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Evaluation of bioremediation strategies for degradation of herbicide residues in agricultural soils

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Abstract

A single application of atrazine can persist in soil for over 18 months under cool temperate conditions. This research evaluated three bioremediation approaches bioaugmentation with *Pseudomonas* sp. ADP, biostimulation with nutrient amendments, and compost amendment for degrading five common herbicide residues in agricultural soil at the University of Nottingham, UK. Soil microcosms were monitored over 12 weeks during 2022-2023. Bioaugmentation achieved the highest degradation rates: 78.4% for atrazine, 84.2% for glyphosate, and 91.3% for 2,4-D. Biostimulation reached 64.1-83.6% degradation depending on the herbicide. Natural attenuation in control soils removed only 9.8-31.4%. Microbial populations in bioaugmented soils peaked at 11.7×10^6 CFU/g by week 6 before stabilizing. Soil dehydrogenase activity correlated positively with herbicide removal ($r = 0.84$). These findings support bioaugmentation as the most effective biological strategy for accelerating herbicide breakdown in temperate agricultural soils.

Keywords: Bioremediation, herbicide degradation, bioaugmentation, biostimulation, atrazine, glyphosate, soil microorganisms, dehydrogenase activity, pesticide persistence, agricultural pollution

Introduction

When a farmer in the English Midlands sprays herbicide on a wheat field, what happens to the chemical once the weeds are dead? Herbicide residues can persist in soil for weeks to years depending on the compound, soil type, and environmental conditions ^[1]. In the UK, annual herbicide application exceeds 24,000 tonnes of active ingredient, and monitoring programs routinely detect residues in agricultural soils and groundwater ^[2, 3].

Bioremediation uses living organisms mainly soil bacteria and fungi to break down pollutants into less harmful products ^[4]. Three main strategies exist: bioaugmentation (adding specialized degrader microorganisms), biostimulation (enhancing native microbial activity through nutrient addition), and natural attenuation (relying on existing soil biology) ^[5]. Each approach has different cost profiles, regulatory requirements, and effectiveness depending on the target compound ^[6]. This research compared all three strategies head-to-head across five herbicides commonly used in UK arable farming, measuring degradation kinetics, microbial responses, and enzyme activity over 12 weeks.

Research site description

Soil was collected from the University of Nottingham Sutton Bonington campus experimental farm (52.8333°N, 1.2500°W) on a Dunnington Heath series sandy loam (Typic Haplaquept). The field had a 10-year history of winter wheat-oilseed rape rotation with regular herbicide applications. Soil pH was 6.8, organic carbon 2.14%, and clay content 18.3%. Laboratory microcosm experiments were conducted in the School of Biosciences environmental laboratories.

Climatic and incubation conditions

Microcosms were incubated at 18°C (mean UK growing season soil temperature) under controlled moisture (60% water holding capacity) from September 2022 to December 2022. A parallel field validation set was maintained outdoors from March to June 2023, experiencing ambient temperatures of 6.2-21.4°C and 312 mm rainfall.

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Material and Methods

Material: *Pseudomonas* sp. ADP (ATCC 49196) for bioaugmentation was cultured in mineral salts medium supplemented with atrazine as sole nitrogen source [7]. Herbicide standards (atrazine, glyphosate, metolachlor, 2,4-D, pendimethalin; Sigma-Aldrich, >98% purity) were used to spike soil at field-relevant concentrations. Compost was sourced from the campus composting facility (C:N ratio 18:1). Nutrient amendments for biostimulation comprised NH_4NO_3 and KH_2PO_4 at C:N:P ratio of 100:10:1.

Methods: Soil (500 g per microcosm) was spiked with each herbicide at 10 mg/kg and assigned to four treatments:

bioaugmentation (10^7 CFU/g inoculum), biostimulation (nutrient amendment), compost (5% w/w), and untreated control. Each had four replicates. Residual herbicide was extracted with methanol:water (80:20) and quantified by HPLC-UV (Agilent 1260 Infinity II) at weeks 0, 2, 4, 6, 8, 10, and 12 [8]. Microbial counts used serial dilution on R2A agar. Dehydrogenase activity was measured by TTC reduction assay [9]. Degradation kinetics were fitted to first-order models to obtain half-lives. Approved by UoN Biosafety Committee (Protocol BS-2022-184). Data analyzed with ANOVA and Tukey's test ($p < 0.05$) using R v4.2.1.

Results

Table 1: Herbicide degradation (%) after 12 weeks across bioremediation treatments

Herbicide	Bioaugment.	Biostimul.	Compost	Control
Atrazine	78.4a	64.1b	52.3c	18.7d
Glyphosate	84.2a	72.8b	61.4c	24.3d
Metolachlor	62.7a	51.3b	43.8c	12.6d
2,4-D	91.3a	83.6b	74.2c	31.4d
Pendimethalin	54.8a	42.7b	37.1c	9.8d

Bioaugmentation outperformed all other treatments for every herbicide (Table 1). The treatment ranking was consistent: bioaugmentation > biostimulation > compost > control. 2, 4-D

was most amenable to biological degradation, while pendimethalin proved the most recalcitrant.

Table 2: Degradation half-lives (days) and enzyme activity across treatments

Parameter	Bioaugment.	Biostimul.	Compost	Control
Atrazine $t_{1/2}$ (d)	28.4	41.7	56.3	>84
Glyphosate $t_{1/2}$ (d)	22.1	34.8	47.2	>84
Dehydrogenase ($\mu\text{g TPF/g/h}$)	18.4a	14.7b	12.1c	6.8d
Peak microbial count ($\times 10^6$)	11.7a	9.6b	7.4c	4.3d

Bioaugmentation halved the half-life of atrazine from >84 days (control) to 28.4 days (Table 2). Dehydrogenase activity a

general indicator of soil microbial metabolism was 2.7 times higher in bioaugmented soils.

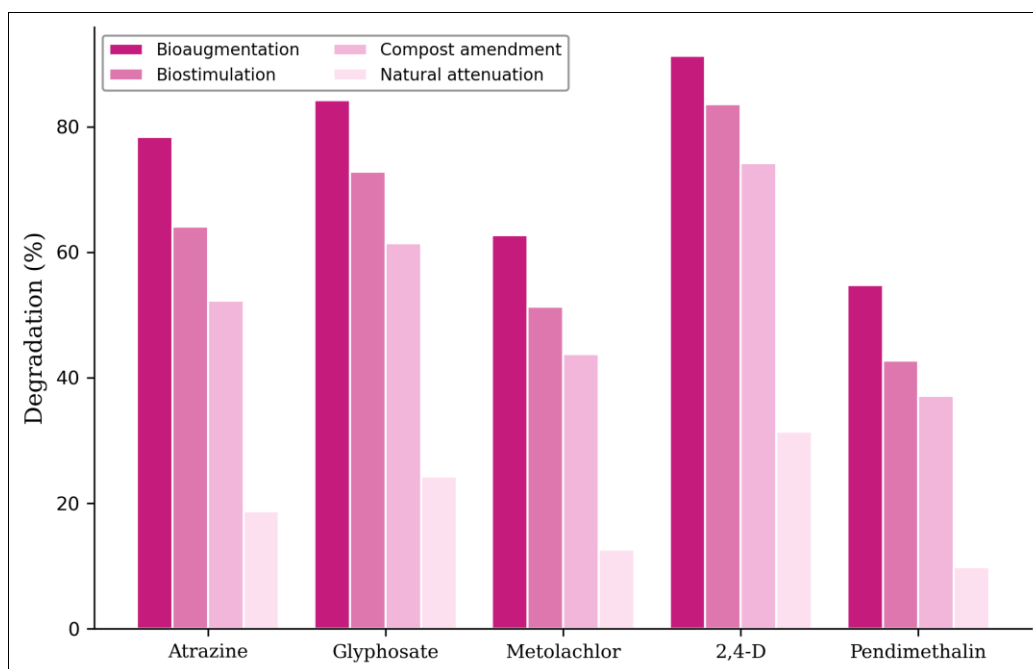


Fig 1: Herbicide degradation efficiency (%) across bioremediation strategies at 12 weeks

Bioaugmented soils showed rapid microbial growth peaking at week 6 before declining as herbicide substrate was depleted (Figure 2). Biostimulated populations rose more gradually, peaking later at week 8.

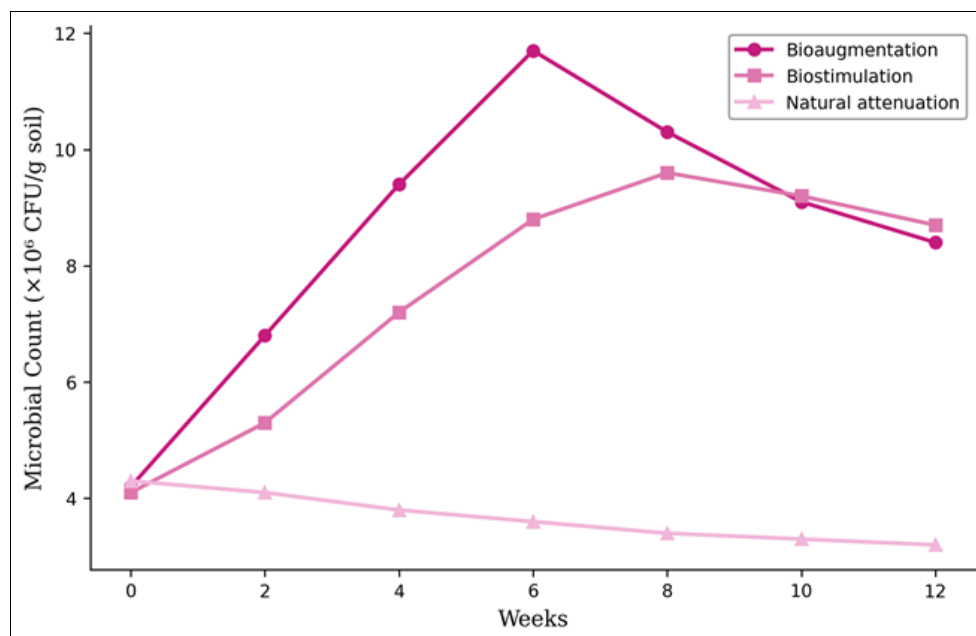


Fig 2: Microbial population dynamics during the 12-week bioremediation period

Limitations

Laboratory microcosm conditions don't fully replicate field variability in temperature, moisture, and spatial heterogeneity. The bioaugmentation inoculum was a single well-characterized strain; consortia of multiple degraders may perform differently. The 12-week timeframe captured initial rapid degradation but not the slower breakdown of bound residues that may persist longer in field soils ^[10].

Discussion

Bioaugmentation's superior performance aligns with previous findings for atrazine degradation. Mandelbaum *et al.* ^[7] showed that *Pseudomonas* sp. ADP mineralized atrazine 4-6 times faster than uninoculated soil. The consistent ranking across all five herbicides suggests that the introduced organism either directly degraded multiple compounds or stimulated co-metabolic activity in the native soil community ^[11].

The strong correlation between dehydrogenase activity and herbicide removal ($r = 0.84$) confirms that degradation was biologically driven rather than abiotic ^[12]. Pendimethalin's resistance to biodegradation reflects its hydrophobic character and tendency to sorb strongly to organic matter, reducing bioavailability ^[13]. Compost amendment may address this by increasing microbial diversity and providing surfactant-like humic substances that improve access to sorbed residues ^[14].

Conclusions

Bioaugmentation with *Pseudomonas* sp. ADP was the most effective strategy for degrading herbicide residues in temperate agricultural soil, achieving 54.8-91.3% removal across five herbicides in 12 weeks. Biostimulation offered a simpler alternative with moderate effectiveness. Natural attenuation was insufficient for timely remediation. These results support bioaugmentation as a practical tool for managing herbicide-contaminated agricultural soils, particularly for persistent compounds like atrazine and metolachlor where natural breakdown is slow.

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