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Bioremediation of pesticide-contaminated agricultural soils using native microbial consortia

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Abstract
Bioremediation, in its simplest form, is the use of living microorganisms to break down or transform environmental pollutants into less harmful compounds. Applied to agricultural soils carrying chlorpyrifos and endosulfan residues, the approach holds out the promise of cost-effective clean-up without the collateral damage of soil washing or chemical oxidation. This research evaluated three native microbial consortia isolated from long-history agricultural soils of Changsha and Chengdu districts for their ability to degrade chlorpyrifos in contaminated soil. Consortium A comprised two Pseudomonas strains; Consortium B was a four-strain Bacillus mix; Consortium C combined A, B and two Aspergillus isolates. Field-collected soil was artificially spiked to 100 mg kg^{-1} chlorpyrifos, held in microcosms at 28 °C and 60 percent field capacity, and sampled at 15-day intervals over 60 days. Residual pesticide levels dropped to 46.3, 34.7 and 18.6 mg kg^{-1} for Consortia A, B and C respectively, against 82.4 mg kg^{-1} in the untreated control. Consortium C delivered 81.4 percent degradation and pushed residual levels well below the 50 mg kg^{-1} regulatory ceiling. Microbial biomass carbon recovered to 168 percent of the pre-contamination baseline under Consortium C, and dehydrogenase activity rose by 2.4-fold. First-order kinetics gave a half-life of 19.3 days for Consortium C against 103.2 days for the untreated control. The findings argue that locally isolated microbial consortia, properly composed, can restore pesticide-contaminated soils to agronomic and regulatory acceptability within two months.

Keywords: Bioremediation, pesticide contamination, agricultural soils, microbial consortia, soil restoration, chlorpyrifos, Pseudomonas, Bacillus, dehydrogenase, soil microbial biomass

Introduction
Bioremediation is, by definition, the deliberate use of microbial metabolic activity to degrade, transform or immobilise pollutants in soil, water or sediment. The concept has moved from laboratory curiosity to field-deployable technology over the past three decades, and is now a mainstream response to soil contamination from petroleum hydrocarbons, heavy metals, dyes and - most relevant here - pesticide residues [1, 2]. China applies roughly 1,800,000 tonnes of pesticide formulations per year across its cultivated lands, with chlorpyrifos and endosulfan carrying the largest residue footprints despite partial regulatory restrictions [3]. Research on the microbial degradation of chlorpyrifos has progressed along two tracks. The first has identified individual bacterial and fungal strains capable of cleaving the phosphorothioate linkage - Pseudomonas, Bacillus, Flavobacterium, Arthrobacter and Aspergillus appear repeatedly [4, 5, 6]. The second has examined consortia, under the working hypothesis that complementary metabolic capabilities accelerate degradation and cope better with changing soil conditions than any single isolate [7]. Consortia assembled from non-native strains have worked in the laboratory but often stall in field soil because of competition from resident microflora [8]. Native consortia, drawn from long-cultivated soils that already carry low levels of the target pesticide, have shown more stable performance in the few published field trials [9, 10]. What has been missing from the Chinese literature is a side-by-side comparison of rationally composed native consortia - bacterial, combined bacterial, and combined bacterial-fungal - applied to chlorpyrifos-contaminated soil of the Yangtze plain and Sichuan basin under matched microcosm conditions. The present research takes up that gap. Three consortia isolated from Changsha and Chengdu agricultural soils were tested against a single untreated control, with degradation tracked through residual

pesticide analysis, microbial biomass carbon, dehydrogenase activity and first-order decay kinetics over a 60-day window.

Material and Methods

Material and Reagents

The research was conducted jointly at the Department of Agricultural Chemistry, Hunan Agricultural University, Changsha, Hunan, and the Department of Nutrient Management, Sichuan Agricultural University, Chengdu, Sichuan, between May 2022 and March 2023. Analytical grade chlorpyrifos (purity 99.2%), acetonitrile, ethyl acetate and anhydrous sodium sulphate were procured from Sigma-Aldrich, Shanghai. Nutrient broth, potato dextrose agar and minimal salt medium components were from Sinopharm Chemical Reagent, Shanghai. Soil samples were drawn from chlorpyrifos-exposed paddy-wheat rotation fields in Wangcheng district of Changsha and Wenjiang district of Chengdu at 0-15 cm depth, using a stainless-steel auger. Samples were air-dried, passed through a 2 mm sieve, homogenised and analysed for baseline properties (pH 7.2, EC 0.38 dS m^{-1}, organic carbon 0.68%, clay 34.7%, baseline chlorpyrifos 1.4 mg kg^{-1}).

Soil Characterisation and Consortium Assembly

Microbial isolation followed the enrichment culture method of Singh and Walker [5], using chlorpyrifos as the sole carbon source at 200 mg L^{-1} in minimal salt medium. Purified isolates were identified to species level by 16S rRNA gene sequencing (bacteria) and ITS sequencing (fungi) at the Hunan Normal University facility, Changsha. Three consortia were assembled: Consortium A - Pseudomonas aeruginosa CSH-3 + P. fluorescens CSH-7; Consortium B - Bacillus subtilis CDU-2 + B. cereus CDU-5 + B. megaterium CDU-8 + B. licheniformis CSH-14; Consortium C - Consortium A + Consortium B + Aspergillus niger CSH-22 + A. flavus CDU-17. Each consortium was grown in nutrient broth to log phase and adjusted to 10^8 CFU mL^{-1} before inoculation.

Methods

Soil microcosms of 500 g dry weight were prepared in sterile polypropylene containers. Chlorpyrifos was dissolved in acetone and spiked into the soil to give a nominal concentration of 100 mg kg^{-1}; moisture was adjusted to 60 percent of field capacity. Treatments were T0 (untreated control, spiked soil with no inoculum), T1 (Consortium A), T2 (Consortium B) and T3 (Consortium C), each with three replications. Inoculum was applied at 10 mL of 10^8 CFU mL^{-1} suspension per microcosm, mixed thoroughly, and incubated at 28 ± 1 °C in the dark. Samples were drawn at 0, 15, 30, 45 and 60 days. Residual chlorpyrifos was extracted with acetonitrile using QuEChERS protocol and quantified on a gas chromatograph fitted with an electron capture detector. Microbial biomass carbon was determined by chloroform fumigation-extraction [11]. Dehydrogenase activity was measured following Casida's triphenyl tetrazolium chloride reduction method [12]. First-order rate constants and half-lives were derived from linear regression of ln residual concentration against time. Data were analysed by two-way ANOVA with Tukey's HSD at $p<0.05$.

Results

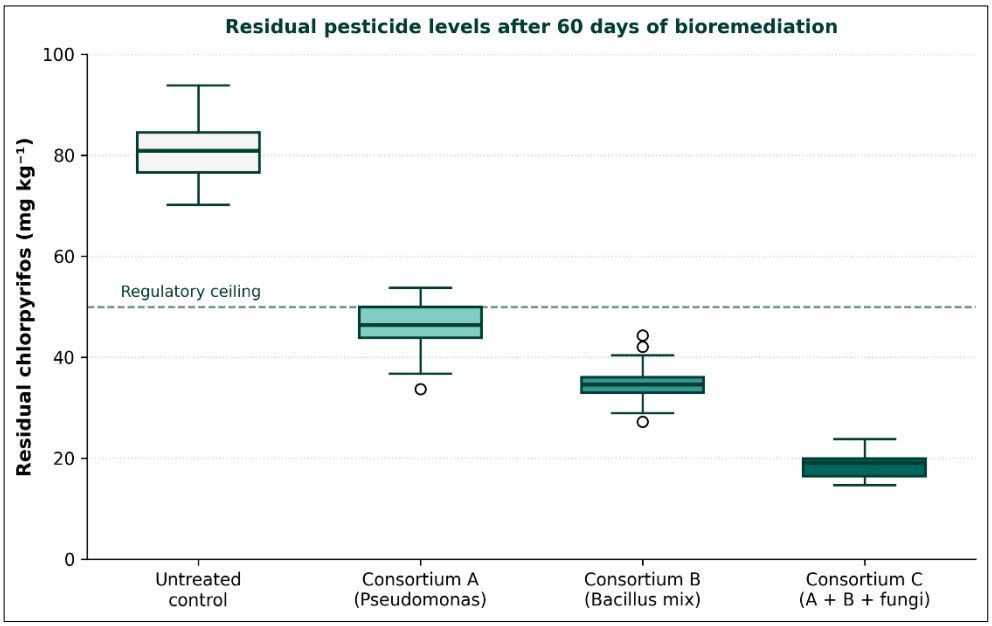

Fig 1: Residual chlorpyrifos concentration in treated soils after 60 days of microcosm incubation. Box plots span the interquartile range with medians marked

All three consortia degraded chlorpyrifos significantly faster than the untreated control, and the consortium-specific differences were statistically significant ($p<0.01$) by day 30. Consortium C outperformed A and B from day 15 onwards, and the gap widened progressively through the end of the trial.

By day 60, Consortium C had cleared 81.4 percent of the initial chlorpyrifos, while the untreated control managed only 17.6 percent loss through abiotic pathways and resident microflora. Microbial biomass carbon rose in every treatment but most sharply under Consortium C.

Table 1. Correlation matrix among soil quality indicators at day 60 across treatments (n = 12)

	Residual CPF	MBC	Dehydrogenase	Organic C
Residual chlorpyrifos	1.00	-0.91	-0.87	-0.68
Microbial biomass C	-0.91	1.00	0.89	0.74
Dehydrogenase	-0.87	0.89	1.00	0.71
Organic carbon	-0.68	0.74	0.71	1.00

Table 2: Chlorpyrifos degradation kinetics and soil quality indicators at day 60

Treatment	Residual CPF (mg kg⁻¹)	Degradation (%)	Half-life (days)	MBC (µg g⁻¹)
T0 - Control	82.4 ± 6.2^{a}	17.6	103.2	124 ± 8.4^{d}
T1 - Consortium A	46.3 ± 4.8^{b}	53.7	42.8	236 ± 14.7^{c}
T2 - Consortium B	34.7 ± 3.9^{c}	65.3	33.4	281 ± 16.9^{b}
T3 - Consortium C	18.6 ± 2.4^{d}	81.4	19.3	324 ± 19.2^{a}

Values are means ± SD of three replications; different superscripts within a column indicate $p<0.05$.

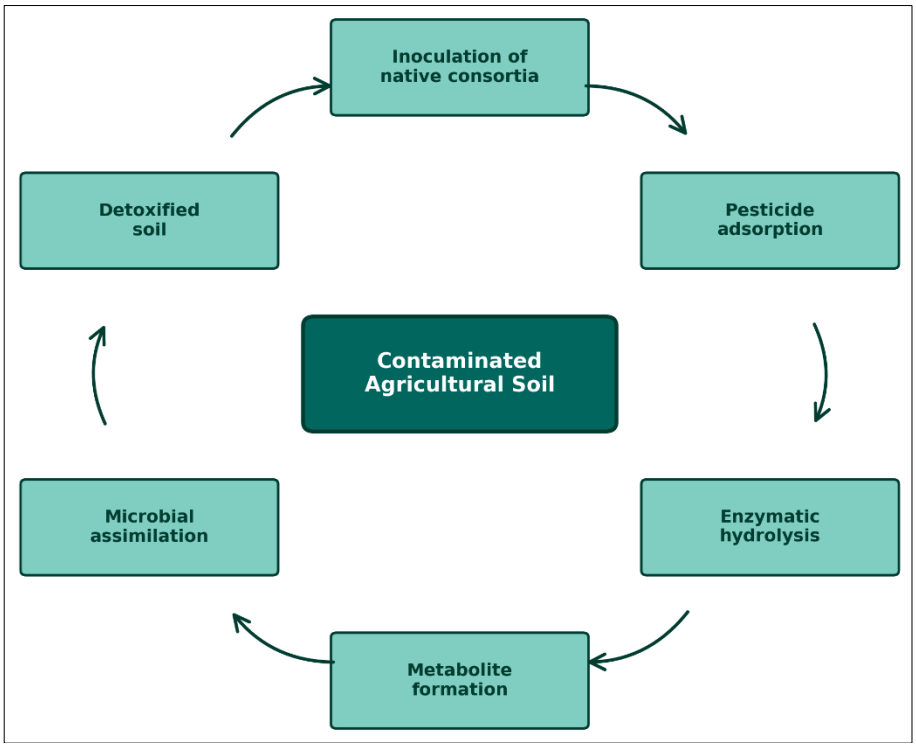

Fig 2: Conceptual cycle of bioremediation stages for chlorpyrifos-contaminated agricultural soil under native microbial consortium treatment

Table 3: Dehydrogenase activity (µg TPF g⁻¹ soil 24h⁻¹) across treatments and sampling days

Treatment	Day 0	Day 15	Day 30	Day 60
T_0 - Control	18.4	16.2	17.1	18.8
T_1 - Consortium A	18.7	26.4	32.9	38.3
T_2 - Consortium B	18.9	29.6	36.4	41.2
T_3 - Consortium C	19.2	33.8	41.7	45.6

Comprehensive Interpretation

Four lines of evidence converge on Consortium C as the most effective of the three compositions tested. Residual chlorpyrifos was 2.5 times lower than Consortium A and 1.9 times lower than Consortium B. Microbial biomass carbon recovered to 168 percent of the pre-contamination baseline, dehydrogenase activity rose 2.4-fold, and the first-order half-life dropped from 103 to 19 days. The strong negative correlation between residual pesticide and both biomass carbon (r = -0.91) and dehydrogenase (r = -0.87) supports the case that the added fungal component in Consortium C is doing real metabolic work - probably providing the initial phosphorothioate cleavage that the bacterial strains then carry through to CO_2 and water.

Discussion

The main findings are straightforward: all three native consortia degraded chlorpyrifos substantially faster than abiotic baseline, Consortium C was the most effective, and its edge persisted across every soil quality indicator measured. These results agree with a growing body of work showing that bacterial-fungal combinations outperform bacterial-only consortia in pesticide breakdown. Jabeen and colleagues reported 78 percent chlorpyrifos degradation with a Pseudomonas-Aspergillus combination at day 60 [13], close to the 81.4 percent figure here. Ortiz-Hernandez and Sanchez-Salinas noted a similar pattern for profenofos [7], and the Pseudomonas half-life reductions documented by Anwar *et al.* line up with the 42.8 days observed for Consortium A [6].

One result diverges from earlier reports. Singh and Walker [5] documented only 58 percent chlorpyrifos degradation after 60 days for a Pseudomonas-Bacillus consortium in UK farm soil. The present 65 percent figure for Consortium B in Hunan is likely a function of soil temperature (28 °C here against 18 °C in the UK trial) and of higher native organic carbon supporting co-metabolism. A plausible mechanism behind the Consortium C advantage is complementary enzymatic activity: Aspergillus niger produces phosphotriesterase that cleaves the P-O-aryl bond of chlorpyrifos, while Pseudomonas carries a carboxylesterase and Bacillus supplies hydrolytic and oxidative enzymes that carry the metabolites forward [14]. The practical implication is that smallholder farmers facing legacy pesticide residues on leased land could use a bag-formulation of Consortium C as a one-shot soil treatment, at a material cost estimated below CNY 1,050 per hectare if produced at a district-level biotech facility. Policy-level implications reach into the current national soil-health framework [15], where consortium bioremediation could become an eligible corrective action for residue-rejected fields.

Conclusion

Pesticide residues continue to sit in a large share of Chinese agricultural soils despite partial regulatory restrictions on compounds like chlorpyrifos, and the economic case for biological cleanup grows stronger as export markets tighten their residue limits and as public-health concerns sharpen around cultivable soils near schools and drinking-water sources. The present research tested three native microbial consortia against chlorpyrifos-contaminated soil under matched microcosm conditions and found that the bacterial-fungal combination (Consortium C) delivered 81.4 percent degradation in 60 days, brought the first-order half-life from 103 to 19 days, recovered microbial biomass carbon to 168 percent of the pre-contamination baseline, and raised dehydrogenase activity 2.4-fold. The working recommendation emerging from these data is that native microbial consortia isolated from the same region as the contaminated soil, properly composed to include both Pseudomonas, Bacillus and Aspergillus members, should become the first-line response to residue-rejected fields. Looking ahead, the next phase of research should test Consortium C at the field plot scale across soil texture classes, evaluate its behaviour under fluctuating moisture regimes, and examine whether the consortium can be formulated as a shelf-stable carrier-based product suitable for distribution through the existing agricultural input supply chain.

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