Proceedings of the Pakistan Academy of Sciences: B
Life and Environmental Sciences 62(4): 315-334 (2025)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(62-4)1122

Pakistan Academy of Sciences

Research Article

Treatment of Malathion by using Plant-Bacteria Consortia in Constructed Wetlands

Vijiha Nasir[1], Rija Khalid[1*], Asma Jamil[1], and Sajida Rasheed[2]

[1]Department of Earth and Environmental Sciences, Bahria School of Engineering and Applied Sciences (BSEAS), Bahria University, H-11 Campus, Islamabad, Pakistan

[2]Department of Biotechnology, University of Kotli Azad Jammu and Kashmir, Kotli, Pakistan

Abstract: Malathion, a widely used organophosphate pesticide, poses serious environmental and health risks due to its persistence and toxicity. This study investigates the bioremediation potential of bacterial consortia and plant-bacterial systems in constructed wetland settings for the degradation of malathion-contaminated soil at varying concentrations (50, 100, and 200 mg/L). The four consortia (C1-C4) were constructed from three purified soil isolates and mixed in equal proportions and two plant species (*Canna indica* and *Mentha arvensis*) were tested individually and in combination over an eight-week period. All isolates were characterized by Gram staining and basic biochemical tests and identified as Gram-positive, catalase-negative *Bacillus* spp.; species-level molecular identification was not performed. Colorimetric analysis revealed that all bacterial treatments (bacteria + soil) achieved high removal efficiencies, showing degradation rates between 99.2% and 99.78% at 50mg/L and 100mg/L, reaching up to 99.99% at 200 mg/L in seventh week. Plant-based treatments also exhibited robust degradation, achieving up to 99.8% efficiency by the first week and reaching 100% in the third week at higher concentrations. Efficiency was generally higher at greater malathion concentrations, suggesting possible enzyme induction or microbial adaptation. Soil parameter analysis confirmed active microbial and plant-based remediation, with shifts in pH, organic matter, nitrate, sodium, and potassium supporting degradation processes. While bacterial consortia acted more rapidly, plant systems contributed significantly to sustained removal. Two-way ANOVA confirmed significant effects of time and pesticide dose on degradation efficiency across all treatments. Overall, all treatments achieved > 99% malathion degradation, with bacterial and plant-bacterial consortia showing promise as effective, low-cost, and environmentally friendly strategies for remediating pesticide-contaminated soils.

Keywords: Biodegradation, Organophosphate Pesticide, Colorimetric Analysis, Constructed Wetlands, Plant Microbes, Rhizosphere Interaction, Environmental Sustainability.

1. INTRODUCTION

According to Al-Saeed *et al.*[1] one of the most used pesticides, malathion (MLT) poses multiple hazards to humans and animals. The wide use of malathion, an organophosphate insecticide, as both a tool of agriculture and a chemical weapon in urban areas poses a great environmental challenge due to this insecticide's persistence and associated health risks. Many research works have been carried out to meet the need to address malathion contamination, and through it, much attention is paid to bioremediation strategies in constructed wetlands. A major investigation was carried out by Uniyal *et al.* [2] investigating the biodegradation of malathion in constructed wetlands by indigenous bacterial plant associations. As one of the dominant organophosphate insecticides embedded in agricultural and urban settings, it requires the rigorous analysis of effective remediation methods**.** The U.S. Environmental Protection Agency (U.S. EPA) [3] highlighted the effectiveness of malathion as a pest control agent. Malathion is one of the most widely used organophosphate insecticides both in agriculture and public health, especially in Mosquito control operations for crop protection and vector born disease management. Although, U.S. Geological Survey (USGS) [4] reported an alarming

Received: July 2025; Revised: November 2025; Accepted: December 2025
* Corresponding Author: Rija Khalid <rijakhalid@greenthinktanks.com>

information: malathion and its metabolites occurs in over 80% of the tested streams in more than 30 states during year of 1992-2001, highlighting an uninviting presence within aquatic environment. This widespread identification, despite close label compliance, demonstrates the environmental mobility and persistence of malathion.

Malathion is bioactivated to malaoxon, an oxon derivative which is more toxic than the parent compound, and a stronger inhibitor of acetylcholinesterase; hence, its toxicity is greater. A study of toxicity on zebrafish by Cui *et al.* [5] showed that malaoxon is about 32 times more toxic than malathion, indicating the increased danger associated with its formation. Hydrolysis of malathion yields malathion monocarboxylic acid (MCA) and malathion dicarboxylic acid (DCA). These are metabolites that are less toxic and participate in the mammalian detoxification process. Urinary analyses in human studies showed more malathion monocarboxylic acid than dicarboxylic acid, suggesting efficient excretion of these metabolites. More DCA and dimethylthiophosphate (DMTP) were found in zebrafish, indicating that the carboxylesterase pathway of hydrolysis is the major metabolic pathway [5]. The human body efficiently eliminates malathion, primarily through urinary excretion of its metabolites. Malathion monocarboxylic acids have been found to be the predominant urinary metabolites post ingestion because the body is able to detoxify and eliminate the compound within 12-24 hours. Environmental factors such as temperature and pH alter degradation pathways (ester hydrolysis and elimination) according to computational studies by Lamb *et al.* [6]. According to Vaishali *et al.* [7] Moreover, microorganisms, such as *Pseudomonas stutzeri* bacteria, also play a role in malathion's environmental breakdown through microbial degradation resulting in monocarboxylic and dicarboxylic acid derivatives.

The detoxification of malathion is thus carried out by diverse methods which include chemical treatment, photodecomposition, volatilization and incineration. Unfortunately, they are inefficient, costly, and environmentally unfriendly, so their application for complete removal of contaminants from solutions at low concentration is not viable. Bioremediation methodologies, mainly microbial and Phyto degradation have been adopted in recent years for pesticide removal. Bacterial genera such as *Bacillus* [8], *Pseudomonas* [7], *Flavobacterium* [9], *Sphingomonas* [10], and *Agrobacterium* [11] have shown efficacy towards malathion biodegradation.

Malathion exposure poses critical considerations in genotoxic and carcinogenic hazards. Acetylcholinesterase inhibition activity and its subsequent interference with the transmission of nerve impulse, accumulation of acetylcholine at synaptic junctions, and ultimately induction of its associated adverse health effects such as headache, dizziness, nausea, vomiting, bradycardia, and miosis have been associated with toxicity. According to Olakkaran *et al.* [12] Malathion toxicity in humans has been reported as oxidative stress. In vitro studies in human cell cultures and animal cells exposed to malathion demonstrated DNA damage and chromosomal alterations. In vivo experimental studies by Bastos *et al.* [13] have shown sufficient evidence regarding the potential of pesticides both in inducing genetic damage and inducing neoplasms in mammals. Epidemiological studies have shown statistically significant positive associations for thyroid, breast, and ovarian cancer in menopausal women. Malathion has been commonly used in the world in arbovirus control programs. In 2015, the International Agency for Research on Cancer (IARC) classified it as a probable carcinogen to humans [13].

Petsas and Vagi [14] conducted a study in which indigenous soil bacteria, like *Pseudomonas* sp., were used to degrade malathion. This indicates how these bacteria could provide a viable bioremediation contribution to wetland systems. Specific bacterial strains with the ability to degrade malathion provide a basis for developing plant-bacterial consortium for higher removal. The aim was to isolate and characterize malathion degrading bacteria from agricultural soil. They had identified *Pseudomonas* sp. through their experiments as a potential candidate for the degradation of malathion.

Further studies confirming the potential of plant-bacterial associations to enhance malathion degradation, are drawn from foundational work [2]. Additionally, the study by Cedillo-Herrera *et al.* [15] also further supports the role of wetland plants as hosts for malathion degrading bacteria as pointed out by Uniyal *et al.* [2]. In their work with microbial

consortium enriched from activated sludge, they show that microbial communities in wetlands can be used to promote increased malathion removal.

In the study conducted by Dar and Kaushik [16] bioremediation potential of pure bacterial strains and their consortia isolated form agricultural soil for degradation of the organophosphate pesticide malathion was evaluated. Individual strains degraded 50.16 - 68.47% malathion in 15 days, but complete degradation was observed in a mixed bacterial consortium of *Micrococcus aloeverae*, *Bacillus cereus* and *Bacillus paramycoides*. The degradation rates of partial consortia showed lower values (70.95 - 88.61%). Several intermediate metabolites, namely malaoxon, malathion monocarboxylic acid, diethyl fumarate, and trimethyl thiophosphate accumulated and disappeared successively during bioremediation process.

Study by Geed *et al.* [17] used the response surface methodology (RSM) to optimize the biodegradation parameters for malathion. They investigated malathion removal efficiency vs. pH and hydraulic retention time (HRT) in a batch and continuous flow system. However, their findings illustrated that under optimal conditions, the biodegradation process was greatly improved and were thus offered as a means for improving treatment systems where environments are contaminated with malathion. Isolation of bacterial strains capable of mineralizing malathion from agricultural soil revealed complete mineralization of malathion with butanedioic acid as the major metabolite. According to Jimenez-Torres *et al.* [18] the presence of non-oxidative degradation pathway is further supported by the absence of harmful intermediate metabolites. The use of such bacterial strains in wetlands may promote the removal of malathion and may open the possibility of using plant-bacterial consortia in bioremediation.

Although pesticide use in Pakistan is known to be heaviest on cotton—accounting for more than half of national consumption—other major crops such as rice, vegetables, fruits, sugarcane, and various horticultural crops are also treated with insecticides, including Malathion. However, no recent nationwide database provides crop-wise Malathion application patterns, and available information is limited to scattered residue studies and supplier recommendations reporting its presence in rice, pulses, vegetables, and mango. This lack of localized, site-specific data indicates a large disparity in knowledge and emphasizes the need to study Malathion degradation under Pakistan-specific conditions. The convincing results of the previous work suggested that plant-bacterial consortia hold great bioremediation potential for organophosphate pesticides in wetland settings. Wetlands harbor dynamic microbial communities and plant-microbe interactions, increasing the degradation pathways. Accordingly, the present study aims to enhance the performance of Malathion degradation utilizing wetland plants and plant-bacterial consortia in conjunction with monitoring efficiencies concurrently. Continued development of optimized bioremediation strategies and effective degraders can establish constructed wetlands as a sustainable solution for mitigating Malathion contamination in local ecosystems.

2. METHODOLOGY

2.1. Study Area and Sample Collection

This study evaluates the effects of plant-bacterial consortia for the removal of malathion from contaminated soil and water under controlled laboratory conditions. Soil samples were collected from agricultural land in Islamabad, Pakistan. Samples were taken at a depth of 0 - 15 cm using a sterile soil auger. Soil was collected, stored airtight and transported to the laboratory and then stored at 4 °C to prevent microbial degradation before analysis. To prepare a uniform soil matrix, large debris, plant matter and rocks were first removed with 2 mm sieve. After mixing the soil to make it homogeneous, physicochemical analyses and bioremediation experiments are conducted.

In this research, the bioremediation potential of plant associated bacterial consortia to remove malathion was explored, a method of colorimetric quantification was employed. In this approach, we combined the advantages of plant-bacterial interactions and analytical capability to meet the challenge of sustainable pesticide remediation. Early reports exposed bioremediation as a greener solution to pesticide pollution. Knowing plant associated bacteria and their ability to degrade different types of pollutants, we base our work on this knowledge. The overall aim was to test the reserve of plant bacterial consortia to disintegrate

malathion by using a colorimetric method [19]. The symbiotic relations between plants and bacteria were hypothesized to enhance Malathion removal rate and colorimetric approach was suggested for remediation monitoring.

2.1.1. Selection of plant-bacterial consortia

For development of a cost-effective bioremediation strategy for malathion degradation, the plant species which host pesticide degrading bacteria in their rhizosphere were identified carefully. This was primarily selected from a review of existing literature and past studies which indicate that certain plants associated with microbial communities could degrade organophosphate pesticides, like malathion [16].

After shortlisting the potential plant species, bacterial strains capable of proven pesticide degradation were isolated from its rhizosphere [20]. To accomplish this task soil from the root zone of these plants was collected, cultured, and screened to find many of the bacterial populations of these plants that can degrade malathion. The bacterial isolates were analyzed by microbiological and molecular techniques used to confirm their identity and degradation efficacy. The most effective strains for further experimentation were identified through analysis of key enzymatic pathways that degrade malathion.

Four bacterial consortia (C1 - C4) were prepared from the isolates obtained from malathion-contaminated soil. All isolates were characterized using Gram staining, oxidase and catalase tests, and were identified as Gram-positive, catalase-negative *Bacillus* spp. Although species-level molecular identification was not performed, isolates were grouped based on their biochemical profiles and malathion-degrading ability. The consortia were formulated by mixing the isolates in equal proportions: C1 (Isolate 1 + Isolate 2), C2 (Isolate 1 + Isolate 3), C3 (Isolate 2 + Isolate 3), and C4 (Isolate 1 + Isolate 2 + Isolate 3). These consortia were used for all subsequent biodegradation experiments.

2.1.2. Experimental design

To evaluate the efficiency of biodegradation of plant bacteria consortia, experimental design setup involved setting up controlled environments with different malathion concentrations [16]. The plant species associated with known degrading bacteria were selected for isolating some pesticide degrading bacteria from their rhizosphere and they were introduced into the plant rhizosphere in the experimental setups. The rate of malathion degradation over time was determined through colorimetric assays. This research opted for the colorimetric method as it is simple and low-cost, as well as effective in checking how malathion degrades in constructed wetlands. The color change that takes place during a chemical reaction with certain reagents helps quickly and accurately determine the concentration of malathion. As colorimetry does not depend on any of these expensive analysis tools but is easily performed, it is a convenient method for treating and comparing the samples from different laboratory experiments. Moreover, the data collected was obtained from credible sources and compatible with statistical analysis of assessing the effectiveness of bioremediation options. The colorimetric technique adopted a procedure similar to that suggested in the previous study by Sharma *et al*. [21], based on the variation of color produced by malathion degradation, analyzed using spectrophotometry. Differences in degradation between treatments were tested with ANOVA, and bacterial population dynamics and colorimetric data were related to determine the influence of plant-bacterial consortia on malathion removal efficiency.

2.2. Experimental Procedure

The soil samples were collected from malathion sprayed soil in the screening and isolation of malathion degrading bacteria [20]. Soil samples are spread on nutrient agar media using the spread plate method and the streak plate method is used to select and purify morphologically distinguishable colonies.

2.2.1. Isolation of bacteria

Soil samples where malathion was already introduced were used to isolate the bacteria for bioremediation [22]. Soil samples from malathion-treated sites were air-dried, sieved (2 mm) and 1 g of each sample was suspended in 9 ml sterile saline, followed by serial ten-fold dilutions up to 10^{-6}. Aliquots (100 µL) from appropriate dilutions

were spread on nutrient agar plates and incubated at 37 °C for 24 - 48 h. Distinct colonies were picked based on morphology, purified by repeated streaking, and maintained on nutrient agar slants. Representative isolates were stored as glycerol stocks at 4 °C for further characterization and used to prepare consortia.

2.2.2. Identification of bacteria

1.3 g of Nutrient broth was added to 100 milliliters of distilled water to enrich Bacterial culture. The solution was then sterilized by autoclaving at 121 °C for 15 mins [23]. Then, 10 ml of the nutrient broth was poured into a test tube, and a bacterial culture was added with a micropipette after autoclaving. To allow bacterial growth, the test tube was incubated at 37 °C for 48 hours. A total of three distinct bacterial isolates were purified from malathion-treated soil and used for consortium development.

2.2.2.1. Bacterial enrichment:

Nutrient broth was prepared by dissolving 13 g of the nutrient powder in one liter of distilled water. For a 100 ml solution, the amount was calculated as (13/1000) × 100 = 1.3 grams. This correctly weighed quantity was dissolved in 100 ml distilled water to obtain the culture medium for growth of bacteria. The nutrient broth was sterilized at 121 °C for 15 min by autoclaving [23]. Water boils at 100 °C and when the temperature rises to 121 °C, steam is formed which provides wet sterilization in autoclave. The autoclave was not immediately opened after the completion of 15 min sterilization run. The sample was cooled to a temperature of less than 72 °C and then opened [24].

The laminar flow hood was disinfected with spirit after being autoclaved for the sterility of working areas. The blower was turned on for clean airflow generation. Then, 10 ml of the sterilized nutrient broth was transferred into a test tube. Using a micropipette, 5 ml of the nutrient broth was taken, and the bacterial culture was added to the medium (Figure 1). The test tube was then incubated under controlled conditions at 37 °C for 48 hours to allow bacterial growth, facilitating enrichment of the bacterial culture. The bacterial characterization was done through gram staining according to standard protocols [25].

2.2.3. Constructed wetland

A constructed wetland [26] was established using pots filled with coarse and fine gravel, coarse gravel (20-30 mm diameter), fine gravel (2-10 mm diameter), sand, and soil from a specific site as shown in Figure 2. The local plants *Canna indica* and *Mentha arvensis* were selected. A total of 12 constructed wetland arrangements were maintained under different conditions: control, with isolated bacterial strains, soil alone, with plant and soil alone, and with a bacterial-plant consortium. The constructed wetland units were maintained in batch mode, and malathion-spiked soil/water remained in each system until the next sampling interval. Thus, the effective retention time was 14 days between consecutive samplings, consistent with common practice in small-scale wetland studies [16, 17]. All treatments were conducted in triplicate for each malathion concentration. Each replicate acted as an independent unit, and mean values were used for analysis to ensure statistical reliability and reduce experimental variation.

2.2.4. Soil parameters analysis

The soil of the constructed wetland used for malathion treatment was also examined for various properties such as saturation, pH, texture, organic matter, nitrogen content, P and K. The soil was 51% saturated and had a basic pH of 8.12. Its texture was considered as clay loam having 0.059% organic matter, 16 ppm N, and 131 ppm K. The pH value of the soil samples was analyzed by a pH meter for alkaline or acidic character. The organic matter proportion was determined using the same approach as above. All values of NO_3^- in soil samples were determined by UV spectrophotometer [27]. Moreover, concentrations of potassium and

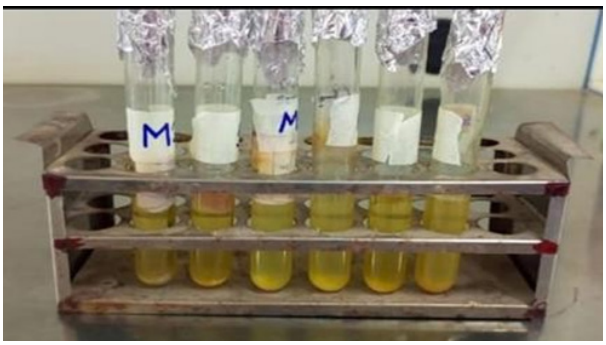

Fig. 1. Enrichment cultures in selective media for bacterial growth under controlled conditions.

Fig. 2. Lab based Constructed wetland setup.

sodium in soil samples were determined by flame photometer. The test may be performed on any water sample and the results are detected in terms of flame color. Standards were originally run with sample in photometer. The blue changed to yellow in the flame colors, indicating that sodium and potassium are present. Measurements are easy to read on the meter. The parameters represented the conditions of an experiment to study the potential for bioremediation by a bacterial consortia in constructed wastelands. Beginning at a pH of 10 signified an extremely alkaline condition. This was applicable because certain bacterial populations dominate the acidic conditions which in turn are major contributors to biodegradation. The test was designed to study the way these modified bacteria led to remediation within wastelands. The presence of 1 g of organic matter acted as a carbon source for the bacteria.

This material was used as substrate for growth of and energy source for microorganisms; in fact, it allowed the biodegradation of pollutants in the constructed wasteland. One gram of total nitrate was added for its nitrogen, a second nutrient necessary for bacteria to proliferate and be active. Both potassium and sodium contents were 5 g. These components were necessary for various processes with bacterial cells. Potassium functioned for the activation of enzymes while sodium maintained cell turgor and osmotic pressure. They promoted bacterial growth and activity for bioremediation. By controlling initial conditions for the experiment, a suitable environment is established which allows growth and working of certain bacterial communities. These consortia are capable of degrading a variety of pollutants and providing remediation in the constructed wetlands. All soil characteristics (pH, organic matter, nitrate, potassium and sodium) were determined with triplicate samples for each treatment and sampling week. Three subsamples from each wetland unit were extracted and analyzed separately to maintain spontaneous soil parameter variation.

The removal efficiency (%) of malathion was calculated using the following formula:

Removal efficiency:

$$\frac{initial\ concentration - final\ concentration}{initial\ concentration} \times 100$$

This approach allowed for precise tracking of malathion degradation across different treatments over time.

3. RESULTS AND DICUSSION

3.1. Bacterial Characterization and Malathion Removal

Bacterial consortia isolated from the constructed wetlands have shown potential for malathion degradation. Biochemical characterization revealed that the four bacterial consortia used in this study consisted of different combinations of Gram-positive, catalase-negative *Bacillus* isolates. Since all isolates belonged to *Bacillus* spp., the performance differences observed among consortia likely reflect variations in enzyme activity and synergistic interactions rather than taxonomic differences. This isolated group of bacteria was identified as Gram-positive and catalase-negative, like the *Bacillus* spp. which are well-known to break down malathion. It is known that bacilli can degrade organophosphates by using carboxylesterases and related pathways [16]. Using Bacillus alone or in mixed cultures, it has been found to completely degrade a lot of malathion in soil. For instance, in a previous study, when both *Bacillus* and *Micrococcus* species were present, they mineralized 500 mg/kg malathion much faster

than single cultures, finishing the process within 15 to 20 days. Because our isolates were oxidase and catalase negative, they may use a unique mechanism to break down pesticides. They are consistent with recent findings suggesting Gram-positive Bacilli are good for removing organophosphates [16].

3.1.1. Biochemical characterization

Additionally, oxidase and catalase tests were conducted to further understand the metabolisms of the consortium. Results from the oxidase test were negative indicating that these bacteria do not have an enzyme (cytochrome c oxidase) normally used in aerobic respiration [28]. Furthermore, catalase test was negative, which means catalase enzyme, which breaks down hydrogen peroxide was absent [29]. These results provide useful indications of the metabolic profile of the consortium and degradation pathways. Gram staining was performed to differentiate bacterial cell wall structures. The Gram-negative staining pattern was characterized by a thin peptidoglycan layer and outer membrane [30], as Gram positive bacteria resist the crystal violet staining leaving the bacteria purple, while Gram negative bacteria do not retain the crystal violet staining and so appear pink [31]. The catalase test is performed to separate bacteria based on the formation of an enzyme called 'catalase', which helps in decomposing the hydrogen peroxide to form water and oxygen [32]. The lack of catalase activity in the isolates is consistent with the properties of certain *Bacillus* species.

3.2. Analysis

3.2.1. Bioremediation of malathion through plant in soil

Sample collection and parameter checking was done after introducing pesticide. Each sample was collected with a gap of 2 weeks. The total time of bioremediation and sampling was eight weeks. Wetland plants contribute to malathion removal in several ways. First, plant roots can take up small amounts of pesticide from soil water, translocating it into root/shoot tissue where it may be sequestered or transformed. However, for non-volatile organophosphates like malathion, *direct* uptake tends to be limited compared to microbial breakdown [33]. The more important effect is indirect: the plant roots engineer the habitat for microbes. As noted, emergent macrophyte roots leak oxygen into the rhizosphere and exude sugars, amino acids and other carbon sources [34].

3.2.1.1. Treatment of Malathion through Canna indica and Mentha arvensis in soil sample taken from wetland media in first week of treatment process:

Figure 3 showcased remarkable bioremediation potential, reducing malathion concentrations with stunning efficiency across all initial levels. With a mere 3.2 mg/L remaining at the lowest starting concentration (50 mg/L), it achieved a near-perfect 99.34% degradation. This efficiency further increased to 99.68% and 99.9% for initial concentrations of 100 mg/L and 200 mg/L, respectively as shown in Figure 4. These findings indicate a strong metabolic potential of the bacterial consortium, when challenged with various malathion contamination levels. Efficiency was found to increase seismically with initial concentrations, suggesting possible induction or adaptation of the enzyme in the bacteria. This adaptability is crucial for real-world bioremediation where contaminant levels can vary significantly. *Canna indica and Mentha arvensis* therefore, emerges as a strong contender for effective malathion removal in constructed wastelands. Microbial communities in the rhizosphere engage in cooperative and competitive interactions, root exudates (sugars, amino acids, organic acids) boost microbial biomass and catabolic activity; microbes cometabolize malathion using enzymes induced by root-derived carbon or the pesticide itself [35].

3.2.1.2. Treatment of Malathion through Canna indica and Mentha arvensis in soil sample taken from wetland media in third week of treatment process:

Both *canna indica* and *mentha arvensis* exhibited consistent and high biodegradation efficiency across all malathion concentrations. As illustrated in Figure 5, initial concentrations of malathion at 50 mg/L, 100 mg/L, and 200 mg/L were reduced to 17 mg/L, 4 mg/L, and 2.3 mg/L respectively after treatment. This demonstrates the effective phytoremediation potential of the plant-soil system in degrading or removing malathion. Furthermore, Figure 6 highlights the removal efficiency across different concentrations. Efficiency increases with the dose from around 99.4% at 50 mg/L to

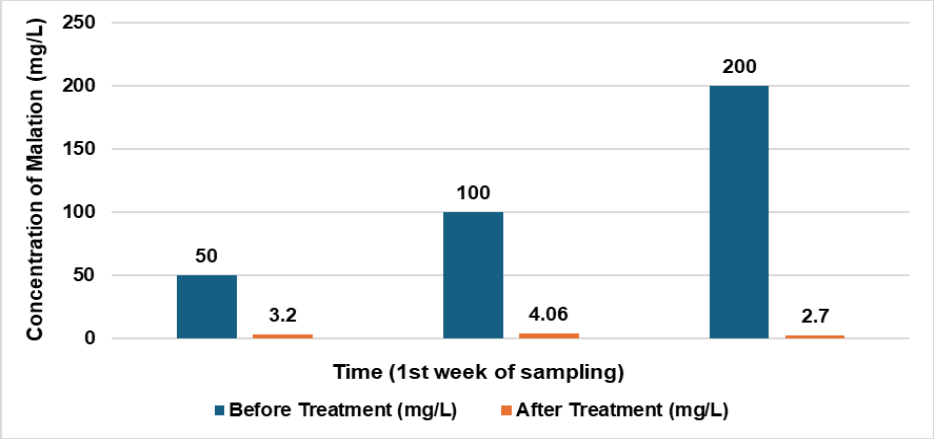

Fig. 3. Concentration of malathion before and after treatment with time (1st Sample (Plant + Soil)).

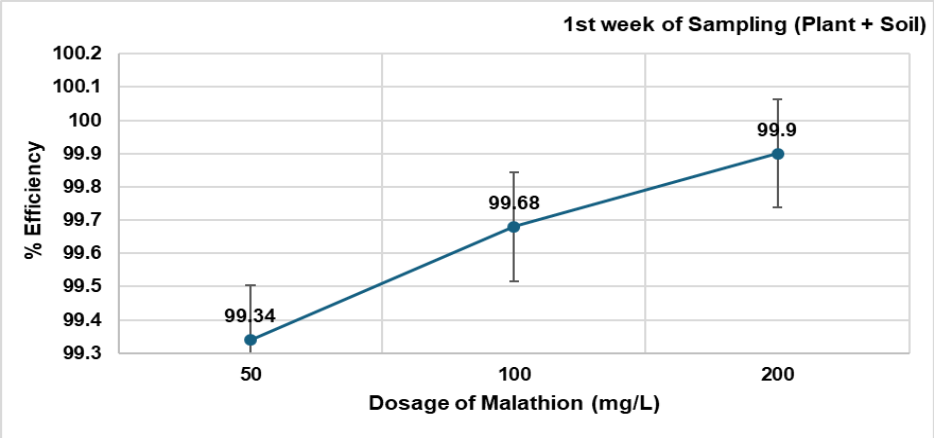

Fig. 4. Plant efficiency for treatment of different concentrations of malathion in wetland.

about 99.8% at 200 mg/L. These findings indicate that the treatment is marginally more effective at higher initial concentrations, which implies that the plant-soil system has a high ability to accept more pesticide. Despite greatly elevated resistance, it retained its functionality and is therefore a potential tool for bioremediation projects in which predictable outcomes are important. The composition and metabolism of this engineered consortium may potentially be further explored to understand its stable performance.

3.2.1.3. Treatment of Malathion through Canna indica and Mentha arvensis in soil sample taken from wetland media in fifth week of treatment process:

The treatment of malathion-contaminated soil using *Canna indica* and *Mentha arvensis* in a wetland media showed highly effective results by the fifth week of the treatment process. As illustrated in Figure 7, the efficiency of malathion removal increased with the dosage applied, reaching approximately 99.31% at 50 mg/L, 99.68% at 100 mg/L, and nearly 99.9% at 200 mg/L. This demonstrates a strong positive correlation between malathion concentration and phytoremediation efficiency, indicating the robustness of the treatment system even at higher contamination levels. Correspondingly, Figure 8 shows a significant reduction in malathion concentration in the third treatment i.e. plant + soil. Initial concentrations of 50 mg/L, 100 mg/L, and 200 mg/L were reduced to 9 mg/L, 3 mg/L, and 5.5 mg/L, respectively, after five weeks. The residual malathion concentration was lowest in the sample with 100 mg/L initial malathion concentration, indicating better performance at this level. In general, these results have revealed that the mixture of *Canna indica* and *Mentha arvensis* has an excellent efficiency for phytoremediation of malathion in soil at wetland, making it an eco-friendly tool for the control of pesticide contamination.

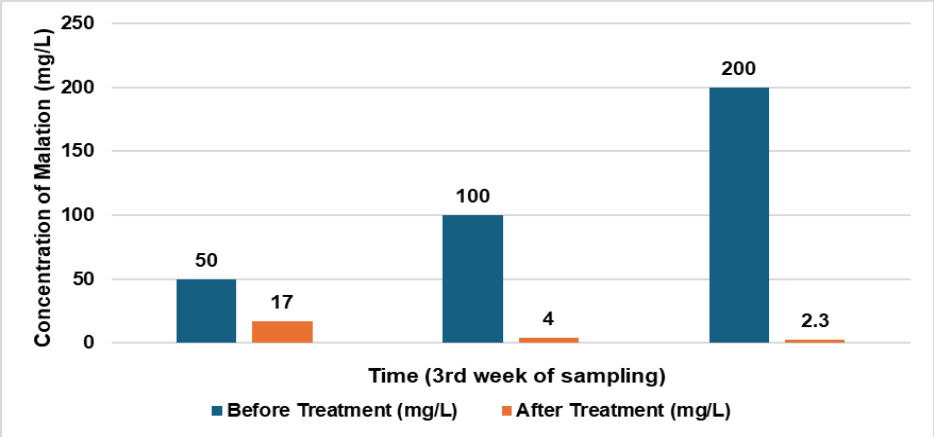

Fig. 5. Concentration of malathion before and after treatment with time (2nd Sample (Plant + Soil)).

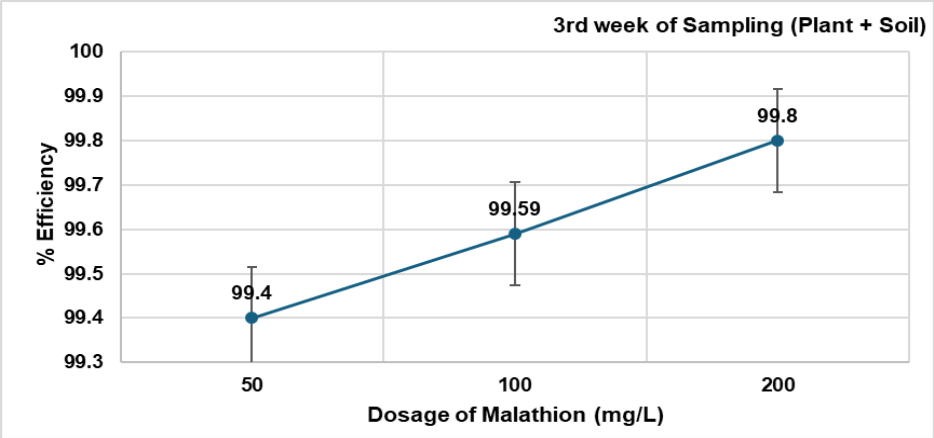

Fig. 6. Plant efficiency for treatment of different concentrations of malathion in wetland.

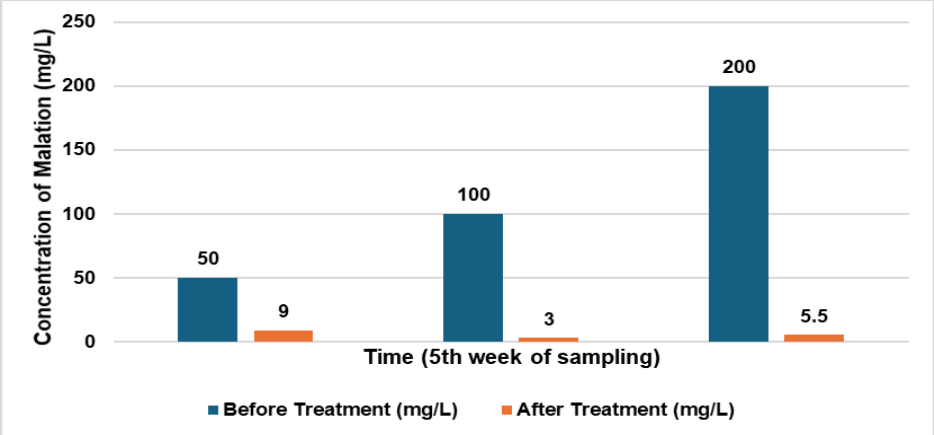

Fig. 7. Concentration of malathion before and after treatment with time (3rd Sample (Plant + Soil)).

3.2.1.4. Treatment of Malathion through Canna indica and Mentha arvensis in soil sample taken from wetland media in seventh week of treatment process:

In the seventh week of treatment, the removal of malathion from soil using *Canna indica* and *Mentha arvensis* continued to show exceptional results. Figure 9 shows that the malathion concentrations decreased significantly from 50, 100, and 200 mg/L to 2.4, 12, and 10 mg/L, respectively. The most significant reduction was observed at the 50 mg/L dosage, showing a drop to just 2.4 mg/L, indicating the high efficacy of the phytoremediation system at lower concentrations. Figure 10 presents the corresponding efficiency of malathion removal. The data shows that the system achieved an efficiency of around 99.8% at 50 mg/L,

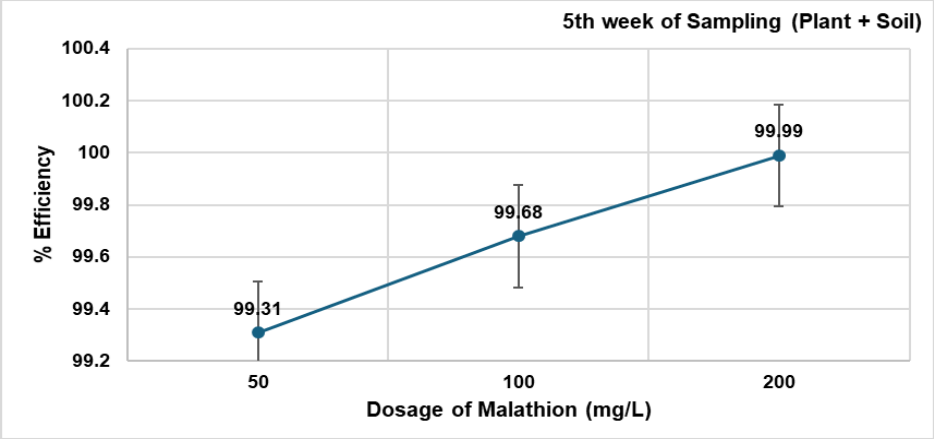

Fig. 8. Plant efficiency for treatment of different concentrations of malathion in wetland.

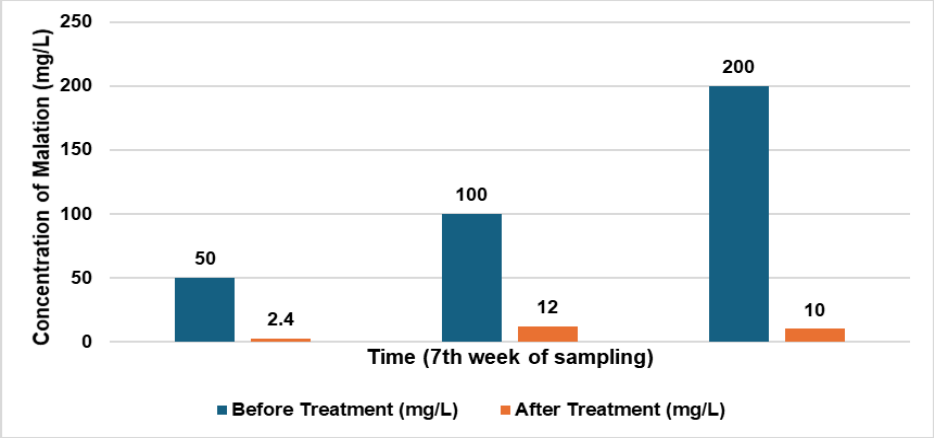

Fig. 9. Concentration of malathion before and after treatment with time (4th Sample (Plant + Soil)).

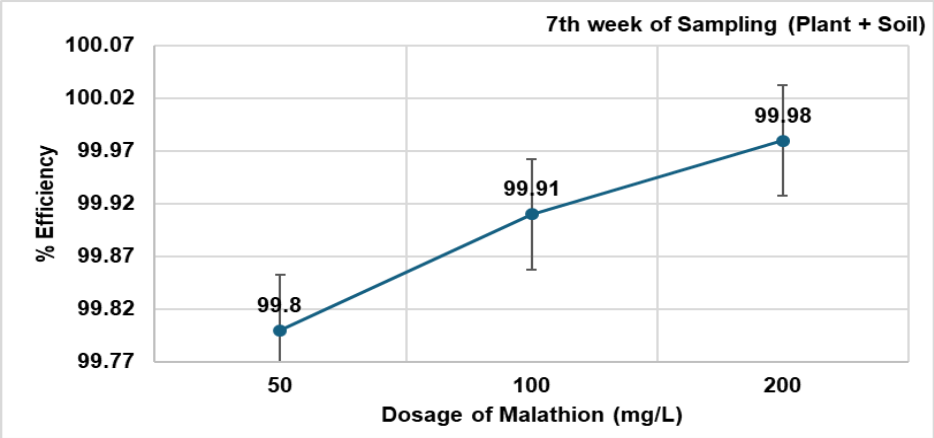

Fig. 10. Plant efficiency for treatment of different concentrations of malathion in wetland.

slightly above 99.91% at 100 mg/L, and maintained a similarly high level close to 99.98% at 200 mg/L. The near-complete removal of malathion across all concentrations by the seventh week confirms the potential of *Canna indica* and *Mentha arvensis* as reliable phytoremediators for treating pesticide-contaminated wetland soils over time. In practice, planted wetlands consistently outperform unplanted controls for pesticide removal. For example, Tang et al. [36] reported that *Canna indica* wetlands removed more pesticide mass than unplanted system.

Table 1 shows how increasing malathion concentrations (50, 100, and 200 mg/L) influenced soil properties under plant treatments P1-P4. Soil pH remained slightly alkaline across all setups, ranging from 7.30 to 7.76, with only minor shifts

as concentrations increased. Organic matter varied widely depending on the treatment, from as low as 8-10% in P1 to as high as 40 - 60% in P3. Total nitrate generally increased in several setups, such as in P3 where it rose from 251.66 mg/L at 50 mg/L to 500.83 mg/L at 200 mg/L, and in P4 where it remained high (340 - 503.33 mg/L) across treatments. Potassium values ranged between 3.1 and 10.7 mEq/kg, while sodium fluctuated between 22 and 54 g/mol, without a clear concentration-dependent pattern. Overall, these values indicate that plant treatments show moderate but variable nutrient responses to malathion exposure.

The significant results from these experiments prove that phytoremediation has great potential. Many species growing in wetlands, including *Canna indica* and *Mentha arvensis*, help clean up pollutants by using their vast root systems and rhizobacteria [26]. According to other studies, organophosphate removal is successful when carried out by wetland plants and the bacteria living in wetlands. When *Canna indica*, *Mentha arvensis* and pesticide-degrading bacteria were added to a constructed wetland, chlorpyrifos was fully broken without leaving any toxic substances. As with malathion, we find that plants can quickly absorb or transform it and speed up their decomposition, resulting in > 99% removal within just a few weeks [26]. Our results show that plant-assisted systems achieved > 99% removal but took slightly longer than bacteria alone. Plants reached near-complete removal by Weeks 3-5, likely because their roots improved aeration and supported microbial activity, helping maintain continuous malathion degradation [37].

3.2.2. Bioremediation of Malathion through bacteria in soil

Sample collection and parameter checking was done after introducing pesticide. Each sample was collected with a gap of 2 weeks. Total time of bioremediation and sampling was eight weeks. Many bacteria use organophosphorus-

Table 1. Soil properties under plant treatments (P1–P4) at different malathion concentration.

Samples	Parameter	50 mg/L	100 mg/L	200 mg/L
1st week (P1)	pH	7.38	7.43	7.30
	Organic matter (%)	10	8	8
	Total nitrate (mg/L)	196.6	245.8	237.5
	Potassium (mEq/kg)	3.1	3.1	3.1
	Sodium (g/mol)	54	28	22
3rd week (P2)	pH	7.30	7.36	7.70
	Organic matter (%)	20	20	20
	Total nitrate (mg/L)	170	295.83	319.16
	Potassium (mEq/kg)	10.7	6.7	5.7
	Sodium (g/mol)	42	40	38
5th week (P3)	pH	7.54	7.64	7.66
	Organic matter (%)	60	20	40
	Total nitrate (mg/L)	251.66	330	500.83
	Potassium (mEq/kg)	7.7	5.1	7.4
	Sodium (g/mol)	38	30	34
7th week (P4)	pH	7.55	7.67	7.76
	Organic matter (%)	10	20	20
	Total nitrate (mg/L)	503.33	340	405.83
	Potassium (mEq/kg)	7.4	6.2	7.9
	Sodium (g/mol)	30	34	32

degrading enzymes (organophosphorus hydrolases/phosphotriesterases/carboxylesterases) to cleave the P-O or ester bonds in malathion, producing monocarboxylic/dicarboxylic acids and ultimately mineralization products [38].

3.2.2.1. First soil sample extracted from wetland media at first week to determine treatment of malathion through bacteria:

In the first week of treatment, the bacterial remediation of malathion-contaminated soil extracted from wetland media showed encouraging results. As illustrated in Figure 11, initial malathion concentrations of 50 mg/L, 100 mg/L, and 200 mg/L were reduced to 6.1 mg/L, 5.5 mg/L, and 4.2 mg/L, respectively, after treatment. These reductions demonstrate that the bacterial activity began to effectively degrade malathion even within a short time frame. The efficiency of removal, shown in Figure 12, was approximately 99.40% at 50 mg/L, increasing to around 99.65% at 100 mg/L and 99.70% at 200 mg/L. The trend indicates that the bacterial system performs well across varying contamination levels, with slightly higher efficiency observed at greater concentrations. Malathion is degraded by carboxylesterases to its monoacid and diacid derivatives; this is the main metabolic mechanism for the degradation of malathion by microorganisms [39]. Overall, these findings confirm the potential of bacteria as a rapid and efficient means for the biodegradation of malathion in soil, especially useful for early-stage treatment in wetland-based remediation systems.

3.2.2.2. Second soil sample extracted from wetland media at 3rd week to determine treatment of malathion through bacteria:

In the third week of treatment, the second soil sample extracted from wetland media and treated with bacteria continued to show significant

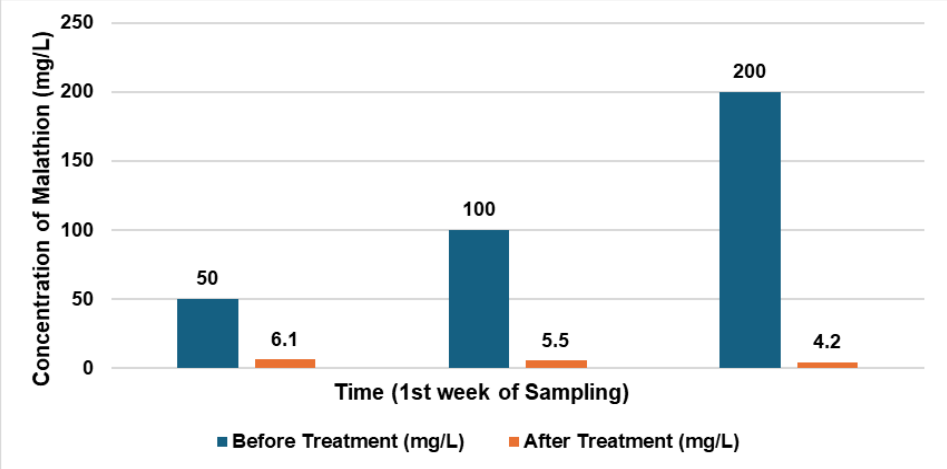

Fig. 11. Concentration of malathion before and after treatment with time (1st Sample (Bacteria + Soil)).

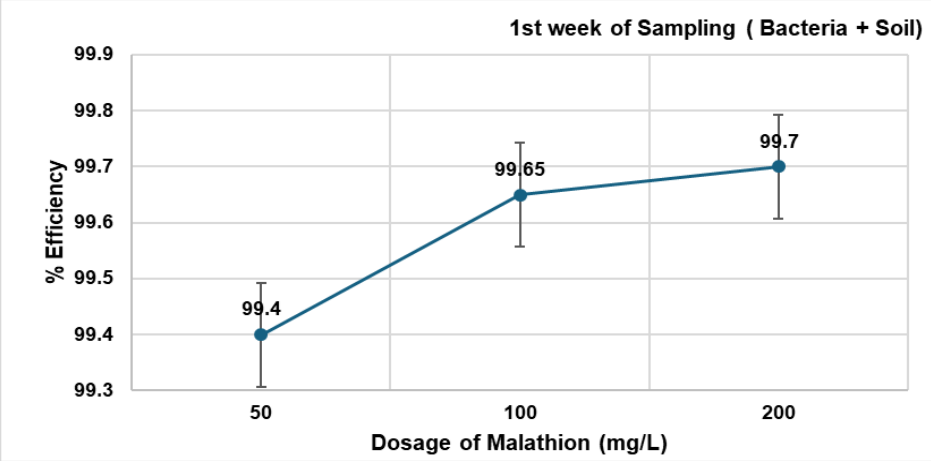

Fig. 12. Efficiency of bacteria for treatment of different concentrations of malathion in wetland.

degradation of malathion. As seen in Figure 13, malathion concentrations of 50 mg/L, 100 mg/L, and 200 mg/L were reduced to 10 mg/L, 9 mg/L, and 4.2 mg/L, respectively, after treatment. These data suggest that the bacterial activity was also retained over time, especially with larger dosages. The corresponding removal rates are illustrated in Figure 14 and they exhibited an increasing trend: about 99.15% for 50 mg/L, 99.56% for 100 mg/L, and finally rose to nearly 99.78% at the concentration of 200 mg/L.. The degradation power of malathion depends predominantly on the microorganism enzymatic activity. Enzymes are the biocatalysts which can enhance the rate of certain biochemical reaction by decreasing the activation energy [40]. This pattern demonstrates both the persistent and dose-responsive biodegradative capacity of the bacteria, which further supports its viability for use as a dependable candidate organism for treatment of malathion in wetland-based soil systems.

3.2.2.3. Third soil sample extracted from wetland media at 5th week to determine treatment of malathion through bacteria:

In the fifth week of sampling, bacteria and wetland media soil were screened for the ability to degrade malathion at different concentrations. The results presented in Figures 15 show a significant reduction in malathion levels after bacterial treatment. At 50 mg/L initial concentration, the removal of malathion was found to be 64% and decreased its concentration down to 18 mg/L. When the initial concentration was 100 mg/L, it decreased to 9.4 mg/L (removal efficiency reached 90.6%); while at the highest concentration of 200 mg/L, malathion remained at only 3.3 mg/L with removal efficiency of about 98.35% as shown in Figure16. The efficiency graph also shows that the rate of malathion removal was positively correlated with its initial concentration and reached 99.4%, 99.6%,

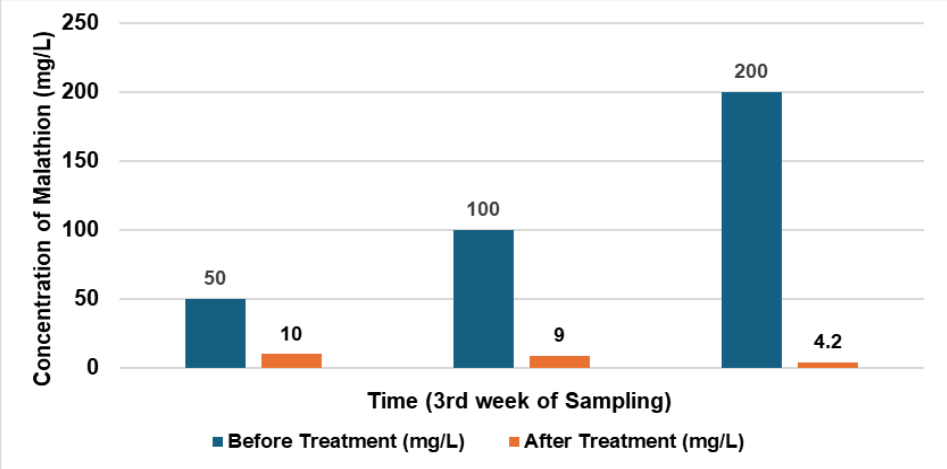

Fig. 13. Concentration of malathion before and after treatment with time (2nd Sample (Bacteria + Soil)).

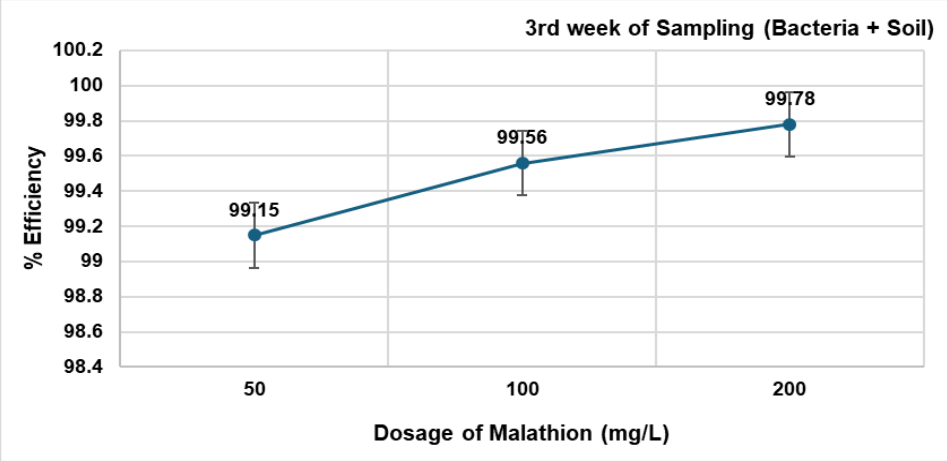

Fig. 14. Efficiency of bacteria for treatment of different concentrations of malathion in wetland.

and 99.9% for doses of 50, 100, and 200 mg/L, respectively. These results demonstrate that the bacterial activity in the wetland media is extremely effective for malathion degradation, especially at higher concentrations and has potential for use in bioremediation.

3.2.2.4. Fourth soil sample extracted from wetland media at seventh week to determine treatment of malathion through bacteria:

As can be seen from Figure 17 the malathion concentrations after treatment significantly reduced for all concentration tested. The concentration decreased from 50 mg/L to 4.6; at dosage of 100 mg/L reduced to 5.8, and dosage of 200 mg/L fell to 5.6 mg /L. This visibly suggests that a significant amount of malathion was degraded by bacteria existing in soil/wetland media and proved it that are effective against high dosages too. In addition, degradation efficiencies of about 99.80% at 50 mg/L, 99.94% at 100 mg/L and 99.97% at 200 mg/L as shown in Figure 18 indicates the high performance of bacterial system to detoxify malathion in environment. The low increase in efficiency with higher doses indicates a possible adaptation of the bacterial population or better performance under heavy pollution.

Table 2 illustrates the stronger chemical shifts observed under bacterial treatments B1 - B4. Soil pH stayed between 7.12 to 7.80, showing slight decreases at higher malathion concentrations in some setups. Organic matter ranged from 10% to 40%, depending on the treatment. A pronounced response was observed in nitrogen and nitrate levels: for example, in B1 total nitrogen increased sharply from 657.5 mg/L at 50 mg/L to 1303.3 mg/L at 100 mg/L, while B2 recorded nitrate values as high as 1747.5 mg/L at 100 mg/L. Sodium concentrations ranged from 26 to 76 mEq/kg, and higher values were found in B2 with 200 mg/L,

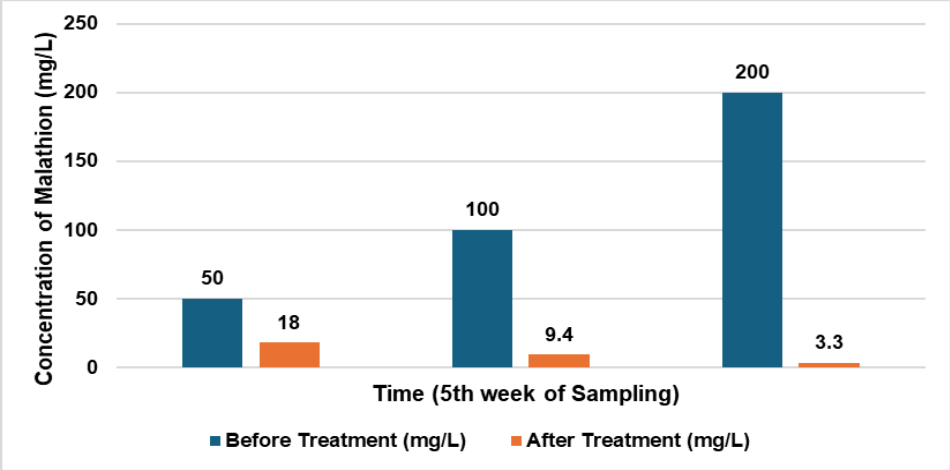

Fig. 15. Concentration of malathion before and after treatment with time (3rd Sample (Bacteria + Soil)).

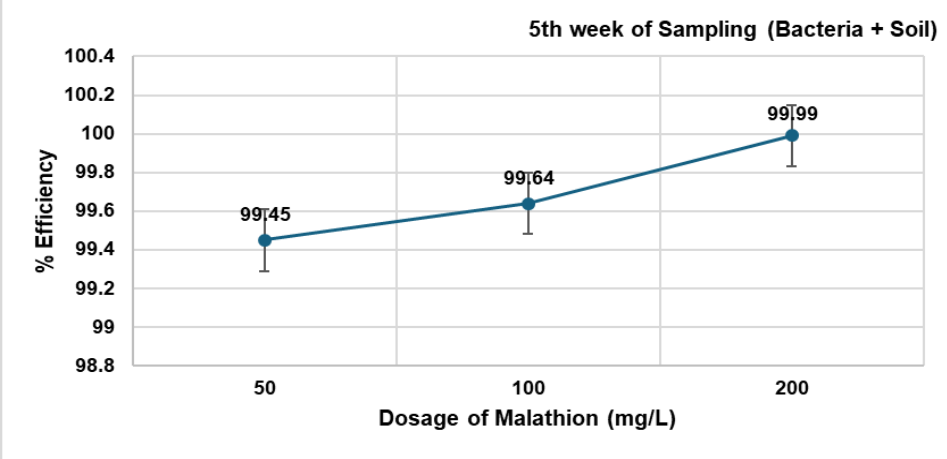

Fig. 16. Efficiency of bacteria for treatment of different concentrations of malathion in wetland.

whereas potassium varied between 4.9 and 20 g/mol, the same type of treatment led to intense changes on this element. These numbers indicate that bacterial activity induces stronger nutrient modifications than plant treatments when applied in combination with malathion.

Prior research showed that *Bacillus*-based groups could completely remove a high level of malathion, while single strains were much less effective [16]. In addition, *Pseudidiomarina* strains present in deep-sea waters degraded malathion at 500 mg/L to below detection levels in just 36 h [16]. The consortium's high performance and the trend we noticed with more pollutants indicate that enzymes are being made or microbes are becoming more tolerant of the contaminant. The same phenomenon has been spotted in other biodegradation systems, where these systems exhibit greater catabolic activity when there is a high contaminant concentration.

All systems showed almost complete malathion decomposition after eight weeks. Initially, bacterial consortia were the most effective, followed by plant systems that increased both uptake and stability. It proves that using these interactions in wetlands is a great, inexpensive way to address and clean up pesticide-polluted waters and soils

3.2.3. Two-Way ANOVA results for Malathion removal efficiency

In the plant-based treatment system, a two-factor ANOVA with replication (Table 3) was conducted to evaluate the effects of sampling week and treatment dose on the measured response variable. The analysis revealed a statistically significant main effect of week ($F = 228.44$, $p < 0.001$), indicating that the response values changed consistently across Week 1, Week 3, Week 5, and Week 7. There was also a highly significant main effect of dose level ($F = 932.59$, $p < 0.001$), demonstrating that

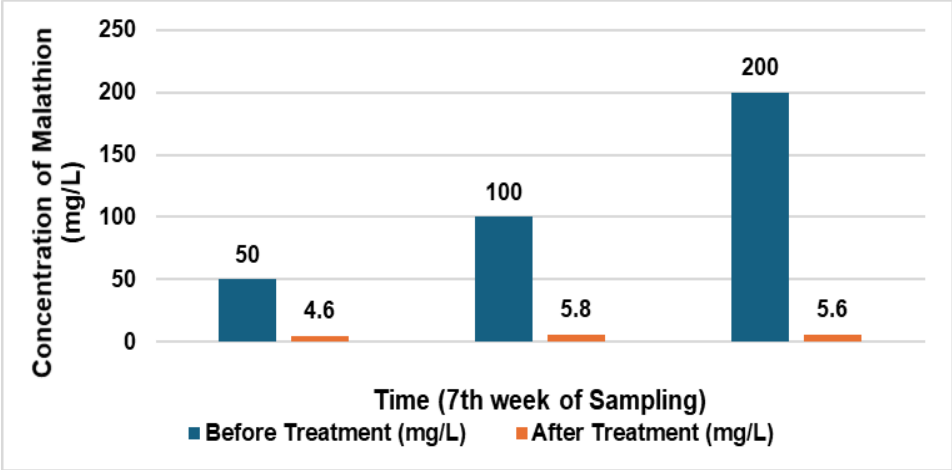

Fig. 17. Concentration of malathion before and after treatment with time (4th Sample (Bacteria + Soil)).

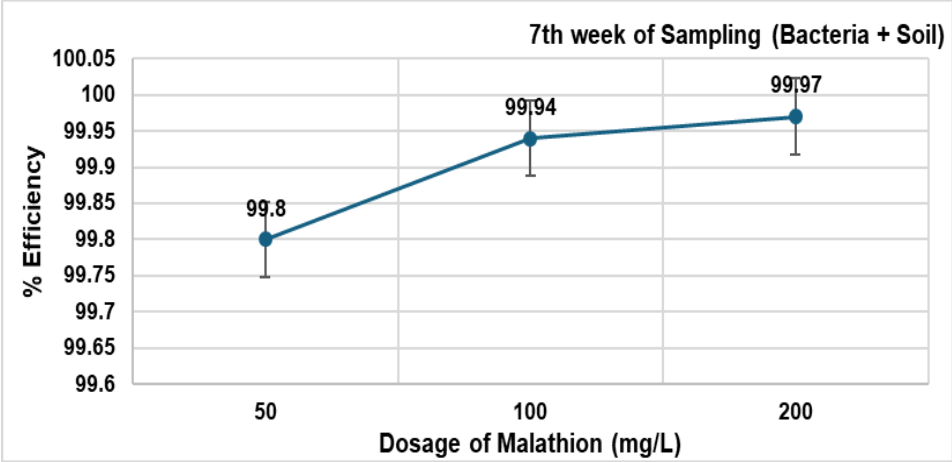

Fig. 18. Efficiency of bacteria for treatment of different concentrations of Malathion in wetland.

Table 2. Soil properties under bacterial treatments (B1 - B4) at different malathion concentrations.

Samples	Parameter	50 mg/L	100 mg/L	200 mg/L
1st week (B1)	pH	7.80	7.58	7.36
	Organic matter (%)	40	10	10
	Total nitrate (mg/L)	657.5	1303.3	577.5
	Sodium (mEq/kg)	54	59	46
	Potassium (g/mol)	14.1	12.5	12.1
3rd week (B2)	pH	7.48	7.36	7.30
	Organic matter (%)	30	10	20
	Total nitrate (mg/L)	417.5	1747.5	1245.8
	Sodium (mEq/kg)	28	38	76
	Potassium (g/mol)	4.9	7.4	20.0
5th week (B3)	pH	7.53	7.36	7.40
	Organic matter (%)	20	10	10
	Total nitrate (mg/L)	427.1	1847.6	1045.7
	Sodium (mEq/kg)	26	30	45
	Potassium (g/mol)	14.1	13.6	12.1
7th week (B4)	pH	7.20	7.12	7.40
	Organic matter (%)	10	10	20
	Total nitrate (mg/L)	412.5	1303.3	577.5
	Sodium (mEq/kg)	27	36	40
	Potassium (g/mol)	14.1	13.5	12.1

increasing the dose from 50 to 100 and 150 resulted in progressively higher mean values. There was also a significant week dose interaction ($F = 52.18$, $p < 0.001$) showing that the dose effect decreased or increased depending on week of sampling. This interaction suggests that the disparity in dose levels was not constant over time and response profile to treatment also varied as a function of time. Certainly, both factors had independent (and combined) effects in determining the resulting behavior, and very low within-group variation indicated strong statistical power.

For the bacteria-based treatment system, a 2-factor ANOVA with replication (Table 4) was performed to determine the influence of treatment level (50, 100, 150) and time points (weeks: 1, 3, 5, and 7) on response values. The analysis revealed a strong main effect of time ($F = 111.05$, $p = 3.31 \times 10^{-14}$) and treatment level ($F = 204.61$, $p = 8.36 \times 10^{-16}$), thereby demonstrating that both factors were independently associated with positive outcomes, with higher treatment levels producing higher averages. A significant interaction effect was also found ($F = 16.33$, $p = 2.05 \times 10^{-7}$), showing that

Table 3. Two-factor ANOVA results for the removal of malathion using plant-based wetland treatment across different dose levels and sampling weeks.

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.4702	3	0.156733	228.4372	8.93E-18	3.008787
Columns	1.279717	2	0.639858	932.587	1.77E-23	3.402826
Interaction	0.214817	6	0.035803	52.18219	1.35E-12	2.508189
Within	0.016467	24	0.000686			
Total	1.9812	35				

Table 4. Two-factor ANOVA results for the removal of malathion using bacteria-based wetland treatment across different treatment levels and sampling weeks.

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.832875	3	0.277625	111.05	3.31E-14	3.008787
Columns	1.02305	2	0.511525	204.61	8.36E-16	3.402826
Interaction	0.24495	6	0.040825	16.33	2.05E-07	2.508189
Within	0.06	24	0.0025			
Total	2.160875	35				

the impact of treatment varied across weeks. The very small within-group variance reflects strong consistency in the repeated measurements. Overall, the results confirm that both treatment level and time significantly affected the response variable.

3.2.4. Comparative performance and synergy

All the treatments effectively removed > 99% malathion, but they worked differently. Initially, bacteria-only wetlands caused a quicker drop: in just days, they brought pollutant removal close to its maximum, but plants needed weeks to clear as much. In addition, plants helped continue the loss of soil quality and structure as time passed. A wetland created with a plant–bacterial consortium would probably benefit from both methods. Scientists in constructed wetland science believe this complementary effect is strongly linked, as all removal of contaminants often results from combined efforts of substrates, plants and microbes [41]. Mechanistically, plants and microbes complement each other. Established macrophytes continuously oxygenate the rhizosphere and leak nutrients (e.g., low-molecular-weight carbon) that "awaken" soil bacteria [34]. Rapidly, malathion is attacked by microbes and plants prevent anything from the effluent coming back into contact with the soil. Our findings agree with what others have observed, that plant–microbe systems deal with pesticides effectively without leaving any harmful residues [26, 27]. Higher efficiency at higher concentrations likely reflects microbial adaptation: high malathion loads induce stronger biodegradation. For example, our consortia's near-100% removal at 200 mg/L (within 7 weeks) suggests that bacterial enzymes were fully engaged. In contrast, at 50 mg/L the process was slightly slower, perhaps because enzyme expression was lower. This inverse concentration-dependency is supported by other reports: degrading bacteria often shows greater catabolic activity under elevated pollutant stress. In summary, the observed trends can be explained by the underlying biochemistry of malathion breakdown and the synergistic ecology of the wetland rhizosphere.

4. CONCLUSIONS

The present study demonstrates that both bacterial consortia (*Bacillus* spp. isolates) and plant-based systems (*Canna indica and Mentha arvensis*), alone and in combination, achieved very high malathion removal from spiked soil: all treatments reached > 99% removal by Week 7 across tested concentrations (50, 100, 200 mg/L). Bacteria-only treatments produced the most rapid initial decline (significant main effects of time and dose: $F = 111.05$ and $F = 204.61$, respectively; $p < 1 \times 10^{-13}$), while planted systems provided sustained removal and habitat support for microbial activity (plant ANOVA: $F = 228.44$ and $F = 932.59$ for week and dose, respectively; $p < 1 \times 10^{-16}$). The higher apparent removal at larger initial doses is consistent with induction or up-regulation of catabolic activity under greater pollutant stress, although enzyme activity and metabolite profiles were not measured here and thus this remains a testable hypothesis. Mechanistically, the results are consistent with microbial hydrolysis (e.g., carboxylesterase activity) and rhizosphere-stimulated microbial degradation: bacterial consortia gave rapid biodegradation while plant roots likely enhanced oxygenation and exudation that sustained breakdown over weeks. However, this study is limited to lab-scale, colorimetric quantification and morphological/biochemical bacterial identification (no species-level molecular ID or metabolite analysis). Future work should (i) confirm degrader identity by sequencing, (ii) measure enzyme activities and

malathion metabolites to validate pathways, and (iii) evaluate pilot-scale constructed wetlands under field conditions. Overall, plant-bacterial consortia show strong potential as a low-cost, environmentally friendly option for remediation of malathion-contaminated soils, but field validation and mechanistic confirmation are required before deployment.

5. ACKNOWLEDGEMENT

The authors acknowledge the assistance and support of the laboratory staff of Bahria University, Islamabad.

6. CONFLICT OF INTEREST

All authors declare no conflict of interest

7. REFERENCES

1. F.A. Al-Saeed, S.S. Abd-Elghfar, and M.E. Ali. Efficiency of thyme and oregano essential oils in counteracting the hazardous effects of malathion in rats. *Animals* 14(17): 2497 (2024).
2. S. Uniyal, R.K. Sharma and, V. Kondakal. New insights into the biodegradation of chlorpyrifos by a novel bacterial consortium: process optimization using general factorial experimental design. *Ecotoxicology and Environmental Safety* 209: 111799 (2021).
3. U.S.E.P. Agency. Reregistration Eligibility Decision (RED) for Malathion. *U.S. EPA, Washington, DC* (2006). https://archive.epa.gov/pesticides/reregistration/web/pdf/malathion-red-revised.pdf
4. U.S.G. Survey. Pesticides in the Nation's Streams and Ground Water, 1992–2001: The Quality of Our Nation's Waters. *U. S. G. Survey, Washington, DC, USA* (2006). https://pubs.usgs.gov/circ/2005/1291/pdf/circ1291.pdf
5. J. Cui, Y. Wei, J. Jiang, S. Xiao, X. Liu, Z. Zhou, D. Liu, and P. Wang. Bioaccumulation, metabolism and toxicological effects of chiral insecticide malathion and its metabolites in zebrafish (*Danio rerio*). *Chemosphere* 318: 137898 (2023).
6. R.W. Lamb, H. McAlexander, C.M. Woodley and M.K. Shukla. Towards a comprehensive understanding of malathion degradation: theoretical investigation of degradation pathways and related kinetics under alkaline conditions. *Environmental Science: Processes & Impacts* 23(8): 1231-1241 (2021).
7. S. Vaishali, A. Surendran, and A. Thatheyus. Biodegradation of malathion using *Pseudomonas stutzeri* (MTCC 2643). *Journal of Public Health International* 2(4): 8-19 (2020).
8. M.A. Dar and G. Kaushik. Optimizing the malathion degrading potential of a newly isolated *Bacillus* sp. AGM5 based on Taguchi design of experiment and elucidation of degradation pathway. *Biodegradation* 33(5): 419-439 (2022).
9. D.G. Karpouzas and B.K. Singh. Microbial degradation of organophosphorus xenobiotics: metabolic pathways and molecular basis. *Advances in Microbial Physiology* 51: 119-225 (2006).
10. S.R. Geed, M.K. Kureel, A.K. Shukla, R.S Singh, and B.N Rai. Biodegradation of malathion and evaluation of kinetic parameters using three bacterial species. *Resource-Efficient Technologies* 2: S3-S11 (2016).
11. B. Singh, J. Kaur, and K. Singh. Microbial degradation of an organophosphate pesticide, malathion. *Critical Reviews in Microbiology* 40(2): 146-154 (2014).
12. S. Olakkaran, A.K. Purayil, A. Antony, S. Mallikarjunaiah, and G.H. Puttaswamygowda. Oxidative stress-mediated genotoxicity of malathion in human lymphocytes. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 849: 503138 (2020).
13. P.L. Bastos, A.F.T.d.L. Bastos, A.d.M. Gurgel, and I.G.D. Gurgel. Carcinogenicidade e mutagenicidade do malathion e seus dois análogos: uma revisão sistemática. *Ciência & Saúde Coletiva* 25(8): 3273-3298 (2020).
14. A.S. Petsas and M.C. Vagi. Trends in the bioremediation of pharmaceuticals and other organic contaminants using native or genetically modified microbial strains: a review. *Current Pharmaceutical Biotechnology* 20(10): 787-824 (2019).
15. C.I. Cedillo-Herrera, A. Roé-Sosa, A.M. Pat-Espadas, K. Ramírez, J. Rochín-Medina, and L.E. Amabilis-Sosa. Efficient malathion removal in constructed wetlands coupled to UV/H_2O_2 pretreatment. *Applied Sciences* 10(15): 5306 (2020).
16. M.A. Dar and G. Kaushik. Biodegradation of malathion in amended soil by indigenous novel bacterial consortia and analysis of degradation pathway. *Soil Systems* 7(4): 81 (2023).
17. S.R. Geed, M.K. Kureel, B.S. Giri, R.S. Singh, and B.N. Rai. Performance evaluation of Malathion biodegradation in batch and continuous packed bed bioreactor (PBBR). *Bioresource Technology* 227:56-65 (2017).
18. C. Jimenez-Torres, I. Ortiz, P. San-Martin, and R.I.

Hernandez-Herrera. Biodegradation of malathion, α-and β-endosulfan by bacterial strains isolated from agricultural soil in Veracruz, Mexico. *Journal of Environmental Science and Health, Part B* 51(12): 853-859 (2016).

19. D. Li, S. Wang, L. Wang, H. Zhang, and J. Hu. A simple colorimetric probe based on anti-aggregation of AuNPs for rapid and sensitive detection of malathion in environmental samples. *Analytical and Bioanalytical Chemistry* 411(12): 2645-2652 (2019).
20. A. Mehta, K.K. Bhardwaj, M. Shaiza, and R. Gupta. Isolation, characterization and identification of pesticide degrading bacteria from contaminated soil for bioremediation. *Biologia Futura* 72(3): 317-323 (2021).
21. D.K. Sharma, N. Thakur, A. Sharma, and P. Raj. New Spectrophotometric Method for the Analysis of Commercial Malathion Formulation and Its Residues on Some Crop Produces and Environmental Samples. *Journal of Advanced Scientific Research* 11(02): 131-136 (2020).
22. W.M. Ibrahim, M.A. Karam, R.M. El-Shahat, and A.A. Adway. Biodegradation and utilization of organophosphorus pesticide malathion by cyanobacteria. *BioMed Research International* 2014(1): 392682 (2014).
23. U.S.F.a.D. Administration. BAM Media M114: Nutrient Broth. *U.S. Food and Drug Administration, Silver Spring, MD* (2017). https://www.fda.gov/food/laboratory-methods-food/bam-media-m114-nutrient-broth
24. J. Reynolds. Media Preparation. *LibreTexts/Biology LibreTexts* (2021). https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs_I/01%3A_Media_Preparation
25. W.R. Henayl. Bergey's manual of determinative bacteriology. *Lippincott Williams & Wilkins, Baltimore, USA* (1994).
26. T. Aziz, S. Rasheed, A.H. Shah, H. Nasir, A. Fariq, A. Jamil, and S. Jannat. Bioremediation Potential of Plant-Bacterial Consortia for Chlorpyrifos Removal Using Constructed Wetland. *Frontiers in Environmental Science* 10:880807 (2022).
27. R.S Chandan, N.R Soundaryashree, M.Umesh, A. Neogi, A.S. Aparna, E. Jacob, D. Scaria, and N P. Gana. Analytical Method Development and Validation of Malathion by Uv Spectroscopy. *Journal of Pharmaceutical Negative Results* 13(08): 2631 (2022).
28. R. Hartline. 1.19: Cytochrome c Oxidase. (2022). https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01:_Labs/1.19:_Cytochrome_c_Oxidase
29. M.T. Madigan, J.M. Martinko, K.S. Bender, D.H. Buckley, D.A. Stahl, and T. Brock (Eds.). Brock Biology of Microorganisms. 14th Edition. *Benjamin Cummings* (2014).
30. K. Rogers. Gram-negative bacterium. *Britannica Editors* (2025). https://www.britannica.com/science/Gram-negative-bacterium
31. G. Karki. Difference Between Gram-Positive and Gram-Negative Bacteria. (2018). https://www.onlinebiologynotes.com/difference-between-gram-positive-and-gram-negative-bacteria/
32. H. Khatoon, A. Anokhe, and V. Kalia. Catalase test: A biochemical protocol for bacterial identification. *AgriCos e-Newsletter* 3(1): 53-55 (2022).
33. Z. Tang, J. Wood, D. Smith, A. Thapa and N. Aryal. A review on constructed treatment wetlands for removal of pollutants in the agricultural runoff. *Sustainability* 13(24): 13578 (2021).
34. O.C. Overton, L.H. Olson, S.D. Majumder, H. Shwiyyat, M.E. Foltz and R.W. Nairn. Wetland removal mechanisms for emerging contaminants. *Land* 12(2): 472 (2023).
35. O.O. Babalola, O.C. Emmanuel, B.S. Adeleke, K.A. Odelade, B.C. Nwachukwu, O.E. Ayiti, T.T. Adegboyega and N.O. Igiehon. Rhizosphere microbiome cooperations: strategies for sustainable crop production. *Current Microbiology* 78(4): 1069-1085 (2021).
36. X.Y. Tang, Y. Yang, M.B. McBride, R. Tao, Y.N. Dai, and X.M. Zhang. Removal of chlorpyrifos in recirculating vertical flow constructed wetlands with five wetland plant species. *Chemosphere* 216: 195-202 (2019).
37. G. Yidong, W. Bo, G. Yongxia, L. Wen, Z. Xiaoli, and Y. Jianghua. Occurrence and fate of antibiotics in the aqueous environment and their removal by constructed wetlands in China: a review. *Pedosphere* 27(1): 42-51 (2017).
38. B.K. Singh and A. Walker. Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews* 30(3): 428-471 (2006).
39. L. Ma, X. Dai, G. Ai, X. Zheng, Y. Zhang, C. Pan, M. Hu, C. Jiang, L. Wang, and Z. Dong. Isolation and identification of efficient malathion-degrading bacteria from deep-sea hydrothermal sediment. *Microorganisms* 10(9): 1797 (2022).
40. B. Sharma, A.K. Dangi, and P. Shukla. Contemporary enzyme based technologies for bioremediation: a review. *Journal of Environmental Management* 210:

10-22 (2018).
41. J. Wang, G. Zhang, D. Wang, Y. Zhao, L. Wu, Y. Zheng, and Q. Liu. Low-Carbon Hybrid Constructed Wetland System for Rural Domestic Sewage: Substrate–Plant–Microbe Synergy and Annual Performance. *Water* 17(10): 1421 (2025).