrichment method. 5 g of soil from the agricultural field is mixed with 100 mL soil enrichment MSM medium supplemented with profenofos (25 mg/L) final concentration. For a week, these enriched cultures have been incubated at 28°C with shaking (150 rpm/min). For four cycles, enrichment culture (5 mL) was subcultured into 100 mL fresh enrichment medium comprising profenofos (25 to 100 mg/L). The enriched cultures were then placed in MSM media, which was supplemented with 25 mg/L profenofos as the sole carbon source. Following four cycles of subculture in MSM with increasing concentrations of profenofos (up to 100 mg/L), pure cultures were obtained by performing appropriate serial dilutions of the enrichment culture in MSM medium and plating them onto MSM plates containing 100 mg/L profenofos. The colonies were inoculated into MSM medium containing 100 mg/L of profenofos and pure colony was selected for further investigation. The morphological features of the isolated strain was analysed.

Genomic DNA isolation and sequencing of 16SrRNA gene

The genomic DNA of the isolated bacterial strain

was extracted using the standard chloroform–isoamyl alcohol extraction method. To identify the strain, partial gene sequencing of 16S rRNA was performed and confirmed using biochemical properties. The 16S rRNA gene was amplified by PCR and sequenced as previously described (Wright *et al.*, 2017). BLAST was used to search for sequence similarities in the NCBI Gene Bank. (<http://www.ncbi.nlm.nih.gov>). Biochemical and molecular characterization of the isolates were performed. For PCR amplification of the 16S rRNA gene, the following primers were used: 27 F(5'-AGAGTTTGATCCTGGCTCAG-3'), and 1492R (5'-GGTTACCTTGTTACGACTT-3'). To create the PCR reactions, we used 50 µL of PCR buffer, 10 mol/µL of each primer, 5 U Taq DNA polymerase, and 10 mol/µL BSA, as well as 2 µL of DNA. 94°C denaturation for three minutes, 28 amplification cycles of 20 s each at 94°C, 58°C for 40 seconds at 72 °C, and a final polymerization for three minutes and 30 seconds was the thermocycling conditions. To check the PCR products, we used Gel Doc 2000 to visualize them on 1.0 % agarose gels. The purified PCR products of 16S rRNA gene were sequenced (Agrigenome labs Pvt Ltd, Kochi).

Biodegradation studies

The capability of the isolated strain towards the biodegradation of profenofos was investigated with a mineral salt medium containing 100 mg/L profenofos as a sole source of carbon. Bacterial cells were pre-cultured in nutrient agar medium at 30°C for 8 minutes with 140 rpm in orbital shaker. 2 mL of aliquots were extracted after 24 -48 hrs and analysed gas chromatography-mass spectroscopy (GC-MS) analysis.

Detection of intermediate metabolites by GC-MS analysis

For the detection of intermediate metabolites, GC-MS system (GCMS-QP 2010 Shimadzu) provided with an auto-sampler and a mass spectrometer detector was used. For compound separation, a Phenomenex ZB 5MS column was used. Helium was used as the carrier gas, with a flow rate of 1 mL /min. The temperature system listed below was used: The temperature of the oven was initially held at 60°C for 1 minute before ramping from 10°C per minute to 290°C with a 20-minute hold time; total run time was 45 minutes. The injector's temperature was kept at 250°C. The ion trap was set to 70 eV and had a scan range of 40 to 800 m/z. In split mode, 1 L of each sample was injected (10:1). Metabolite identification was accomplished by comparing the results to standard compound mass fragmentation patterns as well as instrumental library searches (Herrmann *et al.*, 1999).

RESULTS AND DISCUSSION

Physico-chemical characteristics of the soil sample

The physico-chemical characteristics of the collected soil sample is given in Table 1.

Identification of isolated strain

Under the test conditions, a single bacterial isolate PDB 1 (Fig. 2) was found positive for profenofos degradation in soil samples collected from the Karur district of Tamil Nadu, India. PDB1 bacterium grew significantly in MSM (pH 7.0) supplemented with 100 mg/L profenofos at 28°C, indicating that the bacterium was using profenofos as its sole carbon and energy source. Morphological analysis revealed that the strain PDB1 was rod-shaped and a fuzzy-white in colour. The biochemical characteristics of the PDB1 were listed in Table 2.

Molecular characterization of PDB1 strain by 16S rRNA sequence

The bacterium was identified further through molecular characterization using 16S rRNA gene sequencing. PDB1 nucleotide sequences were compared to other 16S rRNA gene sequences in the NCBI database using BLAST analysis. The search exposed that the 16S rRNA gene sequence of the bacterium PDB1 shared a high degree of similarity with *subtilis* species. The bacterium *Bacillus subtilis* strain showed the highest sequence similarity of 99 %. The phylogenetic tree of the bacterium PDB1 is shown in (Fig. 3) (Ankenbrand *et al.*, 2016).

Biodegradation of profenofos

Degradation in liquid culture media : Aliquots 2 mL of the sample were excluded from the 100 mL liquid culture, mixed with 2 mL ethyl acetate, and vigorously

Table 1 : Properties of soil sample.

S. no.	Parameters	Value
1.	Soil type	Red soil
2.	Density	1.47 g/mL
3.	Water holding capacity	40.39 %
4.	pH	7.2
5.	Electrical Conductivity (EC)	0.32
6.	Organic Carbon (OC)	2.30%
7.	Nitrogen (N)	254.2 kg/ha
8.	Potassium (K)	260.1 kg/ha
9.	Phosphorous (P)	20.4 kg/ha
10.	Sulphur (S)	12.1 mg/kg
11.	Zinc (Zn)	0.85 mg/kg
12.	Boron (B)	1.24 mg/kg
13.	Iron (Fe)	30 g/kg
14.	Manganese (Mn)	6.14 mg/kg
15.	Copper (Cu)	1.21 mg/kg



Fig. 2 : Pure colony of PDB1.

shaken for 2 hours to extract the profenofos residues. For 20 minutes, the organic layer was centrifuged at 9000 rpm. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and then evaporated at room temperature with

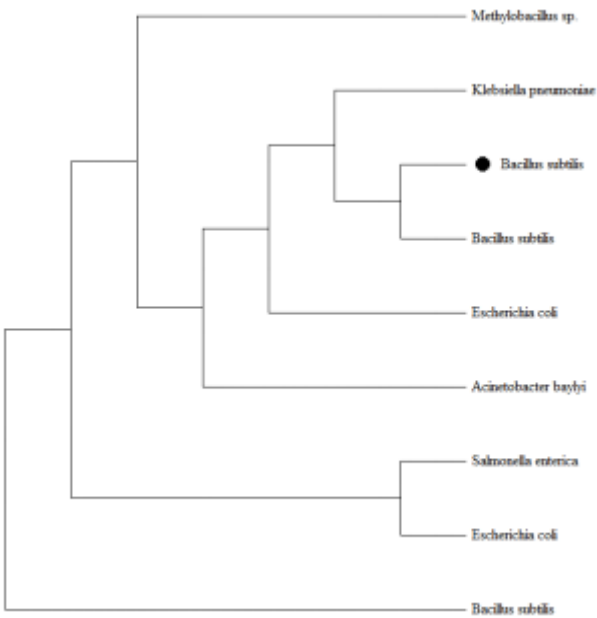


Fig. 3 : The phylogenetic tree of the isolated bacterium PDB1.

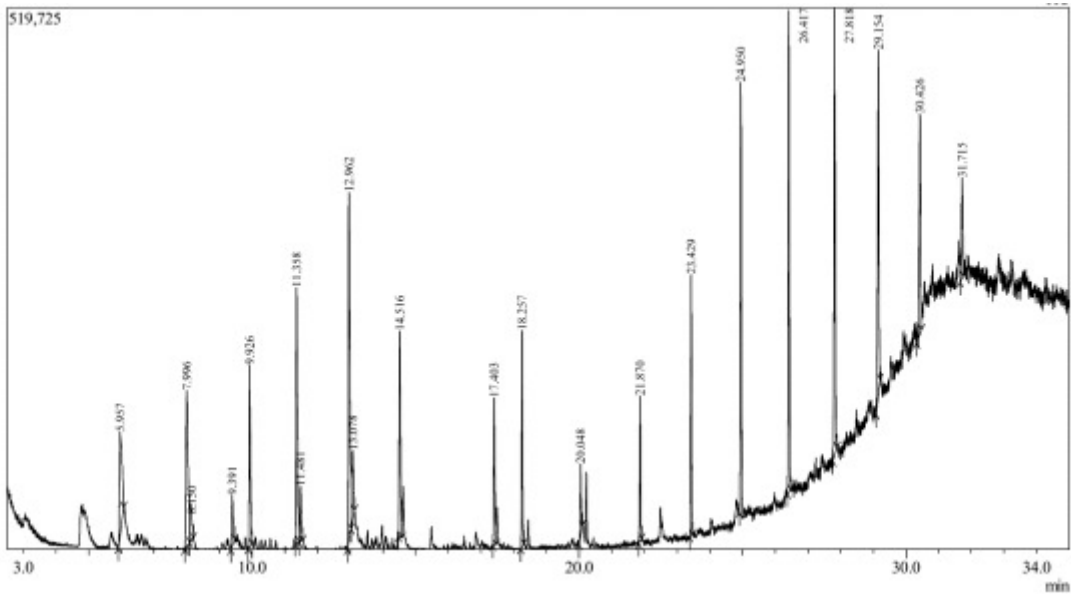


Fig. 4 : GC-MS spectrum showing the degradation of profenofos.

Table 2 : Biochemical test for PDB 1.

S. no	Test	Results
1	Gram Staining	Positive
2	Citrate	Positive
3	Gelatin hydrolysis	Positive
4	Catalase	Positive
5	Indole	Negative
6	Methyl Red	Negative
7	Nitrate Reduction	Positive
8	Pigment production	Negative

The biochemical analysis of the PDB1 exhibited 99 % characteristics of *Bacillus* genus.

a stream of nitrogen. The residues were dissolved in 200 μL of ethyl acetate.

GC-MS analysis of degraded products of profenofos : The GC-MS methodology has been successful identification of profenofos degradation products in the current study. After 24 - 48 hours, sample retrieved in ethyl acetate from bacterium cultures with profenofos pesticide was injected into GC-MS. Fig. 4 shows the GC-MS spectrum showing the degradation of profenofos. The metabolite identified as 4-bromo-2-cholorophenol corresponded to the peak of 5.957 and 6.23 min retention time. The formation of 4-bromo-2-cholorophenol (molecular weight 206 g/mol) demonstrates the breakdown of profenofos at the ester bond linkage of

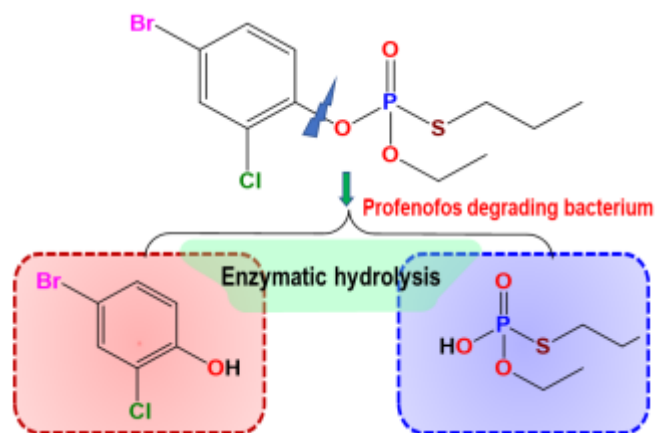


Fig. 5 : Plausible mechanistic representation of profenofos biodegradation.

the parent compound (Palanimanickam *et al*, 2017). Further, the degradation ability of profenofos was further confirmed by the formation of diethyl thiophosphoric acid (peak of 12.962 at 13 min retention time). The results implies that the isolated bacterium contains enzymes with esterase-activity. The plausible mechanistic representation of profenofos biodegradation was portrayed in Fig. 5. Nonetheless, more research is needed to determine the complete degradation pathway and the mineralization of intermediates.

CONCLUSION

Microbial degradation of organophosphorus pesticides and the advancement of bioremediation approaches for contaminated agricultural fields by using potential microorganisms symbolise a rapidly expanding area of research worldwide. In pristine ecosystems and non-exposed agricultural fields, naturally existing profenofos-degrading microorganisms may be scarce. As a result, the enrichment technique for isolating bacterial strains has been widely used in the study of pesticide biodegradation. In the present study, PDB1 strain was isolated from pesticide contaminated agricultural soil. The isolated bacterial strain was identified as *Bacillus subtilis* by various characterization techniques. The PDB1 strain was tested for its ability to degrade the pesticide profenofos. The ability of the PDB1 strain to degrade profenofos pesticide into 4-bromo-2-chlorophenol and diethylthiophosphoric acid metabolites was demonstrated by GC–MS analysis. Microbes mediated degradation of profenofos is a significant step toward the development of new bioremediation strategies for cleaning up the pesticide contaminated sites.

Conflict of the interest

The authors declare no competing financial interest.

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REFERENCES

- Ankenbrand M J and Keller A (2016) bcg Tree: automatized phylogenetic tree building from bacterial core genomes. *Genome* **59**(10), 783-791.
- Diez M C (2010) Biological aspects involved in the degradation of organic pollutants. *J. Soil Sci. Plant Nutr.* **10**(3), 244–267.
- Ghani M U, Asghar H N, Niaz A, Ahmad Zahir Z, Nawaz M F and Häggblom M M (2021) Efficacy of rhizobacteria for degradation of profenofos and improvement in tomato growth. *Int. J. Phytoremediation*, 1-11.
- Herrmann J M, Guillard C, Argüello M, Agüera A, Tejedor A, Piedra L and Fernandez-Alba A (1999) Photocatalytic degradation of pesticide pirimiphos-methyl: Determination of the reaction pathway and identification of intermediate products by various analytical methods. *Catalysis Today* **54**(2-3), 353-367.
- Jabeen H, Iqbal S, Anwar S and Parales R E (2015) Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. *Int. Biodeterioration & Biodegradation* **100**, 89-97.
- Jatoi A S, Hashmi Z, Adriyani R, Yuniarto A, Mazari S A, Akhter F and Mubarak N M (2021) Recent trends and future challenges of pesticide removal techniques—A comprehensive review. *J. Environ. Chem. Engineering* 105571.
- Kushwaha M, Verma S and Chatterjee S (2016) Profenofos, an acetylcholinesterase inhibiting organophosphorus pesticide: A short review of its usage, toxicity and biodegradation. *J. Environ. Quality* **45**(5), 1478-1489.
- Ma C S, Zhang W, Peng Y, Zhao F, Chang X Q, Xing K and Rudolf V H (2021) Climate warming promotes pesticide resistance through expanding overwintering range of a global pest. *Nature Communications* **12**(1), 1-10.
- Mahajan R, Verma S and Chatterjee S (2021) Biodegradation of organophosphorus pesticide profenofos by the bacterium *Bacillus* sp. PF1 and elucidation of initial degradation pathway. *Environ. Tech.* 1-9.
- Malghani S, Chatterjee N, Hu X and Zejiao L (2009) Isolation and characterization of a profenofos degrading bacterium. *J. Environ. Sci.* **21**(11), 1591-1597.
- Milesen B E, Chambers J E, Chen W L, Dettbarn W, Ehrich M, Eldefrawi A T and Wallace K B (1998) Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicological Sciences* **41**(1), 8-20.
- Mu D S, Liang Q Y, Wang X M, Lu D C, Shi M J, Chen G J and Du Z J (2018) Metatranscriptomic and comparative genomic insights into resuscitation mechanisms during enrichment culturing. *Microbiome* **6**(1), 1-15.
- Palanimanickam A and Sepperumal U (2017) Profenofos degradation potential of *Bacillus cereus* and *Aneurinibacillus migulanus* isolated from paddy crop field soil. *J. Pure and Appl. Microbiol.* **11**(1), 221-227.
- Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal A S and Kaushal J (2021) An extensive review on the consequences of chemical pesticides on human health and environment. *J. Cleaner*

- Production* **283**, 124657.
- Salahi Moghadam N, Rezaei R and Kavousi A (2021) Factors affecting farmers' excessive use of application chemical pesticides and analyzing the self-protective behaviour about the use of chemical pesticides (case study: Zanjan province). *Agricultural Extension and Education Research* **13**(4), 95-112.
- Salunkhe V P, Sawant I S, Banerjee K, Rajguru Y R, Wadkar P N, Oulkar D P and Sawant S D (2013) Biodegradation of profenofos by *Bacillus subtilis* isolated from grapevines (*Vitis vinifera*). *J. Agricult. Food Chem.* **61**(30), 7195-7202.
- Sarker A, Nandi R, Kim J E and Islam T (2021) Remediation of chemical pesticides from contaminated sites through potential microorganisms and their functional enzymes: Prospects and challenges. *Environ. Technol. Innovation* **23**, 101777, <https://doi.org/10.1016/j.eti.2021.101777>
- Siripattanakul-Ratpukdi S, Vangnai A S, Sangthean P and Singkibut S (2015) Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. *Environ. Sci. Poll. Res.* **22**(1), 320-328.
- Verma S and Chatterjee S (2021) Biodegradation of profenofos, an acetylcholine esterase inhibitor by a psychrotolerant strain *Rahnella* sp. PFF2 and degradation pathway analysis. *Int. Biodeterioration & Biodegradation* **158**, 105169.
- Willoquet L, Nelson N A, Ficke A and Savary S (2017) Importance of disease and pest losses on key world crops- priorities. *Synthesis and Report* 18-19.
- Wright M H, Adelskov J and Greene A C (2017) Bacterial DNA extraction using individual enzymes and phenol/chloroform separation. *J. Microbiol. Biol. Education* **18**(2), 18-2.

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An environmentally sustainable strategy for the efficient biodegradation of profenofos and plant growth enhancement by indigenous soil bacterium *Bacillus subtilis*

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Abstract

In modern agriculture, the widespread use of organophosphorus pesticides results in environmental pollution and pose severe threats to human, flora and fauna. Hence, the efficient removal of these pesticides from polluted environment remains an enduring inspiration. Bioremediation can be used as a proficient detoxifying strategy for polluted ecosystem since it can breakdown or convert toxic pollutants into less hazardous by-products while also being cost-effective, extremely efficient, and environmental sustainable. Further, a detailed analysis of the plant growth-promoting activity of plant-bacteria relationships could be used to promote sustainable agricultural output as well as the remediation of pesticide-polluted soil and water. Hence, a bacterial strain PDB1 was extracted from the soil sample exposed to pesticide for an extended period of time at Karur district, Tamilnadu, India for the bioremediation. Using morphological, biochemical, and 16S rRNA gene analysis, the bacterium PDB1 was characterized as *Bacillus subtilis*. The impact of *Bacillus subtilis* on several plant - growth indices was studied in the presence and absence of the pesticide profenofos. The capacity of *Bacillus subtilis* to degrade profenofos (PF) was examined using gas chromatography-mass spectroscopy (GC-MS). Since this isolated bacterial strain has (PF) degrading capacity as well as other characteristics that promote plant growth, the isolate could be an attractive opportunity for the advancement of bioremediation technique.

Keywords: bioremediation; 4-bromo-2-chlorophenol; degradation; plant growth parameters; profenofos; soil bacteria

Introduction

Modern agriculture has relied on the constant administration of agrochemicals predominantly pesticides to increase crop productivity and food security [1]. Despite the fact that pesticides play a crucial function in contemporary agriculture, their prolonged use causes severe environmental problems [2]. Continuous pesticide exposure causes immunological issues, immunodeficiency syndromes, and cancer-related problems. The excessive use of pesticides pollutes the soil and water. Furthermore, it has a negative impact on the genetic variation of soil microbiota, accompanying in deteriorated soil fertility and plant development, which together jeopardize the long-term productivity of agricultural field. To complicate the issue further, pesticide residues and their by-products frequently penetrate through the surface soil into groundwater, provoking widespread pollution of aquatic environments [3]. Organophosphates are by far the most frequently used insecticides, accounting for 34% of global pesticides consumption. They are known to inhibit cholinesterase neurotransmitters irreversibly, causing disruption of endocrine activities, nervous system failure, and defects in childbirth, infertility, distortion of growth, development, and reproduction in fauna, birds, and mankind [4]. PF [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl(S)-phosphorothioate)] is a highly active organophosphate insecticide that is commonly used on crops, vegetables and fruits. The molecular structure of PF comprises phenolic and phosphate ester components (Fig. 1). It is one among the most widely used pesticides because of its easy fabrication,

cost-effectiveness and high efficiency. PF has been categorised as moderately dangerous pesticide of toxicity class II by the World Health Organization (WHO), and it has been shown to inhibit acetylcholinesterase. PF is genotoxic to off-target organisms, including terrestrial and aquatic creatures. PF is widely used in agriculture in many countries across the world. It has been widely used to manage and control numerous lepidopterous insects, aphids, whiteflies, spider mites, and hoppers on a variety of crops including tomato, potato, corn, sugarcane, cotton, tobacco, and vegetables. The residual levels of PF have been measured in a variety of plants and food samples. Alarmingly, these organophosphate pesticide residues have been identified in human blood, breast milk, plasma, serum and urine after exposure [5].

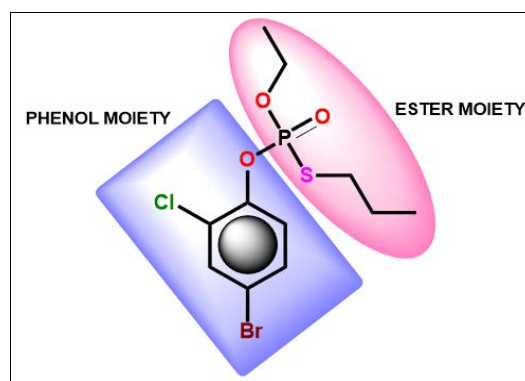


Fig 1: Chemical Structure of Profenofos

To address the ever-increasing demand and over consumption of synthetic pesticides while taking into account their long-term negative impacts on global agro-ecosystems and living organisms, we desperately need to develop, adopt, and promote environmentally sustainable approaches [6]. Traditional methods for removing and/or degrading organophosphorus pesticides, such as chemical treatment, combustion, and landfills, were later shown to be troublesome due to the possibility of secondary exposure. Furthermore, such procedures are prohibitively expensive. As a result, sustainable agriculture is critical in this period since it has the capabilities to fulfil our future agricultural demands [7]. Hence, bio-remediation, bio-mineralization, bio-protection, bio-stimulation have been considered as safe, convenient, environmentally friendly, and economically viable methods for the decontamination of organophosphorus pesticides [8].

Bioremediation is a technique which uses the potential of microbial degradation to provide a cost-effective and dependable way of pesticide diminution. Numerous soil and aqueous atmospheres have effectively recovered by utilising bacteria capable of the degrading the contaminants. PF is removed through hydrolysis, either chemically or through microbial activity, by transforming it to diethylthiophosphoric acid and 4-bromo-2-chlorophenol [5]. Malghani *et al.* described the isolation and characterization of bacterial strains capable of PF decomposition [9]. Salunkhe *et al.* extracted *Bacillus subtilis* from grapevines and examined its ability to degrade PF [10]. Jabeen and colleagues used the surface response technique to analyse the degradation capacity of a bacterial consortium [11]. The research team of Siripattanakul-Ratpukdi explored PF biodegradation by bacterial consortium [12]. Verma and Chatterjee briefly demonstrated a molecular mechanism of PF biodegradation [13].

Plant-assisted bioremediation has a lot of potential for cleaning up of pesticide contaminated soil. Pesticide degrading bacteria may improve plant tolerance to pollutants by detoxifying polluted soils by direct mineralization of contaminants. Furthermore, plant exudates increase the density and efficiency of novel bacteria in the root zone. The ability of microorganisms to detoxify pollutants while also increasing plant development has previously been explored for various organophosphorus insecticides such as malathion, methyl parathion chlorpyrifos and monocrotophos [14]. Despite, the significance of plant-bacteria partnerships towards the remediation of various pollutants has been demonstrated in various studies, research on PF degradation using this strategy is currently limited.

Akbar and Sultan demonstrated that the chlorpyrifos-degrading bacteria *Achromobacter xylosoxidans* and *Ochrobactrum sp.* have the potential to become excellent options for increasing crop productivity in pesticide-contaminated soils [15]. Nivedita and Sundari examined the impact of direct inoculation of specified consortia on plants in the presence of the root disease *Sclerotium rolfsii* and the organophosphate insecticides malathion and methyl parathion [16]. D. M. Dash and J. W. Osborne investigated the bioremediation of monocrotophos by native isolates obtained from cultivable sugarcane soil. The inclusion of *Bacillus aryabhattai* to the rhizosphere of *Liriope muscari* improved plant growth and monocrotophos degradation in soil [17]. Meng Di *et al.* presented the entire genome

sequence of *Bacillus amyloliquefaciens* as well as its capacity to breakdown a variety of organophosphorus insecticides [18]. Fiaz Ahmad's research group portrayed the ability of inoculated external bacteria *Bacillus pumilus* to accelerate the cleanup of chlorpyrifos-contaminated soil and reduce levels of harmful pesticide residues in agricultural crops [19]. Govarthan *et al.* explored the potential for speedy biodegradation of chlorpyrifos and plant growth promoting psychrophilic *Shewanella sp.* cultures were obtained from salt water using the enrichment technique [20]. Ghani *et al.* examined the efficacy of a bacterial strain, *Enterobacter cloacae*, competent of promoting plant growth and biodegrading PF [21]. Jaoti examined the potency of *rhizobacteria* towards the biodegradation of PF and analysed the progress of growth of the plants [22]. Vinay Kumar *et al.* assessed the plant development and PF elimination efficacy of *Acinetobacter* and *Comamonas sp.* bacteria as separate strains and in combination and found that the consortium has greater plant growth-promoting properties than the individual bacterium [23].

Inspired by the facts and results, we attempted to isolate and identify a novel bacterial strain from a pesticide-contaminated soil and succeeded. Further, the metabolites derived from PF degradation was identified by using GC-MS and probable mechanism has been proposed. Furthermore, the isolated bacteria was tested for its ability to promote plant development.

Materials and Methods

1. Sample collection

Technical grade profenofos (99 % purity) was obtained from the Scientific Fertilizer Co Pvt. Ltd, Pesticide Division, Gundur, Tiruchirappalli, Tamil Nadu, India. Profenofos-degrading microorganism has been isolated from agricultural soils from Karur district, Tamil Nadu, India.

2. Medium for isolation

Mineral salt media MSM (in g/ L) (KH_2PO_4 – 3; NaCl – 0.5; Na_2SO_4 – 5.8; NH_4Cl – 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.2) and nutrient broth were purchased from HIMEDIA, India.

3. Isolation of bacterium by Enrichment Method

The bacterial strain was isolated using an enrichment method as reported earlier [24]. 5 g of soil from the agricultural field is mixed with 100 mL soil enrichment MSM medium supplemented with PF (25 mg/L) concentration. For a week, these enriched cultures have been incubated at 28 °C with shaking (150 rpm/min). For four cycles, enrichment culture (5 mL) was subcultured into 100 mL fresh enrichment medium comprising PF (25 to 100 mg/L). Pure cultures were developed by proper serial dilutions of the enrichment culture in MSM medium and plating them onto MSM plates comprising 100 mg/L PF. The colonies were inoculated into MSM medium containing 100 mg/ L of PF and pure colony was selected for further investigation. The morphological features of the isolated strain were analysed.

4. Genomic DNA isolation and sequencing of 16SrRNA gene

The genomic DNA of the isolated bacterial strain was extracted using the standard chloroform-isoamyl alcohol extraction method as per our previous report [24]. To identify the strain, partial gene sequencing of 16S rRNA was performed and confirmed using biochemical properties. The

16S rRNA gene was amplified by PCR and sequenced as previously described. BLAST was used to search for sequence similarities in the NCBI Gene Bank. (<http://www.ncbi.nlm.nih.gov>). Biochemical and molecular characterization of the isolates were performed. For PCR amplification of the 16S rRNA gene, the following primers were used: 27 F(5'- AGAGTTTGATCCTGGCTCAG-3'), and 1492R (5' GGTTACCTTGTTACGACTT-3'). The purified PCR products of 16S rRNA gene were sequenced (Agrigenome labs Pvt Ltd, Kochi).

5. Biodegradation studies

5.1. Plant growth enhancement in *Vigna mungo* by pot culture method

Pot experiments with *Vigna mungo* (L.) Hepper were performed in order to analyse the effects of bacterial inoculation on plant growth parameters and pesticide degradation. Seeds of *V. mungo* were surface sterilized by treatment with 0.1% HgCl₂ solution for 5 min followed by washing with sterilized distilled water. Soil samples (1.0 kg) were spiked with PF to a concentration of 100 mg /kg. Samples were then inoculated with microbial suspension to give final concentration of 1.6×10^7 cells /g. The test was performed in triplicate and sterilized soil sample was used as control. Sterilized seeds of *V.mungo* were sown in the sample soils and then the soil was moistened with water. Three experimental sets of pots with at least six seedlings were grown under the following conditions: (A) normal growth condition: Sterilized soil as control without bacteria and PF; (B) Under pesticide load: Sterilized soil with PF and without bacteria, (C) Under pesticide with isolated strain: Sterilized soil with isolated strain and profenofos. The pots were carefully monitored to ensure that they have been maintained at ambient environmental conditions. The seed germination process was monitored on a regular basis, and plants were allowed to develop for 3 weeks. The following parameters of plant development were documented: % germination, shoot length (cm), root length (cm), leaf length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g) and root dry weight.

5.2. PF degradation analysis by GC-MS

PF degradation was determined after 3 weeks. For the GC-MS analysis, 5 g soil sample was collected from the pots B and C. 2 mL of aliquots were extracted after 3 weeks and analysed gas chromatography-mass spectroscopy (GC-MS) analysis. For the detection of intermediate metabolites, GC-MS system (GCMS-QP 2010 Shimadzu) provided with an auto-sampler and a mass spectrometer detector was used. For compound separation, a Phenomenex ZB 5MS column was used. Helium was used as the carrier gas, with a flow rate of 1 mL /min. The temperature system listed below was used: The temperature of the oven was initially held at 60 °C for 1 minute before ramping from 10 °C per minute to 290 °C with a 20 min hold time; total run time was 45 minutes. The injector's temperature was kept at 250 °C. The ion trap was set to 70 eV and had a scan range of 40 to 800 m/z. In split mode, 1 L of each sample was injected (10:1).

Metabolite identification was accomplished by comparing the results to standard compound mass fragmentation patterns as well as instrumental library searches ^[24].

5.3. Statistical analysis

All the tests were conducted in triplicates and statistical analysis was performed to analyze significant differences. Statistical analysis was performed using a one-way analysis of variance (ANOVA) for % germination, shoot length (cm), root length (cm), leaf length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g) and root dry weight with multiple comparisons. Statistical analysis for plant growth parameters was performed with one-way ANOVA where significant differences at $p \leq 0.05$ levels with multiple comparisons.

Results and Discussion

1. Isolation, identification and characterization of PDB1

By successive sub-culturing of soil samples, PDB 1 was isolated using an enrichment method. A single bacterial strain PDB 1 was isolated from the soil samples from Karur district of Tamil Nadu, India under prolonged exposure of pesticide applications. The morphological analysis, biochemical characteristics and 16S rRNA gene sequencing analysis of the bacterium PDB1 confirmed a high degree of similarity with *Bacillus subtilis* strain ^[24].

2. Growth experiment of *Vigna mungo* (L.) Hepper

Plant growth studies were used to investigate the effect of bacterial activity on plant growth and PF pesticide breakdown. In pot soil experiments, % germination, shoot length (cm), root length (cm), leaf length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g) and root dry weight were determined. The results are given in Table 1 and Fig. 2. PF addition to soil (B) affected a reduction in all parameters analysed as compared to the plants grown without PF exposure (A). However, when compared to pot B, the plants in pot C exhibited comparatively higher plant growth characteristics. This may be due to the presence of *Bacillus subtilis* which utilised the PF pesticide as only carbon source thus causing the degradation of PF.

Table 1: Measurement of growth parameters of *V. mungo* in pots A, B and C

Growth parameters	Control	PF + <i>V. mungo</i>	PFF + <i>V. mungo</i> + PDB1
% germination	90.33 ± 0.88 ^a	66.33±0.66 ^a	75.00±1.15 ^a
Shoot length (cm)	4.64±0.15 ^c	3.13±0.03 ^c	4.16±0.13 ^c
Root length (cm)	7.43±0.37 ^b	5.03±0.21 ^b	6.06±0.16 ^b
Leaf length (cm)	3.63±0.12 ^c	2.5±0.17 ^c	3.11±0.10 ^c
Shoot fresh weight (g)	1.83±0.03 ^d	1.13±0.04 ^d	1.50±0.04 ^d
Root fresh weight (g)	0.80±0.03 ^{de}	0.56±0.01 ^{de}	0.63±0.02 ^e
Shoot dry weight (g)	0.62±0.02 ^e	0.25±0.01 ^e	0.57±0.01 ^e
Root dry weight (g)	0.25±0.01 ^e	0.06±0.00 ^e	0.12±0.02 ^e

The values indicate the mean ± SD of three replicates.

Different letters in same rows indicate significantly different values.

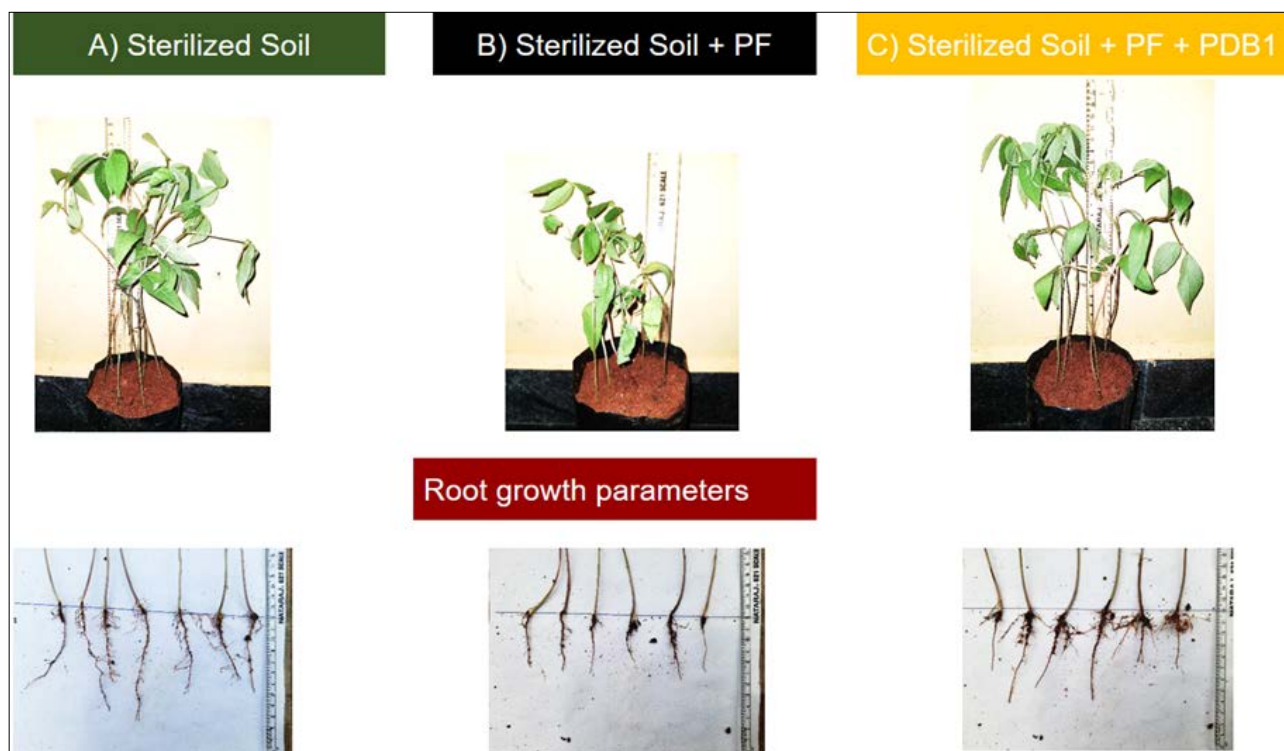


Fig 2: Growth experiment with *V. mungo* in soil supplemented with PF (100 mg /kg).

3. Biodegradation of profenofos

The ability of *Bacillus subtilis* to degrade PF in soil environment was studied using GC-MS. The GC-MS was performed for sterilized soil with PF (B) along with sterilized soil with PF and PDB1 (C). The results of GC-MS are given in Fig. 3 and Fig.4. Fig. 3 displayed a single peak of 20.005 at 19.6 min retention time. The peak is attributed to PF pesticide. Fig. 4 exhibits the GC-MS result of pot C which contain sterilized soil with PF and PDB1. In contrast to Fig.3, The spectrum showed multiple peaks due to the

biodegradation of PF. Specifically, the emergence of two peaks corresponding to the metabolites of PF namely, 4-bromo-2-chlorophenol (peak of 5.978 at 6.3 min retention time) and diethyl thiophosphoric acid (peak of 13.067 at 13.2 min retention time) confirmed the *Bacillus subtilis* mediated biodegradation of PF [5].

Figure 5 depicts a probable mechanistic model of PF biodegradation. Nonetheless, more studies are needed to determine the entire breakdown route and intermediate mineralization.

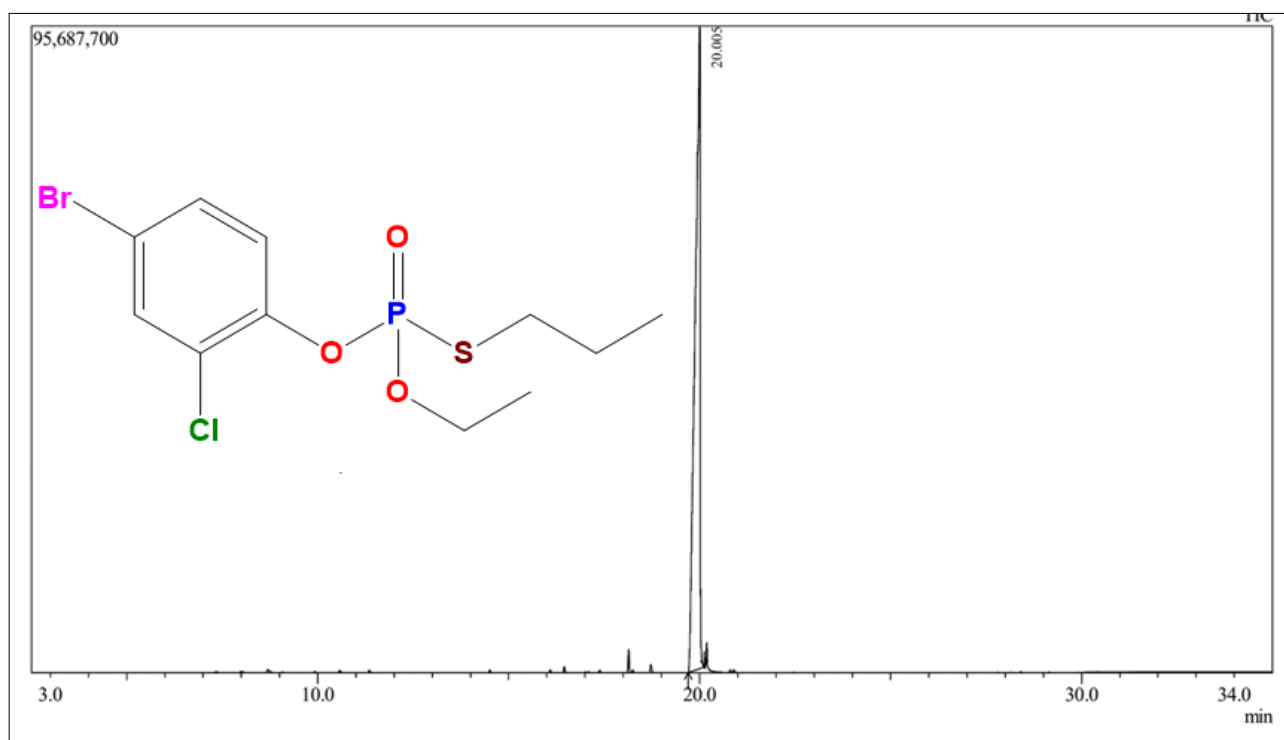


Fig 3: GC-MS result of sterilized soil with PF

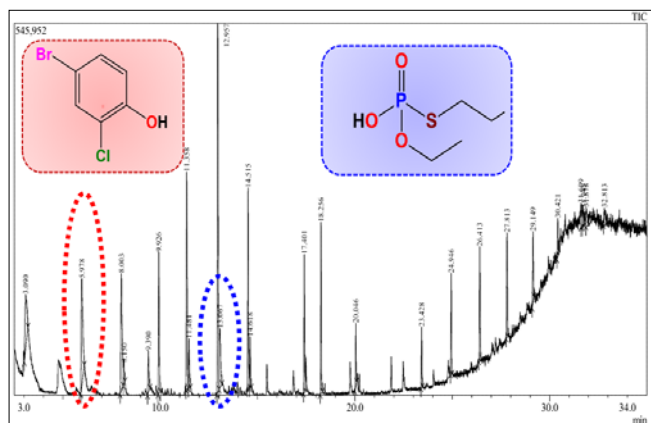


Fig 4: GC-MS result of sterilized soil with PF and PDB1

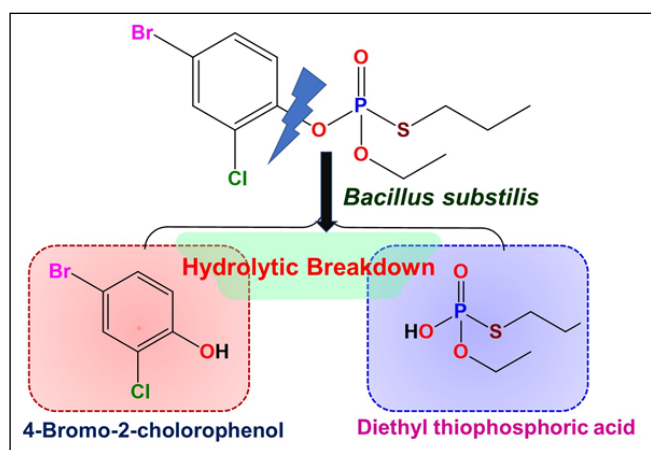


Fig 5: Plausible mechanistic representation of profenofos biodegradation

Conclusion

Plant-bacteria relationships can be used to improve pesticide-contaminated soil remediation. A bacterial strain was isolated using the enrichment methodology for the efficient remediation of PF-contaminated soil. *Bacillus subtilis* was identified using morphological, biochemical, and 16S rRNA gene studies on the bacterium PDB1. *Bacillus subtilis* was tested in the presence and absence of the pesticide profenofos on many plant, *V. mungo* growth variables, including percent germination, shoot length, root length, leaf length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. According to the tested criteria, the pot soil reinforced with profenofos and *Bacillus subtilis* exhibited comparatively better growth of *V. mungo* than the pot soil containing simply profenofos. The hydrolytic breakdown of PF into 4-bromo-2-chlorophenol and diethylthiophosphoric acid metabolites was demonstrated in mechanistic studies of PF degradation using GC-MS. This isolate, *Bacillus subtilis* has the potential to be a key contender for the advancement of bioremediation approach due to its high biodegradation and plant growth stimulating potential.

Conflict of the interest

The authors declare no competing financial interest.

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References

1. Van Zelm R, Larrey-Lassalle P, Roux P. Bridging the gap between life cycle inventory and impact assessment for toxicological assessments of pesticides used in crop production. *Chemosphere*,2014;100:175-181.
2. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang MQ. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*,2021;9(3):42.
3. Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, Kaushal J. An extensive review on the consequences of chemical pesticides on human health and environment. *J. Clean. Prod.*,2021;283:124657.
4. Milesen BE, Chambers JE, Chen, WL, Dettbarn W, Ehrich M, Eldefrawi AT, Wallace KB. Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicol. Sci.*,1998;41(1):8-20.
5. Kushwaha M, Verma S, Chatterjee S. Profenofos, an Acetylcholinesterase-Inhibiting Organophosphorus Pesticide: A Short Review of Its Usage, Toxicity, and Biodegradation. *J. Environ. Qual.*,2016;45(5):1478-1489.
6. Sun S, Sidhu V, Rong Y, Zheng Y. Pesticide pollution in agricultural soils and sustainable remediation methods: a review. *Curr. Pollut. Rep.*,2018;4(3):240-250.
7. Jatoti AS, Hashmi Z, Adriyani R, Yuniarto A, Mazari SA, Akhter F, Mubarak NM. Recent trends and future challenges of pesticide removal techniques—A comprehensive review. *J. Environ. Chem. Eng.*,2021;9(4):105571.
8. Khatoun Z, Huang S, Rafique M, Fakhar A, Kamran M A, Santoyo G. Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems,2020;273: 111118.
9. Malghani S, Chatterjee N, Hu X, Zejiao L. Isolation and characterization of a profenofos degrading bacterium. *J. Environ. Sci.*,2009;21(11):1591-1597.
10. Salunkhe VP, Sawant IS, Banerjee K, Rajguru YR, Wadkar PN, Oulkar DP, Sawant SD. Biodegradation of profenofos by *Bacillus subtilis* isolated from grapevines (*Vitis vinifera*). *J. Agric. Food Chem.*,2013;61(30):7195-7202.
11. Jabeen H, Iqbal S, Anwar S, Perales RE. Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. *Int. Biodeterior. Biodegradation*,2015;100:89-97.
12. Siripattanukul-Ratpukdi S, Vangnai AS, Sangthean P, Singkibut S. Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. *Environ. Sci. Pollut. Res.*,2015;22(1):320-328.
13. Verma S, Chatterjee S. Biodegradation of profenofos, an acetylcholine esterase inhibitor by a psychrotolerant strain *Rahnella* sp. PFF2 and degradation pathway analysis. *Int. Biodeterior. Biodegradation*,2021;158:105169.
14. Pandotra P, Raina M, Salgotra RK, Ali S, Mir ZA, Bhat JA, Upadhyay D. Plant-bacterial partnership: a major pollutants remediation approach. *Modern Age Environmental Problems and their Remediation*, 2018, 169-200.

15. Akbar S, Sultan S. Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. *Braz. J. Microbiol.*,2016;47:563-570.
16. Nivedita M, Sundari KS. Native PGPM Consortium: A Beneficial Solution to Support Plant Growth in the Presence of Phytopathogens and Residual Organophosphate Pesticides. *J. Bioprocess. Biotech.*,2015;5(2):1000202.
17. Dash DM, Osborne JW. Biodegradation of monocrotophos by a plant growth promoting *Bacillus aryabhattai* (VITNNDJ5) strain in artificially contaminated soil. *Int. J. Environ. Sci. Technol.*,2020;17(3):1475-1490.
18. Di MENG, Zhai LX, Tian QP, Guan ZB, Cai YJ, Liao XR. Complete genome sequence of *Bacillus amyloliquefaciens* YP6, a plant growth rhizobacterium efficiently degrading a wide range of organophosphorus pesticides. *J. Integr. Agric.*,2019;18(11):2668-2672.
19. Ahmad F, Iqbal S, Anwar S, Afzal M, Islam E, Mustafa T, Khan QM. Enhanced remediation of chlorpyrifos from soil using ryegrass (*Lolium multiflorum*) and chlorpyrifos-degrading bacterium *Bacillus pumilus* C2A1. *J. Hazard. Mater.*,2012;237:110-115.
20. Govarthan M, Ameen F, Kamala-Kannan S, Selvakumar T, Almansob A, Alwakeel SS, Kim W. Rapid biodegradation of chlorpyrifos by plant growth-promoting psychrophilic *Shewanella* sp. BT05: An eco-friendly approach to clean up pesticide-contaminated environment. *Chemosphere*,2020;247:125948.
21. Ghani, MU, Asghar HN, Niaz A, Ahmad Zahir Z, Nawaz MF, Häggblom MM. Efficacy of rhizobacteria for degradation of profenofos and improvement in tomato growth. *Int. J. Phytoremediation*,2021:1-11.
22. Jatoi AS, Hashmi Z, Adriyani R, Yuniarto A, Mazari S A, Akhter F, Mubarak NM. Recent trends and future challenges of pesticide removal techniques—A comprehensive review. *J. Environ. Chem. Eng.*,2021: 9(4):105571.
23. Kumar V, Sharma N, Maitra SS, Lakkaboyana SK. *In vivo* removal of profenofos in agricultural soil and plant growth promoting activity on *Vigna radiata* by efficient bacterial formulation. *Int. J. Phytoremediation*,2020;22(6):585-593.
24. Arjunan A, Muthuselvam M, Isolation and identification of profenofos degrading bacterium for efficient bioremediation of pesticide contaminated agricultural soil. *Biochem. Cell. Arch*,2021;21(2):5415.