ISOLATION OF ETHOPROPHOS-DEGRADING BACTERIA AND PHYTOREMEDIATION–MICROBIAL REMEDIATION FROM SOIL FOR THE PROJECT OF WATER DIVERSION FROM THE YANGTZE RIVER TO CHAOHU LAKE

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Abstract. Ethoprophos contamination in soils in "the project of water diversion from the Yangtze River to Chaohu Lake" is a wide-ranging and non-negligible environmental problem. This study isolated ethoprophos-degrading microorganisms from typical riparian zones of the project area and conducted pot experiments using native herbaceous plants to evaluate the effectiveness of combined phytoremediation and microbial remediation for ethoprophos-contaminated soils. Five ethoprophos-degrading strains were successfully isolated and identified as *Bacillus badius*, *Serratia liquefaciens*, *Klebsiella aerogenes*, *Stenotrophomonas pavanii*, and *Acinetobacter seifertii*. While the degradation efficiencies of these strains decreased with increasing ethoprophos concentrations (from 10 mg/L to 50 mg/L), supplementation with 0.1 g/L glucose significantly enhanced their degradation capabilities. Among these strains, *Acinetobacter seifertii* demonstrated superior degradation efficiency, reaching a maximum of 80.75%, and was consequently selected for subsequent pot experiments. The combined remediation approach using *Acinetobacter seifertii* with *Polygonum lapathifolium*, *Setaria viridis*, and *Carex dimorpholepis* revealed that, after 30 days of treatment, the degradation efficiency of the combined system significantly outperformed individual microbial or plant treatments. Notably, the combination of *Acinetobacter seifertii* and *Carex dimorpholepis* achieved the highest degradation rate of 93.27%. These findings provide a promising bioremediation strategy for addressing ethoprophos contamination in soils.
Keywords: *ethoprophos, riparian zones, bioremediation, organophosphate pesticides*

Introduction

Ethoprophos is extensively utilized in the cultivation of fruits, vegetables, and a range of agricultural commodities due to its potent insecticidal effects (Witczak et al., 2018). Nevertheless, ethoprophos exhibits high mobility (Dowling et al., 1994), which contributes to its accumulation in soil and poses a risk of leaching into aquatic systems (Li et al., 2021a). As a result, ethoprophos is commonly detected in both soil and freshwater ecosystems. Furthermore, ethoprophos is characterized by its chemical stability, extended environmental half-life (Yuan et al., 2021), and considerable health hazards due to its acute toxicity (Çakır et al., 2024). Consequently, effective remediation of ethoprophos-contaminated environments is essential for ensuring ecological safety and public health.

The degradation of organophosphorus pesticides occurs via both physical–chemical and biological routes in natural settings. Although most organophosphorus pesticides can degrade through physical–chemical processes (Kaushal et al., 2021), these approaches are inefficient and rarely achieve complete degradation. In contrast, bioremediation can

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achieve degradation rates that are more than tenfold faster than those achieved by physical–chemical processes (Kumar et al., 2018). Research suggests that microorganisms play a pivotal role in the degradation of most organophosphorus pesticides in the environment (Lu et al., 2013; Raj et al., 2024). Consequently, the use of microorganisms with rapid degradation capabilities for bioremediation has been extensively studied for remediating organophosphorus-contaminated soils. For example, Singh et al. (2017) isolated *Pseudomonas pseudoalcaligenes* from a contaminated site, achieving a degradation rate of 95% for 100 mg/L acephate within 14 days. Gao et al. (2019) isolated *Bacillus* species from agricultural soils, achieving degradation rates of 73.43% and 70.45% for 1 mg/L methyl parathion and trichlorfon, respectively. However, research on ethoprophos-degrading microorganisms remains limited compared with that on other organophosphorus pesticides.

Microbial degradation of organophosphorus compounds typically involves hydrolyzing P-O alkyl and aryl bonds (Malik et al., 2014; Li et al., 2019; Mishra et al., 2021). However, the viability and functionality of soil microorganisms can be significantly influenced by environmental factors such as surface vegetation and soil moisture (Delgado-Baquerizo et al., 2016; Mason-Jones et al., 2022). Moreover, highly efficient organophosphorus-degrading bacteria isolated under controlled laboratory conditions often exhibit reduced performance in natural applications, potentially due to the complexities of heterogeneous soil environments and intense interspecies competition, which may hinder the successful establishment and proliferation of introduced microbial strains (Khare and Arora, 2015). Plant–microbe synergistic remediation offers a potential solution to these challenges. Through the secretion of amino acids, sugars, and organic acids, plants can create additional ecological niches that increase the degradation capacity of microorganisms (Chu et al., 2016; Zheng et al., 2021). For example, Lin and You (2009) demonstrated that the combination of *Arthrobacter* with *sorghum hybrid sudangrass*, *Medicago sativa*, and *Lolium perenne* significantly improved the degradation of chlorpyrifos-contaminated soil, with synergistic plant–microbe remediation surpassing the effectiveness of either plants or microbes alone. Therefore, plant–microbe co-remediation is considered a promising approach for remediating ethoprophos-contaminated soils, although no comprehensive studies have been conducted to date.

The project of water diversion from the Yangtze River to Chaohu Lake (YC-project) is a strategic water resource engineering project that supports water allocation in Anhui Province, drawing water from the Yangtze River into Caizi Lake and passing through the Kongcheng River, Luobu River, and Baishi River before reaching Chaohu Lake (*Figure 1*). Previous studies have detected varying levels of ethoprophos accumulation in water and sediments along the project route, indicating high ecological risks in certain areas (Song et al., 2022). To date, only a few bacteria, such as *Bacillus cereus*, *Sphingomonas* sp., and *Pseudomonas* sp., have been reported to efficiently degrade ethoprophos, all of which were isolated from agricultural ecosystems (Karpouzas et al., 2000; Karpouzas and Walker, 2000). As a transitional zone between land and water, riparian zones differ significantly from agricultural ecosystems. However, whether efficient ethoprophos-degrading bacteria exist in riparian zones remains unknown. To address ethoprophos contamination in soils along the YC project route, this study collected rhizosphere soils from riparian zones along the project route to isolate highly efficient ethoprophos-degrading bacteria. Five bacterial strains were successfully isolated, and pot experiments were conducted to investigate the plant–microbe synergistic

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degradation of ethoprophos in soil. The findings of this study have important implications for remediating ethoprophos-contaminated soils along the project route and for expanding the repository of ethoprophos-degrading bacteria.

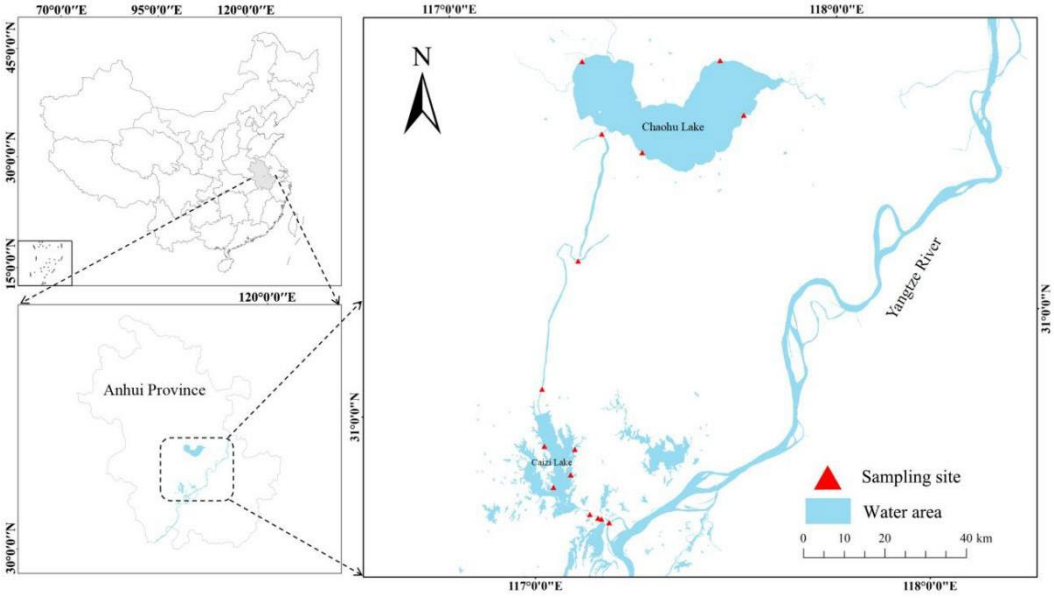

Figure 1. *Distribution of soil sampling sites*

Materials and methods

Soil sample collection

In October 2022, soil samples were collected from 15 sites within the riparian zones along the YC project route (*Figure 1*). Each sampling site was representative of the typical vegetation types of the region and was evenly distributed along the project route. At each site, three to six (1 m×1 m) quadrats were established perpendicular to the river or lake riparian zones. Rhizosphere soil samples were collected to a depth of 20 cm using a soil auger (1.0 m×50 mm) within areas of dense vegetation within each quadrat. The samples were then sealed in polyethylene bags, stored in drikold containers, and transported to the laboratory on the same day for storage at −4 °C.

Screening of ethoprophos-degrading bacteria

Ethoprophos-degrading bacteria (EDB) were isolated and purified following the method described by Lin and You (2009). The procedure was as follows: (1) A 10 g soil sample was combined with 50 mL of sterile water in a centrifuge tube and agitated vigorously. The supernatant was then transferred to 100 mL of beef extract peptone broth (3 g of beef extract, 5 g of NaCl, 10 g of peptone, 1000 mL of deionized water, pH 6.8) and incubated for 24 hours at 30 °C with shaking at 150 rpm. (2) The culture was streaked onto beef extract peptone agar plates supplemented with 30 mg/L ethoprophos using an inoculating loop and incubated at 30 °C for 48 hours. Colonies were then transferred to plates with increasing ethoprophos concentrations of 60 and 120 mg/L. (3) The acclimated colonies were streaked onto inorganic salt agar plates containing 240 mg/L or 500 mg/L ethoprophos as the sole carbon source (Na_2HPO_4, 6.34 g; KH_2PO_4, 1.33 g;

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$(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4$, 0.001 g; $CaCl_2$, 0.04 g; agar, 15 g; deionized water, 1000 mL; pH 6.8) and incubated at 30 °C for 10 days. (4) Bacterial cultures were propagated from isolated colonies, diluted in sterile water to 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}, and plated for single colony isolation. The resulting colonies were stored in glycerol stocks at −60 °C for long-term preservation (Jiang et al., 2006).

Physiological characteristics and DNA sequencing of ethoprophos-degrading strains

After the isolation of the EDB, the colony morphology, including shape, edge, and color, was inspected on solid medium following a 24-hour incubation at 30 °C. Subsequently, Gram staining was carried out to determine their cell wall characteristics. The physiological attributes of the EDBs were assessed in a series of tests: the methyl red staining (MR), Voges–Proskauer tests (VP), gelatin hydrolysis tests, starch hydrolysis tests, nitrate reduction tests, and catalase tests. The EDB was inoculated into beef extract peptone medium (pH=6.8) using an inoculation loop and cultured at 20 °C, 25 °C, 30 °C, 35 °C, or 40 °C at 150 rpm. The OD_{600} value of the bacterial suspension was measured 12 hours later to determine the growth status of the strain at different temperatures. The EDB was inoculated into beef extract peptone at pH values of 5, 6, 7, 8, and 9 using an inoculation loop and cultured at 30 °C and 150 rpm. The OD_{600} was measured every 4 h to determine the growth status of the strain under different pH conditions. Following the measurement of OD_{600} values under varying temperature and pH conditions, we constructed comprehensive growth curves for each parameter set. The specific temperature and pH corresponding to the maximum OD_{600} value were identified as the optimal growth conditions for EDBs.

The EDBs were analyzed by 16S rRNA gene sequencing. Genomic DNA was extracted and amplified using the forward primer B341F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer B785R (5′-GACTACHVGGGTATCTAAT-3′). The polymerase chain reaction (PCR) protocol involved initial denaturation at 95 °C for 3 minutes, followed by 25 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 54 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was performed at 72 °C for 5 minutes. The PCR products were confirmed by 2% agarose gel electrophoresis and subsequently sequenced by Sangon Biotech Co., Ltd. (Jiangsu, China). Homologous sequences were determined through a BLAST search in the National Center for Biotechnology Information (NCBI) database. Phylogenetic trees were constructed using MEGA11 software.

Measurement of ethoprophos degradation efficiency

Ethoprophos at different concentrations of 10, 20, and 50 mg/L were prepared in an inorganic salt medium and poured into 150 mL conical flasks. Then, the flasks were inoculated with 4% (v/v) EDB suspension, and a filter membrane with pores was placed over the mouth of each flask to allow air circulation while preventing microbial contamination. Sterile water was used in place of the EDB suspension for the blank controls. Each treatment was replicated three times. All the treatments were incubated in a shaker at 30 °C and 150 rpm for 7 days. After the incubation period, 5 mL of culture broth was collected and centrifuged at 4,000 rpm for 3 minutes. One milliliter of the supernatant was filtered through a 0.22 μm aqueous filter membrane, followed by extraction with 10 mL of acetonitrile after vigorous shaking for 2 minutes and ultrasonic extraction for 20 minutes. Then, 1 g of anhydrous sodium chloride was added, and the

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mixture was allowed to stand for 30 minutes to facilitate phase separation. The upper organic layer was collected for determination of the ethoprophos concentration. An additional set of experiments was conducted with the addition of 0.1 g/L glucose as an exogenous carbon source to the medium under the same conditions as described above to investigate the effect of the added carbon source on the degradation of ethoprophos.

Ethoprophos concentration determination

The concentration of ethoprophos was quantified using a gas chromatography–mass spectrometry (GC–MS) system, specifically a GCMS-TQ8040 triple quadrupole instrument equipped with an SH-Rxi-17Sil MS polar column (30 m × 0.25 mm × 0.25 μm). The operational parameters were as follows: the inlet temperature was set at 250 °C, the interface temperature was 280 °C, and the ion source temperature was 240 °C. High-purity helium (99.999%) served as the carrier gas, with a flow rate of 1.97 mL/min. For sample injection, a volume of 1 μL was used in selected ion monitoring (SIM) mode. The temperature program for the GC oven was structured as follows: initial hold at 65 °C for 1 minute, followed by a ramp to 130 °C at a rate of 20 °C/min, then to 280 °C at 10 °C/min with a 10-minute hold, and finally, a ramp to 300 °C at 10 °C/min with another 10-minute hold.

The standard curve was established by plotting ethoprophos concentration (x-axis) against corresponding peak area (y-axis), generating a linear regression equation of $y = 670146x - 116701$. The calibration curve demonstrated excellent linearity across the concentration range of 0.1, 0.5, 2, 5, and 10 mg/L, as evidenced by a correlation coefficient (R^2) of 0.9997. Method sensitivity was determined with a limit of detection of 0.01 mg/L in liquid matrices and 0.6 μg/kg in soil samples. Method validation in inorganic salt medium spiked with varying ethoprophos concentrations showed satisfactory recovery rates ranging from 95.41% to 105.78%, with relative standard deviations between 2.76% and 6.16%, fulfilling the established criteria for pesticide residue analysis.

Plant–bacteria co-degradation of ethoprophos

The soil employed in the potting experiment was a 1:1 blend of peat and riparian soil, which was sterilized and then distributed into plastic pots (9.8 cm in height, 9.8 cm in width, containing 300 g of soil per pot). The moisture content was adjusted to 35%. Healthy and uniformly growing plants from the riparian zone, including *Polygonum lapathifolium*, *Setaria viridis*, and *Carex dimorpholepis*, were chosen as the plant species for the combined degradation experiment. In this study, the selected *Setaria viridis, Polygonum lapathifolium*, and *Carex dimorpholepis* were 20–30 cm, 30–40 cm, and 10–20 cm in height, respectively. The EDB with the highest degradation efficiency found in this study was selected as the microorganism used in the combined degradation experiment. The selected EDB was activated and propagated in beef extract peptone medium and evenly inoculated into each pot according to the experimental setting. Four treatments were established: microorganism-only, plant-only, plant+microorganism, and blank control (CK). Each treatment was replicated five times. All the treatments were incubated at 30 °C for 30 days, with bacterial inoculation every 7 days (10 mL per inoculation) and irrigation with deionized water every 3 days to ensure consistent soil moisture.

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Data analysis

The data were processed using Microsoft Excel 2016, and two-way ANOVA was conducted with SPSS 24.0. Significant differences were identified using Tukey's HSD test with a significance level of $p < 0.05$. Figures were generated using the "ggplot2" package in R (version 4.0.1) (R Core Team, 2022).

Results

Morphological and physiological characteristics of ethoprophos-degrading bacteria

Five EDBs were isolated from soil samples through a series of expansion, acclimatization, and purification steps and were named DE-1, DE-2, DE-3, DE-4, and DE-5 (*Figure 2*). The physiological and biochemical results are presented in *Table 1*. DE-1 was identified as gram-positive, whereas the remaining strains were gram-negative, DE-2 displayed irregular colony edges, whereas the other four strains presented smooth edges. In the methyl red (MR) test, DE-4 and DE-5 exhibited positive reactions, whereas DE-1, DE-2, and DE-3 were negative. In the Voges–Proskauer (VP) test, DE-2 and DE-3 had positive results, whereas DE-1, DE-4, and DE-5 had negative results. In the gelatin hydrolysis test, DE-3 and DE-5 were negative, whereas DE-1, DE-2, and DE-4 were positive. In the starch hydrolysis test, DE-2 was positive, whereas DE-1, DE-3, DE-4, and DE-5 were negative. In the nitrate reduction test, DE-4 and DE-5 were negative, whereas DE-1, DE-2, and DE-3 were positive. In the catalase test, DE-3 was negative, whereas DE-1, DE-2, DE-4, and DE-5 were positive.

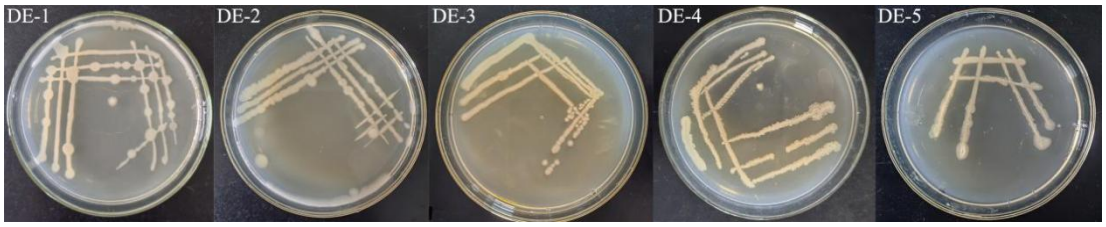

Figure 2. *Morphology of Strains in Culture Medium*

Table 1. *Physiological and Biochemical Identification and morphological description*

Strain	Physiological and biochemical tests							
	MR	**VP**	**Gelatin hydrolysis**	**Starch hydrolysis**	**Nitrate reduction**	**Catalase**	**Gram stain**	**Morphology**
DE-1	-	-	+	-	+	+	+	Milky white, smooth edge
DE-2	-	+	+	+	+	+	-	White, Irregular edge
DE-3	-	+	-	-	+	-	-	Yellow, Smooth edge
DE-4	+	-	+	-	-	+	-	Gray, Smooth edge
DE-5	+	-	-	-	-	+	-	White, Smooth edge

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The temperature had a significant influence on the growth of the five EDBs, with growth rates initially increasing before stabilizing or decreasing across a temperature range of 20–40 °C, and the optimal temperature for all EDBs was 35 °C (*Figure 3*). In contrast, pH had a relatively minor effect on growth (except for DE-1), with stable optical density (OD_{600}) values between 16 and 20 h, and the optimal pH values for each strain were determined to be 8, 8, 7, 7, and 6 (*Figure 4*).

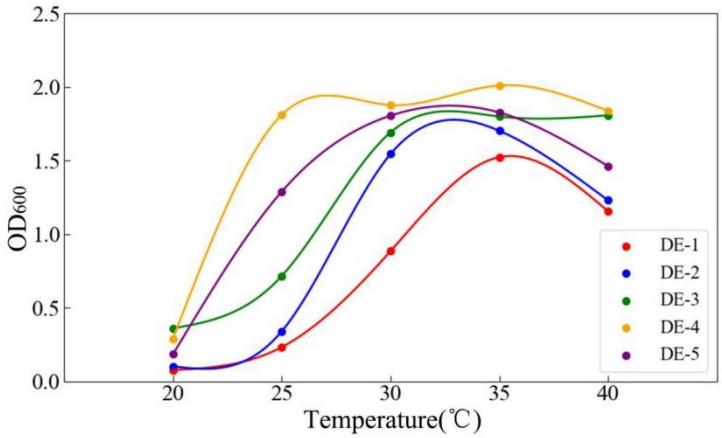

Figure 3. *Effects of Different Temperatures on the Growth of Degrading Strains*

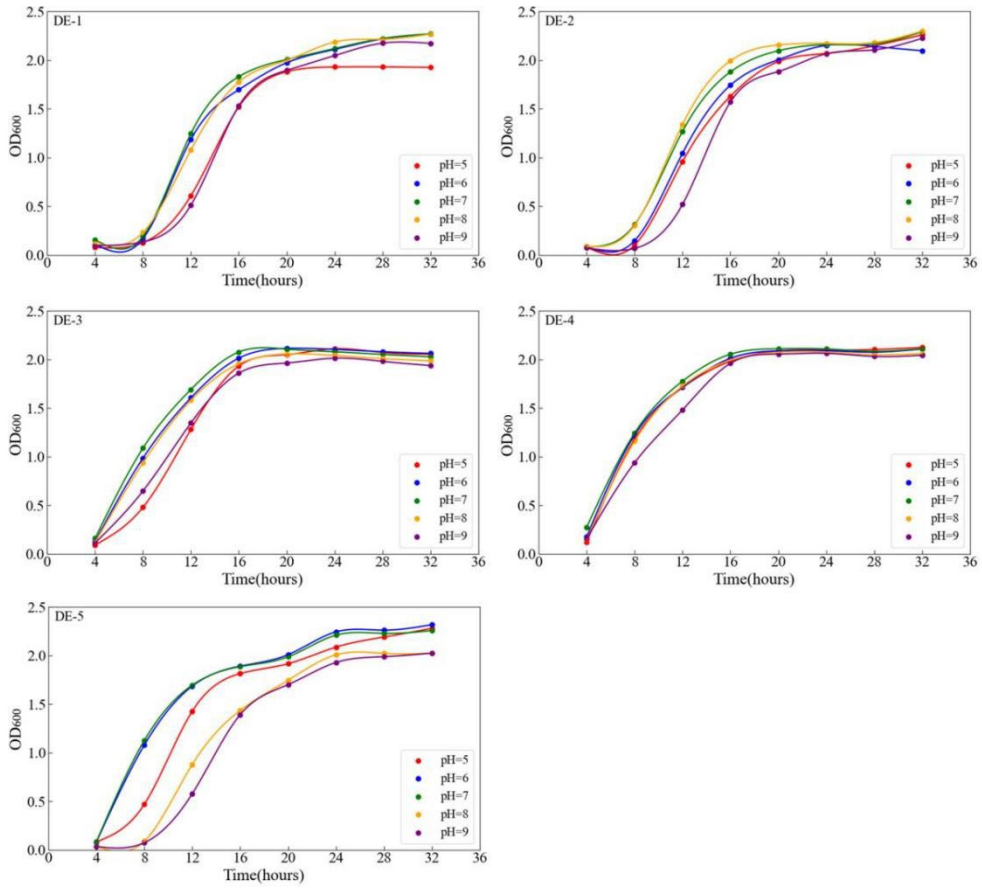

Figure 4. *Growth status of ethoprophos-degrading bacteria at different pH*

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Molecular identification of the degrading strains

The 16S rRNA gene sequences of the five EDBs were analyzed using the Basic Local Alignment Search Tool (BLAST) within the National Center for Biotechnology Information (NCBI) database. These sequences were evaluated in conjunction with their respective physiological and biochemical properties. The identified EDBs were as follows: DE-1 was identified as *Bacillus badius*, DE-2 as *Serratia liquefaciens*, DE-3 as *Klebsiella aerogenes*, DE-4 as *Stenotrophomonas pavanii*, and DE-5 as *Acinetobacter seifertii* (*Figure 5*). DE-1 belongs to the phylum Firmicutes, whereas the other four strains belong to the phylum Proteobacteria. Among the Proteobacteria, DE-2 and DE-3 are members of the family Enterobacteriaceae, DE-4 belongs to the family Xanthomonadaceae, and DE-5 belongs to the family Moraxellaceae.

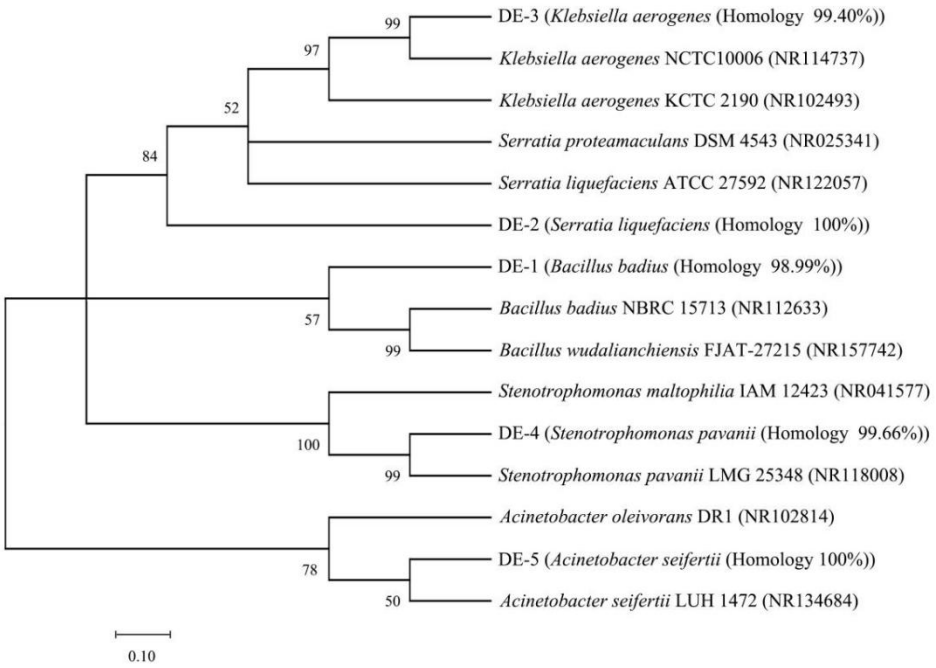

Figure 5. *Phylogenetic relationship of isolated ethoprophos-degrading bacteria. DE-1, DE-2, DE-3, DE-4, and DE-5 were the ethoprophos-degrading bacteria isolated in this study, and other microorganisms were known to be closest to the phylogenetic relationship of ethoprophos-degrading bacteria in the NBCI database. The phylogenetic tree was constructed using the neighbor-joining method*

Degradation efficiency of ethoprophos-degrading bacteria

In this study, all five isolated EDBs displayed ethoprophos-degrading potential, albeit with notable disparities in their degradation capacities (*Figure 6*). However, as the concentration of ethoprophos increased, the degradation rates of all the EDBs tended to decrease. At an ethoprophos concentration of 10 mg/L, the degradation rates of DE-1, DE-2, and DE-5 exceeded 50%, with DE-5 having the highest degradation rate at 63.75%, which was significantly higher than those of DE-2, DE-3, and DE-4, but there was no significant difference compared with DE-1. At an ethoprophos concentration of 20 mg/L, DE-1 exhibited the highest degradation efficiency at 46.18%, followed by DE-5 (41.68%) and DE-2 (38.13%). These degradation rates were significantly higher than those

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observed for DE-3 and DE-4. A similar trend was observed at an ethoprophos concentration of 50 mg/L, where DE-1 and DE-5 maintained its superior degradation performance.

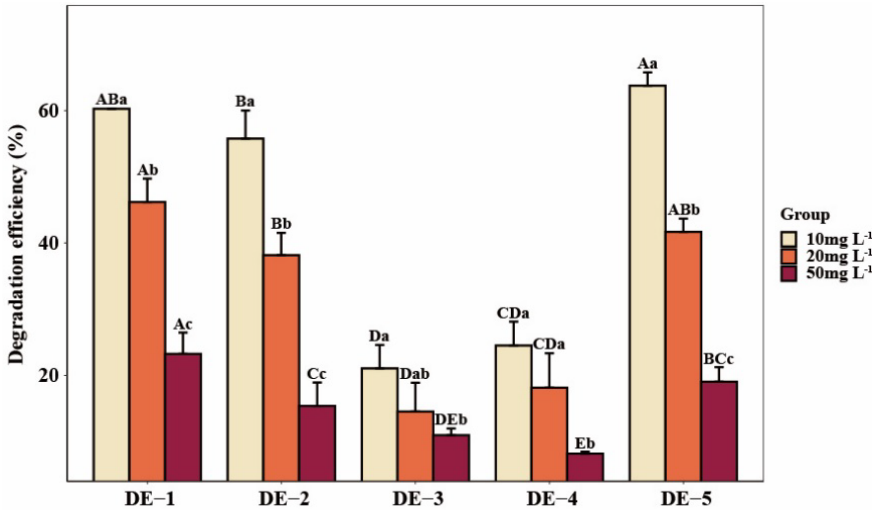

Figure 6. *The degradation ability of ethoprophos-degrading bacteria under different concentrations of ethoprophos. Different uppercase letters indicate significant differences in degradation ability between different ethoprophos-degrading bacteria under the same concentration of ethoprophos, and different lowercase letters indicate significant differences in the degradation ability of the same ethoprophos-degrading bacteria under different concentrations of ethoprophos. $p<0.05$ was considered a significant difference*

The addition of 0.1 g/L glucose to the inorganic salt medium increased the degradation efficiency of ethoprophos by the EDBs, with a more pronounced effect at higher ethoprophos concentrations (*Figure 7*). Moreover, in the treatments with glucose added, DE-5 presented the highest degradation rate, reaching 80.75%, which was significantly greater than those of DE-1, DE-2, DE-3, and DE-4 (10 mg/L ethoprophos). When the ethoprophos concentration was 20 mg/L, the degradation rates of DE-1, DE-2, and DE-5 exceeded 50%, with DE-1 having the highest rate of 57.63%, followed by DE-5 (54.31%). At an ethoprophos concentration of 50 mg/L, strain DE-5 demonstrated the highest degradation efficiency, achieving a rate of 45.13%, which was significantly greater than those of DE-2, DE-3, and DE-4.

Plant–bacteria synergistic degradation of ethoprophos

Pot experiment results showed that, after 30 days of incubation, regardless of the plant species, the combined plant–microbe treatment resulted in significantly higher ethoprophos degradation efficiency than either the plant-only or microbe-only treatments. Furthermore, in comparison with the control (CK), the *Carex dimorpholepis* and DE-5 combination achieved the highest degradation rate of 93.27%, significantly surpassing the *Polygonum lapathifolium* and DE-5 treatment (73.35%). However, the degradation rate of the *Setaria viridis* and DE-5 treatment (77.90%) did not significantly differ from the other two combination treatments (*Figure 8*).

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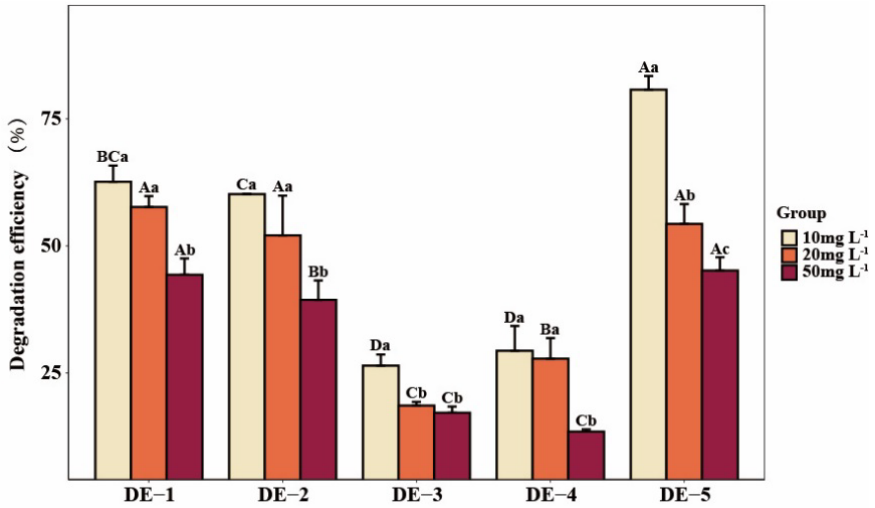

Figure 7. *The degradation ability of ethoprophos-degrading bacteria under different concentrations of ethoprophos under the condition of glucose addition. Different uppercase letters indicate significant differences in degradation ability between different ethoprophos-degrading bacteria under the same concentration of ethoprophos, and different lowercase letters indicate significant differences in degradation ability of the same ethoprophos-degrading bacteria under different concentrations of ethoprophos. $p < 0.05$ was considered a significant difference*

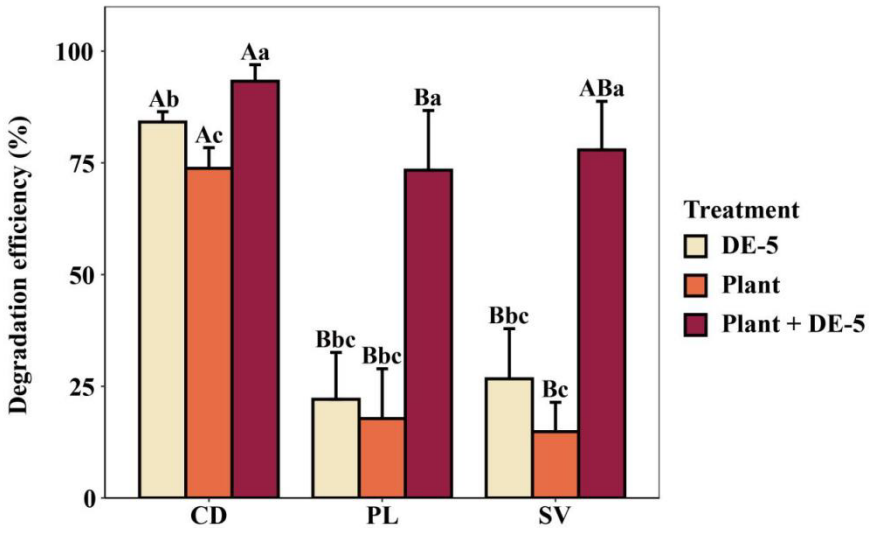

Figure 8. *Degradation of soil ethoprophos by plant-microbial combined remediation. CD: Carex dimorpholepis, PL: Polygonum lapathifolium. SV: Setaria viridis. Different lowercase letters indicate a significant difference ($p < 0.05$). Different uppercase letters indicate significant differences in degradation ability between the same treatment, and different lowercase letters indicate significant differences in degradation ability of the same plant type. $p < 0.05$ was considered a significant difference*

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Discussion

Different ethoprophos-degrading microorganisms and their degradation capabilities in riparian zones

In this study, five EDBs were isolated from the riparian zones of the YC project. Although all five EDBs were isolated from the same ecosystem, the phylogenetic relationships between them are different. DE-5 (*Acinetobacter seifertii*), a typical oligotrophic microbe, has a significantly different life strategy from the other four EDBs (Koch, 2001). Furthermore, the EDBs isolated in this study differ from those previously reported, as the former have predominantly been isolated from agricultural soils, including *Bacillus cereus*, *Sphingomonas* sp., and *Pseudomonas* sp. (Karpouzas et al., 2000; Karpouzas and Walker, 2000). Moreover, except for DE-1 (*Bacillus badius*), which belongs to the same genus as a previously reported EDB (*Bacillus cereus*), the EDBs identified in this study are phylogenetically distinct from previously reported EDBs. Consequently, our findings indicate substantial differences in the types of EDBs across different habitats, thereby expanding the repository of known ethoprophos degraders. Although the isolated EDBs in this study were reported for the first time, their respective genera have been previously associated with the degradation of various organic pollutants. For example, *Acinetobacter calcoaceticus* has been demonstrated to degrade chlorpyrifos (Zhao et al., 2014), *Serratia marcescens* can degrade diazinon (Abo-Amer, 2011), *Klebsiella aerogenes* can degrade endosulfan (Rani et al., 2019), *Stenotrophomonas pavanii* can degrade glyphosate (Zhao et al., 2024), and *Bacillus badius* can degrade the organic pollutant aniline (Sarwade and Gawai, 2014). These findings suggest that the EDBs identified in this study may also have the potential to degrade multiple types of organic pollutants.

Further investigation into the degradation capabilities of the isolated EDBs revealed that DE-1 and DE-5 had significantly higher degradation efficiencies than did the other three EDBs (*Figure 6*). The efficiency of microbial degradation of pesticides is regulated by many factors, including the type of degrading microorganism, pesticide properties, temperature, light, soil properties, and other environmental factors (Bose et al., 2021). For example, Chishti et al. (2021) studied the degradation of dimethoate by *Acinetobacter calcoaceticus* under different environmental conditions and reported that the best degradation effect was achieved when the pH was 7, and Li et al. (2018) reported that *Lactobacillus* species degraded phorate most efficiently at 30 °C. These findings are consistent with our results (*Figures 3 and 4*). Numerous investigations have demonstrated that under controlled laboratory conditions, the degradation rates of organophosphorus pesticides by microorganisms can exceed 90%. For example, *Brevibacterium* sp. (Jiang et al., 2019), *Pseudomonas* sp. (Ramu and Seetharaman, 2014), and *Burkholderia* sp. (Wang et al., 2010) have demonstrated degradation efficiencies of over 95% for methamidophos and dichlorvos. In the present study, DE-5 (*Acinetobacter seifertii*) presented the most substantial ethoprophos degradation rate, reaching 80.75%. This impressive degradation efficiency may be influenced by the duration of incubation or the pesticide type (Guerrero Ramírez et al., 2023). However, our results demonstrate a higher ethoprophos degradation efficiency (80.75%) than the 51.6% reported by Li et al. (2010) for *Bacillus cereus* at the same concentration. This superior performance is likely due to the phylogenetic differences between *Acinetobacter seifertii* (phylum Proteobacteria) and *Bacillus cereus* (phylum Firmicutes) because, compared with Firmicutes, Proteobacteria are known for their higher metabolic rates, which could account for the increased

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degradation efficiency of DE-5 (Ma et al., 2022). Additionally, we observed that increased ethoprophos concentrations reduced the degradation efficiency, whereas the addition of glucose mitigated this trend, especially at higher ethoprophos concentrations. These findings suggest that the availability of a carbon source may stimulate microbial growth, enhancing ethoprophos degradation and indicating that nutrient stress can impact microbial degradation processes (Li et al., 2021b).

Plant–microbe synergistic remediation of ethoprophos

In natural environments, plants contribute to the degradation or immobilization of organophosphorus pesticides by secreting organic acids and other compounds from their roots; this process mitigates the toxicity of these pesticides (Sun et al., 2010). However, this plant-mediated degradation is often gradual and limited by the low tolerance of plants to pesticides (Li and Fantke, 2023). Conversely, microbial degradation is generally rapid but can be limited by nutrient availability (Zhou et al., 2020). Plants can alleviate this constraint by supplying carbon sources to the soil through root turnover and exudates, thereby promoting microbial growth and increasing their ability to degrade pesticides (Yu et al., 2022). Consequently, the collaborative degradation of pesticides by plants and microbes can significantly increase the overall efficiency of pesticide removal. For example, Vaishnavi and Osborne (2024) employed *Proteus myxofaciens* and *Chrysopogon zizanioides* to remediate monocrotophos-contaminated soil, achieving a degradation rate exceeding 90% within 45 days. Similarly, Zhang et al. (2022) integrated a synthetic bacterial community (including *Pseudomonas* sp., *Achromobacter* sp., and *Variovorax* sp.) with *Dolichos lablab* to remediate bensulfuron-contaminated soil, achieving an 81% degradation rate after 25 days. Our results revealed that the combination of DE-5 with each of the three plant species significantly enhanced ethoprophos degradation, which is consistent with previous research (Xie et al., 2018). Therefore, our findings demonstrated that plant–microdegradation strategies can accelerate the degradation of ethoprophos in soil.

Furthermore, our findings indicate that the coinoculation of DE-5 with *Carex dimorpholepis* led to the highest ethoprophos degradation rate in soil (93.27%), surpassing the degradation rates achieved with the other two plant combinations. This may be attributed to the more extensive root system of *Carex dimorpholepis*, which secretes greater amounts of organic carbon, thus promoting DE-5 growth and increasing its activity, thereby accelerating ethoprophos degradation (Sebastian and Dinneny, 2017; Gajbhiye and Singh, 2024). Similarly, Jabeen H. reported complete chlorpyrifos degradation within 45 days using a combination of *Lolium multiflorum* and *Mesorhizobium* sp. (Jabeen et al., 2016). The superior degradation capability in this case may be attributed to the deeper and more extensive root structure of *Lolium multiflorum* than that of *Carex dimorpholepis*, which offers a larger surface area for microbial attachment and supports a more diverse microbial community, thereby creating a more conducive environment for microbes to remove organophosphorus pesticides (Zhou et al., 2011). Our results underscore the importance of selecting suitable plant species for the microbial codegradation of organophosphorus pesticides. Although plant–microbe co-remediation holds promise for ethoprophos degradation, the influence of field conditions on microbial growth should be considered. Consequently, future research should integrate field experiments to investigate the optimal conditions for plant–microbe co-remediation.

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Conclusion

This study isolated five ethoprophos-degrading microorganisms from rhizosphere soils in riparian zones along the project of water diversion from the Yangtze River to the Chaohu Lake route: *Bacillus badius*, *Serratia liquefaciens*, *Klebsiella aerogenes*, *Stenotrophomonas pavanii*, and *Acinetobacter seifertii*. The results of culture experiments demonstrated that *Acinetobacter seifertii* and *Bacillus badius* presented the greatest ethoprophos degradation ability. However, the efficiency of microbial degradation decreased with increasing ethoprophos concentration, an effect that was mitigated by the introduction of glucose. The results of the plant-microbe co-remediation experiments demonstrated that the combination of *Acinetobacter seifertii* and *Carex dimorpholepis* resulted in the highest ethoprophos degradation rate of 93.27%. These findings contribute to the development of a scientific basis for the bioremediation of ethoprophos-contaminated soils.

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Data availability statement. All the data that support the findings of this study are available.

REFERENCES

[1] Abo-Amer, A. E. (2011): Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. – Journal of Microbiology and Biotechnology 21(1): 71-80.

[2] Bose, S., Kumar, P. S., Vo, D.-V. N., Rajamohan, N., Saravanan, R. (2021): Microbial degradation of recalcitrant pesticides: a review. – Environmental Chemistry Letters 19: 3209-3228.

[3] Çakır, B., Klobučar, G., Çömden, E. A. (2024): Investigating the toxic effects of ethoprophos on Eisenia fetida: Integrating light microscopy, scanning electron microscopy, and biochemical analysis. – Chemosphere 350: 141019.

[4] Chishti, Z., Ahmad, Z., Zhang, X. Z., Jha, S. K. (2021): Optimization of biotic and abiotic factors liable for biodegradation of chlorpyrifos and their modeling using neural network approaches. – Applied Soil Ecology 166: 103990.

[5] Chu, H. Y., Sun, H. B., Tripathi, B. M., Adams, J. M., Huang, R., Zhang, Y. J., Shi, Y. (2016): Bacterial community dissimilarity between the surface and subsurface soils equals horizontal differences over several kilometers in the western Tibetan Plateau. – Environmental Microbiology 18(5): 1523-1533.

[6] Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdugo, M., Campbell, C. D., Singh, B. K. (2016): Microbial diversity drives multifunctionality in terrestrial ecosystems. – Nature Communications 7(1): 10541.

[7] Dowling, K. C., Costella, R. G., Lemley, A. T. (1994): Behaviour of the insecticides ethoprophos and carbofuran during soil—water transport. – Pesticide science 41(1): 27-33.

[8] Gajbhiye, R., Sarma, S. S., Kumar, D., Singh, S. (2024): The treasure trove of the genus Carex: A phytochemical and pharmacological review. – Health Sciences Review 10: 100151.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_44574472

[9] Gao, M. M., Bai, J. Y., Cheng, S. M., Huo, S. Y. (2019): Screening and Identification of Organophosphorus Pesticides Degradation Strain L-1 (In Chinese). – Agrochemicals 58(05): 336-339.
[10] Guerrero Ramírez, J. R., Ibarra Muñoz, L. A., Balagurusamy, N., Frías Ramírez, J. E., Alfaro Hernández, L., Carrillo Campos, J. (2023): Microbiology and biochemistry of pesticides biodegradation. – International Journal of Molecular Sciences 24(21): 15969.
[11] Jabeen, H., Iqbal, S., Ahmad, F., Afzal, M., Firdous, S. (2016): Enhanced remediation of chlorpyrifos by ryegrass (*Lolium multiflorum*) and a chlorpyrifos degrading bacterial endophyte Mezorhizobium sp. HN3. – International Journal of Phytoremediation 18(2): 126-133.
[12] Jiang, Y. J., Deng, Y. J., Liu, X. R., Xie, B. G., Hu, F. P. (2006): Isolation and identification of a bacterial strain JS018 capable of degrading several kinds of organophosphate pesticides. – Wei Sheng wu xue bao= Acta Microbiologica Sinica 46(3): 463-466.
[13] Jiang, B., Zhang, N. N., Xing, Y., Lian, L. N., Chen, Y. T., Zhang, D. Y., Li, G. H., Sun, G. D., Song, Y. Z. (2019): Microbial degradation of organophosphorus pesticides: novel degraders, kinetics, functional genes, and genotoxicity assessment. – Environmental Science and Pollution Research 26: 21668-21681.
[14] Karpouzas, D. G., Walker, A. (2000): Factors influencing the ability of *Pseudomonas putida* strains epI and II to degrade the organophosphate ethoprophos. – Journal of Applied Microbiology 89(1): 40-48.
[15] Karpouzas, D. G., Morgan, J. A. W., Walker, A. (2000): Isolation and characterisation of ethoprophos-degrading bacteria. – FEMS Microbiology Ecology 33(3): 209-218.
[16] Kaushal, J., Khatri, M., Arya, S. K. (2021): A treatise on Organophosphate pesticide pollution: Current strategies and advancements in their environmental degradation and elimination. – Ecotoxicology and Environmental Safety 207: 111483.
[17] Khare, E., Arora, N. K. (2015): Effects of Soil Environment on Field Efficacy of Microbial Inoculants. – In: Arora, N. (ed.) Plant Microbes Symbiosis: Applied Facets. Springer, New Delhi.
[18] Koch, A. L. (2001): Oligotrophs versus copiotrophs. – Bioessays 23(7): 657-661.
[19] Kumar, S., Kaushik, G., Dar, M. A., Nimesh, S., Lopez-Chuken, U. J., Villarreal-Chiu, J. F. (2018): Microbial degradation of organophosphate pesticides: a review. – Pedosphere 28(2): 190-208.
[20] Li, Y. F., Song, X. L., Ji, J., Liu, F., Mu, W. (2010): Identification and characterization of an ethoprophos-degrading bacteria DS-1 and its degradation characteristics (In Chinese). – Scientia Agricultura Sinica 43(08): 1594-1600.
[21] Li, C. K., Ma, Y. Z., Mi, Z. H., Huo, R., Zhou, T. T., Hai, H., Kwok, L., Sun, Z. H., Chen, Y. F., Zhang, H. P. (2018): Screening for Lactobacillus plantarum strains that possess organophosphorus pesticide-degrading activity and metabolomic analysis of phorate degradation. – Frontiers in Microbiology 9: 2048.
[22] Li, X. K., Li, H., Qu, C. T. (2019): A review of the mechanism of microbial degradation of petroleum pollution. – In: IOP Conference Series: Materials Science and Engineering 484(1): 012060.
[23] Li, M. F., Yu, T. T., Lai, J. L., Han, X., Hu, J. H., Deng, Z. Y., Li, D. M., Ye, Z. C., Wang, S. H., Hu, C. Y., Xu, X. W. (2021a): Ethoprophos induces cardiac toxicity in zebrafish embryos. – Ecotoxicology and Environmental Safety 228: 113029.
[24] Li, T. P., Wang, R. Z., Cai, J. P., Meng, Y. N., Wang, Z. R., Feng, X., Liu, H. Y., Turco, R. F., Jiang, Y. (2021b): Enhanced carbon acquisition and use efficiency alleviate microbial carbon relative to nitrogen limitation under soil acidification. – Ecological Processes 10(1): 32.
[25] Li, Z. J., Fantke, P. (2023): Considering degradation kinetics of pesticides in plant uptake models: proof of concept for potato. – Pest Management Science 79(3): 1154-1163.
[26] Lin, C., You, M. S. (2009): Plant-microorganism combined bioremediation of chlorpyrifos-contaminated soil (In Chinese). – Entomological Journal of East China 18(2): 081-087.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_44574472

[27] Lu, P., Li, Q. F., Liu, H. M., Feng, Z. Z., Yan, X., Hong, Q., Li, S. P. (2013): Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by Cupriavidus sp. DT-1. – Bioresource Technology 127: 337-342.
[28] Ma, X. Y., Wang, T. X., Shi, Z., Chiariello, N. R., Docherty, K., Field, C. B., Gutknecht, J., Gao, Q., Gu, Y. F., Guo, X., Hungate, B. A., Lei, J. S., Niboyet, A., Roux, X. L., Yuan, M. T., Yuan, T., Zhou, J. Z., Yang, Y. F. (2022): Long-term nitrogen deposition enhances microbial capacities in soil carbon stabilization but reduces network complexity. – Microbiome 10(1): 112.
[29] Malik, D. K., Bhatia, D., Rathi, M. (2014): Bacterial degradation of some organophosphate compounds. – In: Kharwar, R., Upadhyay, R., Dubey, N., Raghuwanshi, R. (eds.) Microbial Diversity and Biotechnology in Food Security. Springer, New Delhi, pp. 531-541.
[30] Mason-Jones, K., Robinson, S. L., Veen, G. F., Manzoni, S., van der Putten, W. H. (2022): Microbial storage and its implications for soil ecology. – The ISME Journal 16(3): 617-629.
[31] Mishra, S., Pang, S., Zhang, W., Lin, Z., Bhatt, P., Chen, S. (2021): Insights into the microbial degradation and biochemical mechanisms of carbamates. – Chemosphere 279: 130500.
[32] R Core Team (2022): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria.
[33] Raj, A., Kumar, A., Khare, P. K. (2024): The looming threat of profenofos organophosphate and microbes in action for their sustainable degradation. – Environmental Science and Pollution Research 31(10): 14367-14387.
[34] Ramu, S., Seetharaman, B. (2014): Biodegradation of acephate and methamidophos by a soil bacterium Pseudomonas aeruginosa strain Is-6. – Journal of Environmental Science and Health, Part B 49(1): 23-34.
[35] Rani, R., Kumar, V., Gupta, P., Chandra, A. (2019): Effect of endosulfan tolerant bacterial isolates (Delftia lacustris IITISM30 and Klebsiella aerogenes IITISM42) with Helianthus annuus on remediation of endosulfan from contaminated soil. – Ecotoxicology and Environmental Safety 168: 315-323.
[36] Sarwade, V., Gawai, K. (2014): Biodegradation of aniline by alkaliphilic strain *Bacillus badius* D1. – IOSR J Environ Sci Toxicol Food Technol 8(5): 71-78.
[37] Sebastian, J., Dinneny, J. R. (2017): Setaria viridis: a model for understanding panicoid grass root systems. – In: Doust, A., Diao, X. (eds.) Genetics and Genomics of Setaria. Plant Genetics and Genomics: Crops and Models 19: 177-193.
[38] Singh, S., Kumar, V., Upadhyay, N., Singh, J., Singla, S., Datta, S. (2017): Efficient biodegradation of acephate by Pseudomonas pseudoalcaligenes PS-5 in the presence and absence of heavy metal ions [Cu (II) and Fe (III)], and humic acid. – 3 Biotech 7: 1-10.
[39] Song, J., Lü, D., Ding, R., Zhang, X. K. (2022): Distribution characteristics and risk Assessment of organophosphorus pesticides in Caizihu Lake Line of the leading water project from Yangtze River to Huaihe River (In Chinese). – Journal of Shanghai Ocean University 31(06): 1502-1513.
[40] Sun, T. R., Cang, L., Wang, Q. Y., Zhou, D. M., Cheng, J. M., Xu, H. (2010): Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. – Journal of Hazardous Materials 176(1-3): 919-925.
[41] Vaishnavi, J., Osborne, J. W. (2024): Biodegradation of monocrotophos, cypermethrin fipronil by Proteus myxofaciens VITVJ1: A plant-microbe based remediation. – Heliyon 10(18).
[42] Wang, L., Wen, Y., Guo, X. Q., Wang, G. L., Li, S. P., Jiang, J. D. (2010): Degradation of methamidophos by Hyphomicrobium species MAP-1 and the biochemical degradation pathway. – Biodegradation 21: 513-523.
[43] Witczak, A., Pohoryło, A., Abdel-Gawad, H., Cybulski, J. (2018): Residues of some organophosphorus pesticides on and in fruits and vegetables available in Poland, an

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_44574472

assessment based on the European Union regulations and health assessment for human populations. – Phosphorus, Sulfur, and Silicon and the Related Elements 193(11): 711-720.
[44] Xie, H., Zhu, L. S., Wang, J. (2018): Combined treatment of contaminated soil with a bacterial Stenotrophomonas strain DXZ9 and ryegrass (*Lolium perenne*) enhances DDT and DDE remediation. – Environmental Science and Pollution Research 25: 31895-31905.
[45] Yu, L., Zi, H. Y., Zhu, H. G., Liao, Y. W., Xu, X., Li, X. G. (2022): Rhizosphere microbiome of forest trees is connected to their resistance to soil-borne pathogens. – Plant and Soil 479(1): 143-158.
[46] Yuan, X., Lee, J., Han, H., Ju, B., Park, E., Shin, Y., Lee, J., Kim, J. H. (2021): Translocation of residual ethoprophos and tricyclazole from soil to spinach. – Applied Biological Chemistry 64: 1-10.
[47] Zhang, Y. N., Wang, X., Liu, W. R., Ge, L. (2022): Plant and microorganism combined degradation of bensulfuron herbicide in eight different agricultural soils. – Agronomy 12(12): 2989.
[48] Zhao, L., Wang, F., Zhao, J. (2014): Identification and functional characteristics of chlorpyrifos-degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus*. – Journal of Basic Microbiology 54(5): 457-463.
[49] Zhao, S. C., Xu, Z. T., Wang, J. H. (2024): *Stenotrophomonas pavanii* MY01 induces phosphate precipitation of Cu (II) and Zn (II) by degrading glyphosate: performance, pathway and possible genes involved. – Frontiers in Microbiology 15: 1479902.
[50] Zheng, H. P., Yang, T. J., Bao, Y. Z., He, P. P., Yang, K. M., Mei, X. L., Wei, Z., Xu, Y. C., Shen, Q. R., Banerjee, S. (2021): Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics. – Soil Biology and Biochemistry 157: 108230.
[51] Zhou, X. H., Wang, G. X., Yang, F. (2011): Characteristics of growth, nutrient uptake, purification effect of Ipomoea aquatica, *Lolium multiflorum*, and *Sorghum sudanense* grown under different nitrogen levels. – Desalination 273(2-3): 366-374.
[52] Zhou, Z. H., Wang, C. K., Luo, Y. Q. (2020): Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. – Nature communications 11(1): 3072.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_44574472