Applied Environmental Research
- RESEARCH FOR SUSTAINABLE PLANET -

Research Article

Effects of Herbicides on 2,4-D-Resistant Soil Microorganisms in Maize Cultivation in Lopburi Province, Thailand

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Abstract

This study aimed to assess the impact of herbicide application on soil microorganism density and to identify microorganisms resistant to 2,4-D-dimethylammonium (2,4-D) for potential biodegradation. Research was conducted in maize plots in Lopburi Province, where 2,4-D was applied at a rate of 120 g of active ingredient per 160 m^2. The soil samples were collected at various intervals: before application; 2 and 4 hours postapplication; and 1, 3, and 7 days after application. The analyses included soil property and microorganism population analyses. The results indicated that 2,4-D applications significantly increased bacteria and fungi in the treated plots compared with those in the control plots, likely due to the ability of certain microorganisms to use 2,4-D as a carbon and nitrogen source. 2,4-D residues are associated with microorganism density and are also correlated with soil organic matter, clay content, and cation exchange capacity. The study confirmed that all three bacterial isolates could degrade 2,4-D, with *Bacillus albus* achieving the highest degradation rate of 23.84%, followed by *Nocardioides aromaticivorans* and *Bacillus cereus*. Among the fungi, *Penicillium shearii* had the highest degradation rate at 50.42%, followed by *Penicillium pimiteousiense*. This research reveals the adaptive capabilities of specific microorganisms in herbicide-impacted environments and their potential for bioremediation.

ARTICLE HISTORY

Received: 18 Jan. 2025
Accepted: 12 Jun. 2025
Published: 17 Jun. 2025

KEYWORDS

2,4-D;
Herbicides;
Soil microorganisms;
Biodegradation;
Agricultural chemicals

Introduction

The agricultural sector is dependent on pesticides for controlling pests such as weeds, insects, and pathogenic microorganisms, with a particular focus on herbicides. As a result, herbicides have become the most imported category of hazardous substances, surpassing all other pesticide types in 2023 [1]. Among these herbicides, the leading imported herbicides based on chemical composition include glufosinate-ammonium, glyphosate-isopropyl ammonium, 2,4-D-dimethylammonium, abamectin, propineb, emamectin benzoate, and atrazine. A notable example is 2,4-D, a chlorophenoxyacetic acid herbicide widely used in postemergence applications to control broadleaf weeds. Upon absorption through the leaves, 2,4-D moves to the meristem at the shoot tip, where it disrupts plant growth by inducing uncontrolled cell proliferation, leaf curling, and ultimately plant death. Commercially, 2,4-D is available not only in its acidic form but also as a salt and ester, increasing its versatility and range of applications across various agricultural practices.

The degradation of 2,4-D occurs through both physical and biological mechanisms. physically degrades via processes such as photodecomposition, hydrolysis, fusion, vaporization, and oxidation [2]. Biologically, 2,4-D undergoes biodegradation under both aerobic and anaerobic conditions. Research has shown that 2,4-D has an environmental half-life of approximately seven days [3], whereas other studies indicate that the half-life of 2,4-D in its

https://doi.org/10.35762/AER.2025021

acidic, diethylamine salt, and ester forms is approximately ten days in soil [4]. Despite both physical and biological degradation processes, 2,4-D residues can persist in soil, largely due to factors influencing adsorption and desorption, such as the soil organic matter content, pH, particle composition, and microbial abundance [3]. An empirical study has shown that 2,4-D residues are more likely to associate with clay particles than with sand, as clay possesses surface charges that increase its adsorption capacity [5 6]. Further research by Ismail et al. [7] compared 2,4-D adsorption in clay loam and clay soils and revealed that clay loam adsorbs more 2,4-D due to its higher organic matter content, which enhances its binding capacity. The findings of this study highlight the complexity of 2,4-D soil interactions and the role of soil composition in its environmental persistence.

The World Health Organization (WHO) reported that in developing countries, there are up to three million cases of agrochemical poisoning. The continuous and indiscriminate use of agrochemicals negatively affects soil biodiversity, agricultural sustainability, and food safety, potentially leading to long-term adverse effects on nutritional security and the health of both humans and animals. Most agrochemicals have detrimental impacts on the functions of soil microorganisms and on biochemical processes. The diversity and composition of beneficial microbial communities may shift in unfavorable ways, which can hinder plant growth either by reducing nutrient availability or increasing the risk of plant diseases [8].

Herbicides often lead to a reduction in the total microbial population within 7 to 30 days after application, depending on the type of herbicidal molecules. They also indirectly impact microbial biodiversity by altering physiological processes or biosynthetic mechanisms [9 10]. Following application, herbicides undergo both physical and biochemical transformations, resulting in the formation of various secondary metabolites. These metabolites may be more toxic or more persistent in the environment, thereby affecting nontarget microbial communities. One example is the impact of 2,4-D and its metabolites on *Burkholderia cepacia*, a group of gram-negative bacteria [11]. The application of 2,4-D can significantly alter the structure of soil microbial communities. Research indicates that the genetic structure of bacterial populations in soil changes in response to 2,4-D application, particularly during the active degradation phase. This effect diminishes within seven days after treatment. Additionally, the genetic potential for 2,4-D degradation, assessed through the presence of specific genes, increases following application, correlating with increased mineralization rates [12]

Thus, this research focuses on examining the impact of herbicides on soil microbial diversity and population dynamics, assessing the persistence of herbicide residues in soil, and exploring the potential of soil microorganisms in biodegrading pesticide residues. By analyzing both the types and abundance of soil microorganisms affected by herbicides, this study aims to provide insights into microbial adaptations to chemical contaminants and their application in bioremediation efforts to reduce pesticide accumulation in soils.

Materials and methods

1) Spraying of 2,4-D maize plots and soil sampling

This research was conducted in a forage corn cultivation area at the Lopburi Seed Research and Development Center, Mueang District, Lopburi Province, located at coordinates 14°79'83.8"N, 100°80'15.5"E", in May 2023. The field was divided into two 400-m^2 subplots. One subplot was treated exclusively with 2,4-D herbicide at the recommended rate of 120 g of active ingredient per 160 m^2, with no additional pesticides; 2,4-D herbicide was sprayed for weed control at the 1-month growth stage of the forage corn, whereas the second subplot served as a control and received no 2,4-D treatment. Both subplots were planted with Pacific 339 forage corn, which was harvested after 110–115 days. The forage corn reached 1 month of age. Soil samples were collected from each subplot at 6 intervals: before herbicide application; 2 and 4 hours postapplication; and 1, 3, and 7 days after herbicide application. Sampling was performed at a depth of 20 cm, with soil collected randomly from 11 locations within each plot to ensure a representative sample. The collected samples were analyzed to quantify populations of fungi, actinomycetes, and bacteria, which were isolated via specific culture media: glucose ammonium nitrate agar (GAN) for fungi; starch casein agar (SCA) for actinomycetes; and nutrient agar (NA) for bacteria. Microorganisms were enumerated via the plate count method, and the data were statistically analyzed via a t test.

Subsequent soil sampling involved collecting composite samples at a depth of 20 cm [13]. The process involved random sampling from 20 locations within each plot to accurately represent the soil conditions of the entire plot. These composite samples were then analyzed for a range of physical and chemical properties, including particle size distribution, pH, organic matter content, available phosphorus, exchangeable potassium, and cation exchange capacity. Moreover, the residual 2,4-D content in the soil was quantified via high-performance liquid chromatography (UHPLC). The analysis utilized an Agilent Technology Model 1290 UHPLC system equipped with a diode array detector (DAD) set to a wavelength of 230 nanometers, allowing precise detection of 2,4-D residues. The experiment consisted of two operational methods: (1) a control plot, where no 2,4-D herbicide was applied, and (2) a treatment plot, where 2,4-D herbicide was sprayed at the recommended application rate.

2) 2,4-D degradation test through selected micro-orgasisms

The purpose of this study was to isolate bacteria and fungi that are capable of degrading 2,4-D. The degradation of 2,4-D was evaluated in selected bacterial and fungal strains under controlled conditions; bacteria were cultured in inorganic salt media, and fungi were cultured in rose bengal media for selection. For bacterial strains, the degradation test was conducted in 2,4-D inorganic salt broth (pH 7.0), which was modified by excluding the carbon source. The media included KH_2PO_4 (0.4 g), K_2HPO_4 (1.6 g), NH_4NO_3 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.025 g), $FeSO_4 \cdot 7H_2O$ (8 mg), and 2,4-D (50 mg). The cultures were incubated at 30 °C in the dark with continuous shaking for 7 days [14]. For fungal strains, the degradation of 2,4-D was evaluated in 2,4-D- Czapek Dox broth modified by excluding the nitrogen source. This medium consisted of sucrose (30 g), peptone (5 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), and 2,4-D (100 mg). Similarly, the cultures were incubated at 30 °C in the dark under continuous shaking for 14 days [15]. After the incubation period, the liquid media from both tests were analyzed to measure the residue content of 2,4-D. The microorganisms responsible for 2,4-D degradation were subsequently identified via molecular biology techniques via polymerase chain reaction (PCR) and DNA sequencing for comparison with the NCBI GenBank database [16–17].

3) Determination of 2,4-D in soil via high-performance liquid chromatography (HPLC)

The objective is to quantify the residual amounts of 2,4-D in the soil. A sample weighing 10±0.02 g was placed into a 50 mL centrifuge tube, followed by the addition of 20 mL of a solvent mixture consisting of methanol:water:acetic acid at a ratio of 80:20:2.5 (v/v/v). The mixture was extracted via an ultrasonic cleaner (sonicator) for 30 minutes. After extraction, the sample was centrifuged at 4,000 rpm for 20 minutes. The extraction process was repeated once. The combined extracts were adjusted to a final volume of 50 mL. The extract was analyzed via an Ultra-High Performance Liquid Chromatograph (UHPLC), Agilent Technologies, Model 1290, equipped with a Diode Array Detector (DAD) set at a detection wavelength of 230 nm. The mobile phase consisted of 5 mM ammonium formate (pH 4.0) and methanol. A Thermo Scientific™ Hypersil™ BDS C18 column was used as the stationary phase [18–19].

4) Determination of 2,4-D in culture broth via HPLC

The purpose was to quantify the residual amounts of 2,4-D in the culture broth. To quantify residual 2,4-D, a 500 mL sample of culture broth was subjected to solid-phase extraction (SPE) via a C18 SPE cartridge (500 mg, 6 mL). The analytes were eluted with 5% w/v ammonium formate in methanol (5 mL) and subsequently analyzed via UHPLC system (Agilent Technology, Model 1290) equipped with a DAD set at a wavelength of 230 nm. Chromatographic separation was performed using a Thermo Scientific™ Hypersil™ BDS C18 column as the stationary phase, while the mobile phase comprised 5 mM ammonium formate solution (pH 4.0) and methanol [20–21]. The percentage of 2,4-D biodegradation was calculated via the following equation (Eq.1).

$$B = (a-b)/a \times 100 \quad \text{(Eq.1)}$$

Where;

B = percentage of biodegradation (%)

a = Initial concentration of 2,4-D on day 0

b = Residual concentration of 2,4-D in the culture medium on day 7 and day 14

Results and discussion

1) Chemical and physical properties of the soil

Postexperiment soil analysis of the maize plots revealed distinct chemical differences between the control and 2,4-D-treated plots. In the control plot, the pH ranged from strongly acidic to moderately acidic, with consistently low organic matter contents. The available phosphorus levels were moderate (21.46–24.83 mg kg^{-1}), showing a temporary increase at 4 h after spraying. The exchangeable potassium levels were high, ranging from 98.78 to 118.94 mg kg^{-1}, and the cation exchange capacity (CEC) was moderate. In contrast, the pH of the 2,4-D-treated plots ranged from very strongly acidic to moderately acidic, with a low organic matter content throughout. The available phosphorus levels were generally high (27.15–30.07 mg kg^{-1}) but temporarily decreased to moderate levels 4 hours after spraying. The exchangeable potassium levels fluctuated from moderate to high, and the CEC remained moderate, as presented in Table 1.

The soil analysis results obtained before and after the experiment serve as basic data for examining the relationships between soil microorganism populations and 2,4-D residues. The changes in nutrient levels observed after the experiment, whether they increased or decreased, may be correlated with the 2,4-D content and its influence on the metabolic processes of soil microorganisms. This, in turn, can impact nutrient cycling and diversity in the soil [15, 23].

Table 1 General characteristics of the soils after the experiment

Treatments	pH (1:1)	OM (g kg^{-1})	Avail. P (mg kg^{-1})	Exch. K (mg kg^{-1})	CEC (cmol kg^{-1})	Particle size distribution (%)			Textural class
						Sand	Silt	clay	
Control									
2 H	5.64	12.19	24.83	100.00	10.27	41.56	35.71	22.72	Loam
4 H	5.46	14.15	27.62	118.94	11.15	39.83	37.71	22.47	Loam
1 D	5.77	14.01	23.57	104.71	10.75	35.08	40.49	24.43	Loam
3 D	5.64	13.60	21.46	98.78	10.37	37.41	38.82	23.77	Loam
7 D	5.66	12.24	23.37	110.13	10.35	40.09	40.45	19.46	Loam
2,4-D									
2 H AS[1]	5.64	12.96	28.93	90.31	11.52	29.05	44.69	26.26	Loam
4 H AS	5.42	14.30	21.07	80.46	11.58	32.22	31.28	26.5	Loam
1 D AS	5.03	13.56	27.96	94.82	11.56	29.44	44.4	26.16	Loam
3 D AS	5.29	12.81	27.15	94.87	11.51	31.37	42.5	26.13	Loam
7 D AS	5.16	12.44	30.07	99.07	11.41	29.87	47.11	23.02	Loam

Remark: [1] AS = After spraying 2,4-D

2) Counts of soil microorganisms

The analysis of microorganism counts via the plate count method [24] in the maize plots in Lopburi Province revealed notable differences in bacterial, fungal, and actinomycete counts between the control plots and those sprayed with 2,4-D (Figure 1). In terms of bacterial counts, the control plots had average bacterial counts of 6.61 and 6.70 Log_{10} CFU at 2 and 4 hours, respectively, with counts of 6.63, 6.40, and 6.32 Log_{10} CFU at 1, 3, and 7 days, respectively. In contrast, the 2,4-D sprayed plots consistently presented higher bacterial counts, with 7.18 and 7.21 Log_{10} CFU recorded at 2 and 4 hours postspraying, respectively. Statistical analysis via an independent sample t test at the 95% confidence level confirmed that the bacterial counts in the 2,4-D sprayed plots were significantly greater than those in the control plots. This enhanced bacterial growth is likely due to the ability of certain bacteria to use 2,4-D as a carbon source [25]. Similarly, Smith and Mortensen [26] reported that *Pseudomonas testosteroni*, a bacterium frequently found in areas with year-round 2,4-D application, could utilize 2,4-D to support bacterial growth. Köksoy and Uraz [27] reported that various strains of Pseudomonas, including *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, have been isolated for their ability to degrade 2,4-D. These bacteria can utilize 2,4-D as a carbon source, contributing to its breakdown in contaminated soils.

For fungal counts, the control plots presented averages of 4.60 and 4.53 Log_{10} CFU at 2 and 4 hours, with values decreasing to 4.55, 4.22, and 4.33 Log_{10} CFU at 1, 3, and 7 days, respectively. In contrast, the 2,4-D-sprayed plots presented higher fungal counts, with averages of 4.95 and 4.94 Log_{10} CFU at 2 and 4 hours postspraying, respectively. T test analysis revealed statistically significant differences between the control and 2,4-D-treated plots, indicating that 2,4-D application promoted fungal growth. The increase could be attributed to certain fungi utilizing 2,4-D as a carbon and nitrogen source, facilitating their proliferation [14]. The study by Nguyen et al. [28] examined three fungi for their ability to degrade 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid and reported that fungi use 2,4-D (2,4-dichlorophenoxyacetic acid) as a nitrogen source. Additionally, Serbent et al. [29] focused on the metabolic breakdown of the herbicide rather than its utilization as a nitrogen source by bacteria and fungi.

In the case of actinomycete counts, the control plots recorded 6.03 and 6.67 Log_{10} CFU at 2 and 4 hours, with slightly higher counts of 6.86, 6.83, and 6.77 Log_{10} CFU at 1, 3, and 7 days, respectively. The percentages of actinomycetes in the 2,4-D sprayed plots were 5.74 and 6.63 Log_{10} CFU at 2 and 4 hours postspraying, respectively. Unlike the bacterial and fungal populations, the actinomycete counts did not significantly differ between the control and 2,4-D sprayed plots at most time points, as confirmed by the t test. Nonetheless, a significant reduction in actinomycete counts was observed in the 2,4-D sprayed plots 2 hours after spraying, suggesting a temporary inhibitory effect of 2,4-D on actinomycetes.

3) Residual 2,4-D levels in the soil

The analysis of residual 2,4-D in the soil samples from the experimental plots revealed clear differences between the control and treated plots. In the untreated control plots, no residual 2,4-D was detected throughout the experiment. However, in the plots sprayed with 2,4-D at a rate of 30 grams of active ingredient per 400 m^2, the residual 2,4-D levels were initially measured at 0.53 mg kg^{-1} and 0.19 mg kg^{-1} of soil at 2 and 4 hours post-application, respectively. No detectable residues remained in the soil samples taken 1, 3, and 7 days after spraying (Figure 2). These findings align with reports by Fu et al.

[30] that analyzed the degradation of 2,4-D in natural agricultural soils of Fuzhou, China, via capillary electrophoresis. The results indicated that 2,4-D degraded rapidly in these soils, with a calculated half-life of 4.6 days at 27 °C. These findings suggest that in certain agricultural soils, 2,4-D can be efficiently decomposed by soil microorganisms under optimal conditions. Vogue et al. [4] reported that the half-life of 2,4-D in soil is approximately 10 days, although it can be shorter in water depending on the environmental conditions. Similarly, Gonod et al. [31] examined the impact of 2,4-D on bacterial communities and their degradation in soil and reported that no residues were detectable after 7 days, which is consistent with the rapid dissipation observed in this study.

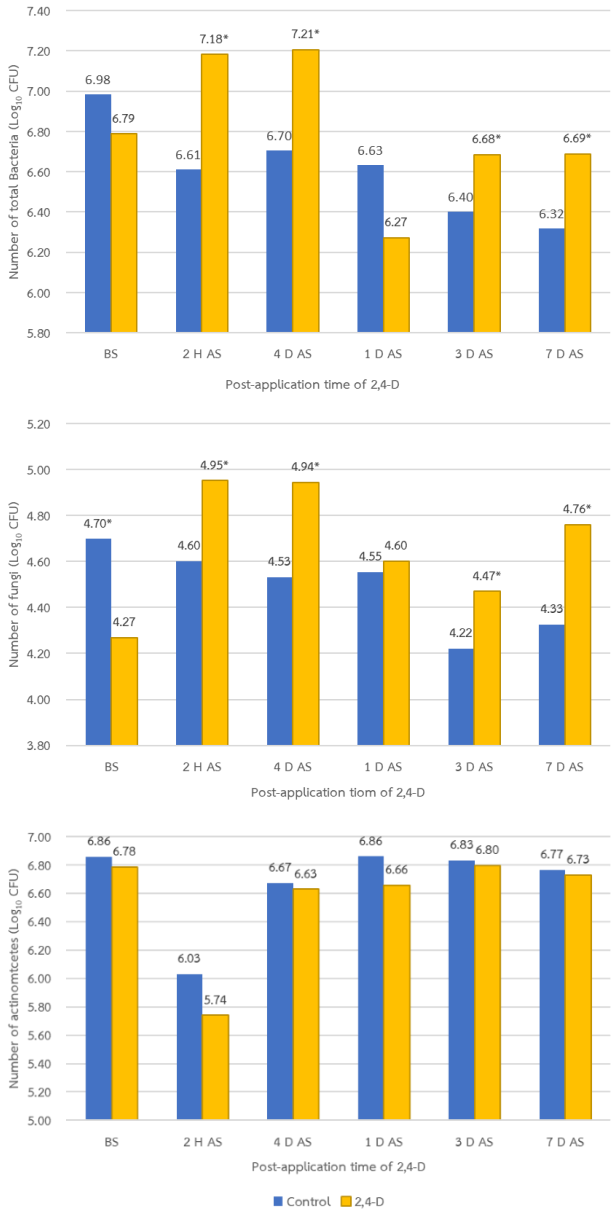

Figure 1 Counts of microorganisms after spraying 2,4-D.
Remarks: BS = before spraying 2,4-D, AS = after spraying 2,4-D.
* Significantly different at the 0.05 level.

The half-life of 2,4-D in soil is influenced by various factors, including the soil type, microbial activity, temperature, and moisture content. While the compound generally degrades within a few days to weeks in many soils, its persistence can be longer in environments with lower microbial activity or less favorable conditions for degradation.

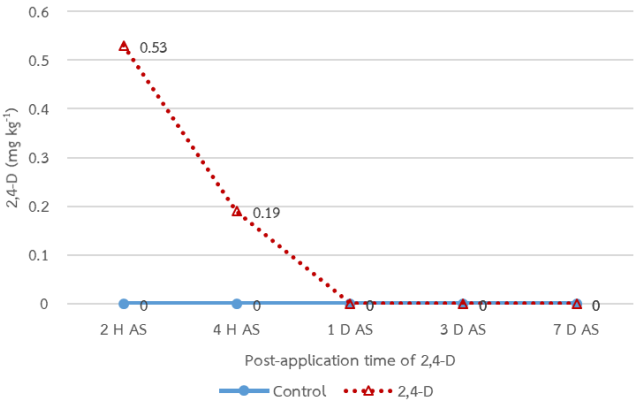

Figure 2 Quantities of 2,4-D in soil.
Remarks: AS = After spraying 2,4-D, 0 = Not detected (< limit of detection (LOD) = 0.03 mg kg^{-1})

4) Factors affecting the soil microbial population

The soil properties, microbial populations, and 2,4-D concentrations were analyzed via Statistica 8 (Figure 3), revealing that the total variance explained was 59.77%, which was divided across two principal components. The first principal component (PC1) included the 2,4-D concentration, bacterial and fungal populations, organic matter, clay content, and cation exchange capacity (CEC), accounting for 38.61% of the variance. In the 2,4-D-treated plot, 2- and 4-h postapplication, the 2,4-D concentration was positively correlated with microbial populations, promoting bacterial and fungal growth due to the ability of certain bacteria and fungi to use 2,4-D as a carbon source [14, 25]. This finding is consistent with that of Han et al. [32], who reported that *Cupriavidus campinensis* can use 2,4-D as a carbon and energy source. Similarly, Magnoli et al. [33] reported that fungi are capable of degrading herbicides to fulfill their diverse nutritional requirements. In the absence of alternative sources, these compounds can be utilized as sources of carbon and energy. Furthermore, the 2,4-D concentration was correlated with the organic matter content, clay content, and CEC. With their charged clay particles, fine-textured soils can adsorb more 2,4-D particles than can sand or silt particles [5]. This finding supports the findings of Boivin et al. [3], who reported that the organic matter content and soil pH significantly influence 2,4-D adsorption and degradation. Similarly, Jamshidi et al. [6] investigated the sorption and degradation of 2,4-D in citrus orchard soils in Mazandaran, Iran. Research has revealed that soils with relatively high clay contents and organic carbon contents exhibit increased adsorption of 2,4-D, with relatively high degradation rates observed in soils with elevated organic carbon contents.

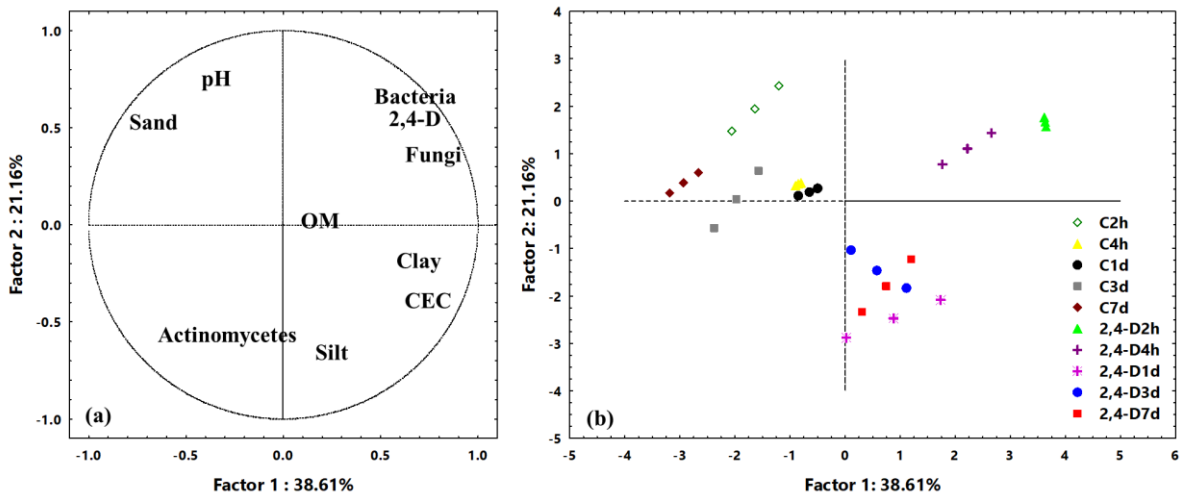

Figure 3 Correlations of the principal component analysis results of the quantities of microorganisms with the quantities of 2,4-D and the soil properties.

5) Testing microorganisms for 2,4-D degradation

Bacteria capable of degrading 2,4-D were isolated via inorganic salt media supplemented with 2,4-D at a concentration of 50 mg L^{-1}, whereas fungi were isolated via rose bengal media supplemented with 2,4-D at a concentration of 100 mg L^{-1} (Figure 4). The selected microorganisms were subsequently purified and identified. Two bacterial isolates, *Bacillus cereus* and *Bacillus albus*, and one actinomycete isolate, *Nocardioides aromaticivorans, were used*. Three isolates were selected. Two fungal isolates, *Penicillium pimiteouiense* and *Penicillium shearii*, were obtained. All five microbial strains exhibited robust growth in media supplemented with 2,4-D, as shown in Table 2. Similarly, Magnoli et al. [33] isolated organochlorine-tolerant fungi from herbicide-contaminated soils and assessed their ability to remove 2,4-D in synthetic wastewater and reported that *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. were the most frequently isolated genera that were able to tolerate 2,4-D contamination in wastewater. Silva et al. [34] isolated microorganisms from contaminated soil and reported that *Serratia marcescens* and *Penicillium* sp. were effective at degrading 2,4-D, demonstrating significant potential for environmental bioremediation. Kizilkaya [36] investigated bacterial growth in media supplemented with varying concentrations of 2,4-D and reported that higher concen-trations inhibited the growth of bacteria, including *Bacillus cereus* var. *mycoides*. However, at a lower concentration of 0.2 μg g^{-1}, both general bacterial populations and *B. cereus* var. *mycoides* were able to thrive. In contrast, Huong et al. [37] isolated bacteria capable of degrading 2,4-D from Vietnamese soils and identified three predominant bacterial groups: *Burkholderia* spp. (43.3%), *Sphingomonas* spp. (40.2%), and *Ralstonia* spp. (15.3%).

Table 2 Microbial species for the degradation test of 2,4-D

No.	Code	Microorganism species
1	LB-B8	*Bacillus cereus*
2	NR-B3	*Nocardioides aromaticivorans*
3	NR-B5	*Bacillus albus*
4	NR-F5	*Penicillium pimiteouiense*
5	NR-F14	*Penicillium pimiteouiense*
6	NR-F15	*Penicillium shearii*

The biodegradation of 2,4-D by bacterial isolates was tested in inorganic salt broth (pH 7.0) at a concentration of 50 mg L^{-1} without an added carbon source, and the bacteria were incubated in the dark at 30°C with shaking for 7 days. The 2,4-D concentration was subsequently measured via HPLC-DAD. The results indicated that all three isolates could degrade 2,4-D, with *Bacillus albus* achieving the highest degradation rate of 23.84%, followed by *Nocardioides aromaticivorans* at 19.74% and *Bacillus cereus* at 13.32% (Table 3). In support of this, Matafonova et al. [35] reported that *Bacillus cereus* BIP507 can degrade 2,4-dichlorophenol at concentrations up to 560 μM. Similarly, Vanitha et al. [38] reported that three bacterial strains, identified as *Arthrobacter* sp. SVMIICT25, *Sphingomonas* sp. SVMIICT11, and *Stenotrophomonas* sp. SVMIICT13, were isolated from agricultural soil and demonstrated the ability to degrade 2,4-D. Over a 12-day incubation period, these strains achieved 81–90% degradation of 100 mg L^{-1} 2,4-D at a 2% inoculum concentration. Udompratyaporn [39] tested the 2,4-D degradation abilities of bacteria from soils with and without a history of 2,4-D exposure. At a concentration of 5,000 ppm, three isolates (*Klebsiella oxytoca*, *Klebsiella pneumonia*, and *Pseudomonas mendocina*) could grow, but when tested at 1,500 ppm in liquid medium, only *Pseudomonas mendocina* was capable of degrading 2,4-D, achieving a degradation rate of 12%. These findings highlight the potential of certain bacterial species for the bioremediation of 2,4-D-contaminated environments.

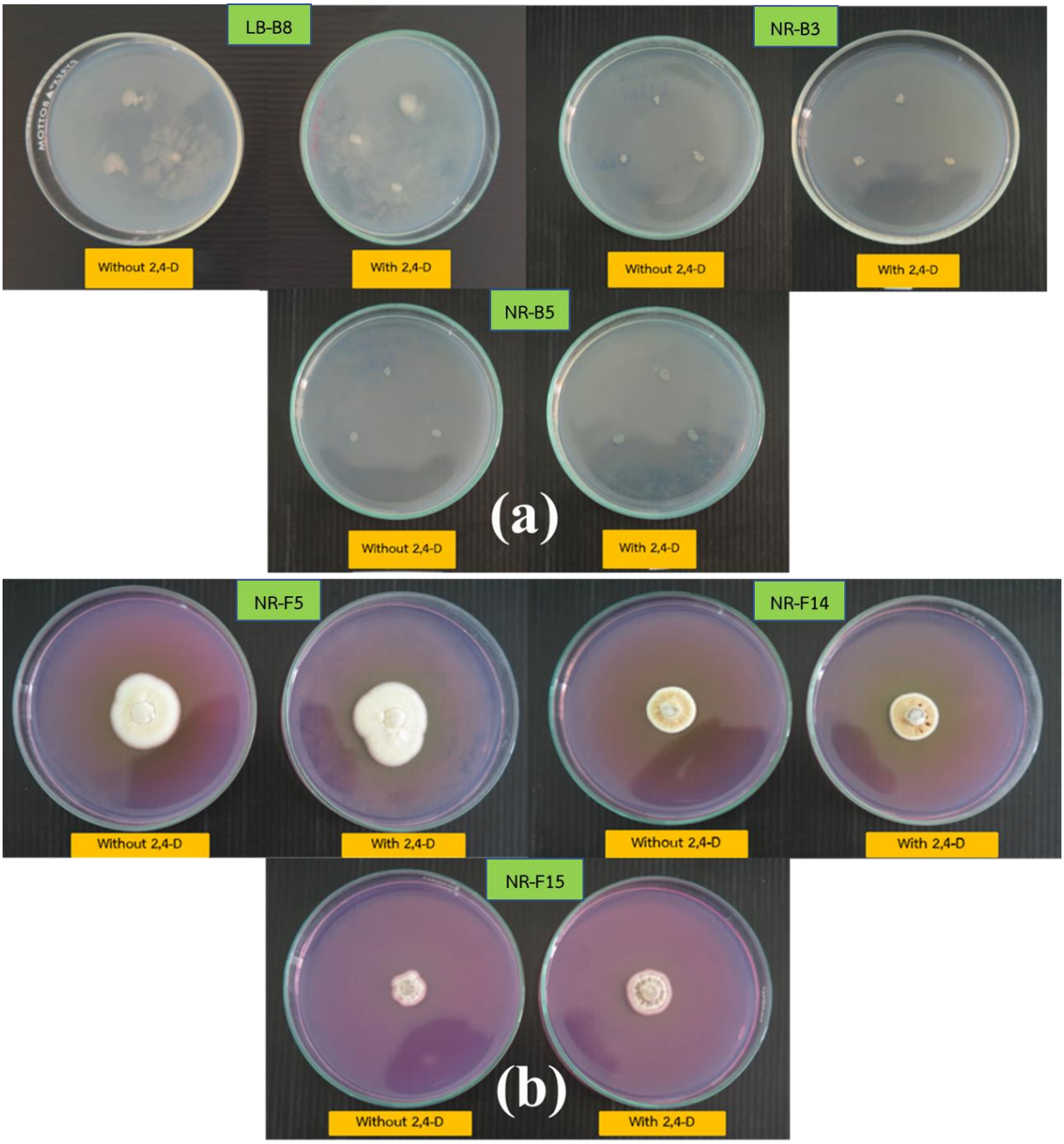

Figure 4 Degradation of 2,4-dichlorophenoxyacetic acid herbicide by bacteria isolated from soil in (a) inorganic salt media and (b) fungi isolated from soil in rose bengal media.

Table 3 Biodegradation of 2,4-D by bacteria after 7 days.

Treatments	Code	Concentration of 2,4-D (mg L^{-1})	Biodegradation of 2,4-D (%)
ISB*	-	44.74	-
ISB + *Nocardioides aromaticivorans*	LB-B8	40.13	19.74
ISB + *Bacillus albus*	NR-B3	38.08	23.84
ISB + *Bacillus cereus*	NR-B5	43.34	13.32

Remarks: * ISB = 50 mg L^{-1} -2,4D-inorganic salt broth.

The biodegradation of 2,4-D by fungi was tested in Czapek Dox broth (100 mg L^{-1}) without a nitrogen source and incubated in the dark at 30°C with shaking for 14 days. The concentration of 2,4-D was then measured via HPLC-DAD. The results indicated that all three fungal isolates were capable of degrading 2,4-D, with *Penicillium shearii* achieving the highest degradation rate at 50.42%, followed by *Penicillium pimiteouiense* at 47.25%, and a third isolate at 44.22% (Table 4). In support of these findings, [33] isolated fungi from 2,4-D-contaminated soils and reported that *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. could tolerate

and degrade 2,4-D, with six *Penicillium* isolates showing tolerance at a concentration of 25 mmol L^{-1}. *Penicillium* strains were particularly effective, with two strains degrading over 50% of 2,4-D within 7 days and up to 75% after 14 days, indicating their potential for bioaugmentation strategies aimed at reducing 2,4-D contamination in wastewater. Bhosle and Thore, [22] also demonstrated effective 2,4-D degradation in soil by *Trichoderma viride*, *Trichoderma koningii*, *Penicillium chrysogenum*, and *Rhizopus stolonifer*. Similarly, Ferreira-Guedes et al. [40] studied 2,4-D degradation by a halotolerant strain of *Penicillium chrysogenum* and reported that it could grow on solid media with 2,4-D concentrations of 100–1,000 mg L^{-1}, using the compound as a carbon and energy source. The findings of these studies indicate the capacity of various fungi to tolerate and degrade 2,4-D, suggesting their potential in bioremediation applications.

The presence of 2,4-D in soil can alter the population structure and functionality of soil microorganisms, thereby influencing nutrient cycling and microbial diversity. As 2,4-D can serve as a carbon and energy source, it facilitates microbial metabolic activities [15, 41]. In examining this effect, [25] isolated soil bacteria to assess the impact of 2,4-D on their metabolism and reported that *Cupriavidus necator* had the highest capacity to metabolize 2,4-D in soil in the Sauce Grande River Basin (Argentina). Similarly, Silva et al. [34] isolated *Serratia marcescens* and *Penicillium* sp. from Brazilian soil contaminated with 2,4-D herbicide as microorganisms capable of significant 2,4-D degradation, indicating their potential utility in bioremediation. These findings suggest that specific microbial populations can not only adapt to the presence of 2,4-D but also contribute to its breakdown, supporting soil health and mitigating its environmental impact.

Table 4 Biodegradation of 2,4-D by fungi after 14 days

Treatments	Code	Concentration of 2,4-D (mg L^{-1})	Biodegradation of 2,4-D (%)
CDB*	-	60.79	-
CDB + *Penicillium pimiteouiense*	NR-F5	52.75	47.25
CDB + *Penicillium pimiteouiense*	NR-F14	55.78	44.22
CDB + *Penicillium shearii*	NR-F15	49.58	50.42

Remarks: * CDB = 100 mg L^{-1} -2,4D- Czapek DOX broth.

Conclusion and recommendation

The experiment revealed that 2,4-D application had a statistically significant effect on bacterial and fungal counts in both the control and 2,4-D sprayed plots, as measured on culture media. The increased bacterial and fungal counts may be attributed to their ability to utilize 2,4-D as a carbon source, thereby promoting their growth. In contrast, actinomycete counts in the culture media were not significantly different between the control and treated plots. The residual 2,4-D levels in the soil were 53.0 mg kg^{-1} and 0.19 mg kg^{-1} at 2 and 4 hours post-spraying, respectively. However, no detectable 2,4-D residues were detected in the soil samples collected at 1, 3, or 7 days after spraying. Analysis of the soil properties, microorganism counts, and 2,4-D levels revealed signi-ficant correlations. The 2,4-D levels were closely associated with the number of microorganisms. In addition, 2,4-D levels were correlated with the soil organic matter content, clay content, and cation exchange capacity.

Microorganism colonies grown on culture media with and without 2,4-D were classified, and three isolates each of fungi and bacteria resistant to 2,4-D were selected for further testing in a 2,4-D degradation assay. The results revealed that all three bacterial isolates were capable of degrading 2,4-D, with *Bacillus albus* achieving the highest degradation rate of 23.84%, followed by *Nocardioides aromaticivorans* and *Bacillus cereus* at 19.74% and 13.32%, respectively. Similarly, all three fungal isolates demonstrated 2,4-D degradation abilities, with *Penicillium shearii* achieving the highest degradation rate at 50.42%, followed by *Penicillium pimiteouiense* at 47.25% and a third isolate at 44.22%. These findings indicate that 2,4-D supports the growth of certain bacterial and fungal populations by serving as a carbon and energy source, thereby promoting microbial proliferation and biodegradation capabilities.

Acknowledgement

This work was supported by the Thailand Science Research and Innovation for financial support.

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