Amendment-Specific Proactive Immobilization of Diffuse Metals During Fertility Recovery: A Micro-Plot Screening with *Lolium perenne*

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01 · BACKGROUND

Diffuse accumulation of heavy metals in agricultural soils from fertilizers, atmospheric deposition, and irrigation is increasingly concerning. Unlike point-source contamination, it is persistent and hard to detect, threatening soil biology, crop productivity, and food safety through gradual entry into the food chain.

Research Gap

Comparative evidence on organic amendment performance under low-fertility, diffusely contaminated soils remains limited. Amendment-assisted phytoremediation offers a nature-based alternative, but optimal amendment-to-metal pairing remains unclear.

Objective

Identify which organic amendment most effectively promotes **diffuse metal immobilization** while recovering soil fertility in degraded low-CEC soils within a single 45-day crop cycle.

02 · EXPERIMENTAL DESIGN

Factorial micro-plot · 1 kg soil · 1% w/w · n=3 · 45 days · Soil spiked at 10 mg·kg⁻¹ with B, Cd, Co, Cu, Li, Mn

- A — Biochar (P-enriched, pyrolysis 500°C)
- B — Compost (commercial)
- C — Humus (commercial)
- D — Poultry manure compost
- E — Vermicompost (lab-produced)
- F — Control (unamended)

Lolium perenne · Days 0, 15, 30, 45 · $CaCl_2$ extraction · ICP-OES

03 · STATISTICAL FRAMEWORK

Shapiro-Wilk normality; Levene's homoscedasticity. Kruskal-Wallis and Mann-Whitney U where needed. Cohen's d with a priori agronomic thresholds:

- ΔCEC ≥ 20% — agronomic relevance
- ΔBioavailable metal ≥ 30% — safety margin
- Root:Shoot ≥ 3 — phytostabilization confirmed
- ΔEC ≥ 50% — salinity risk flag

04 · AMENDMENT-METAL PAIRING GUIDE

Amendment	Primary soil effect	Target
Biochar	↑ CEC +32%, alkalizing (d=1.4)	Cd · Li
Compost	↑ TKN ×2.5 (d=2.0), Co/B complexation	Co · B
Humus	Organic functional groups, Co/B retention	Co · B
Poultry M.	↑ WRC 23%, variable metal effect	Variable
Vermicompost	Mn complexation, microbial support	Mn

WHY PHYTOSTABILIZATION OVER PHYTOEXTRACTION?

Classical phytoextraction risks increasing metal mobility and shoot accumulation. Combining *L. perenne* with organic amendments shifts toward **in situ immobilization** — improving fertility while retaining metals below the food chain, a safer approach for diffusely contaminated agricultural soils.

05 · SOIL PHYSICOCHEMICAL RESPONSE

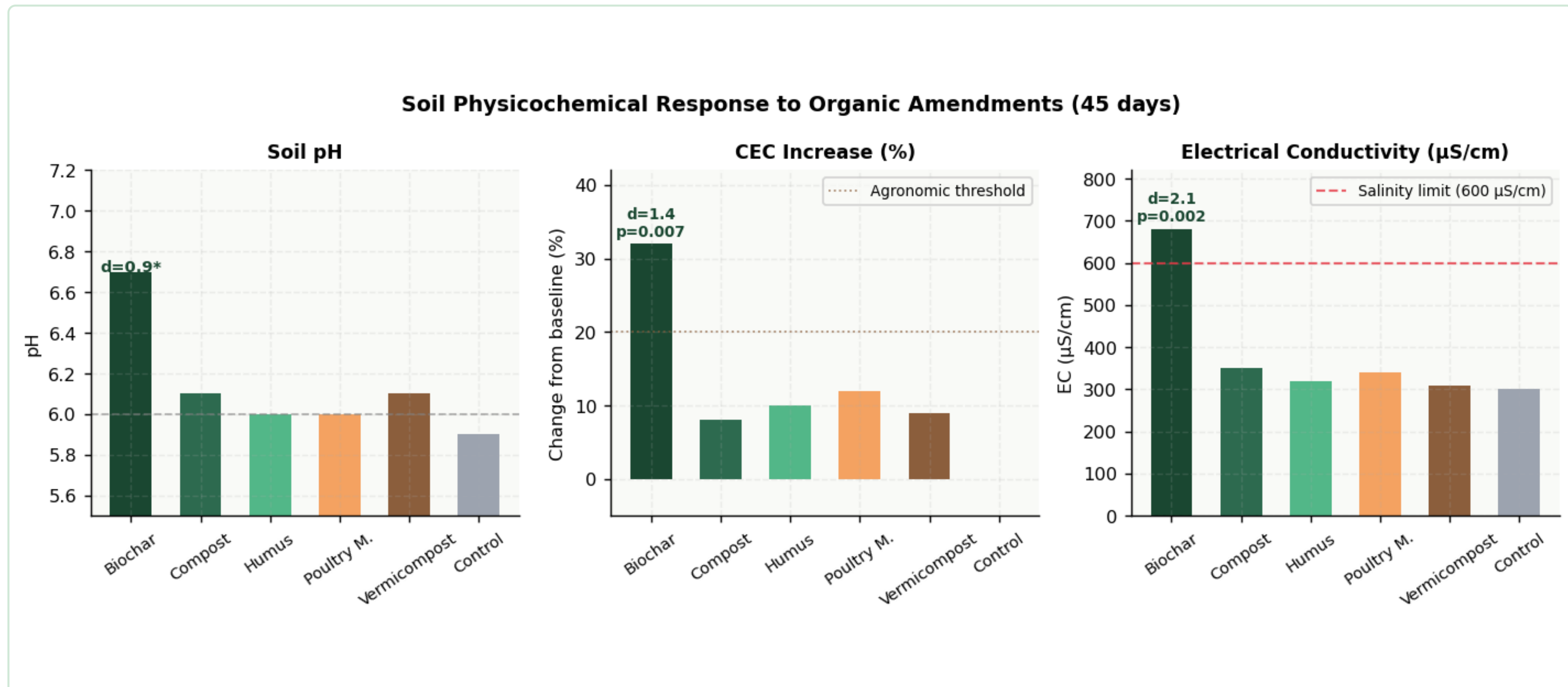

Fig. 1 — pH, CEC change (%), and EC by amendment. Biochar uniquely elevated pH (d=0.9) and CEC (+32%, d=1.4, p=0.007), while doubling EC above the local salinity limit (d=2.1, p=0.002) — careful dosage management required in salt-sensitive soils.

06 · NITROGEN SUPPLY & WATER RETENTION

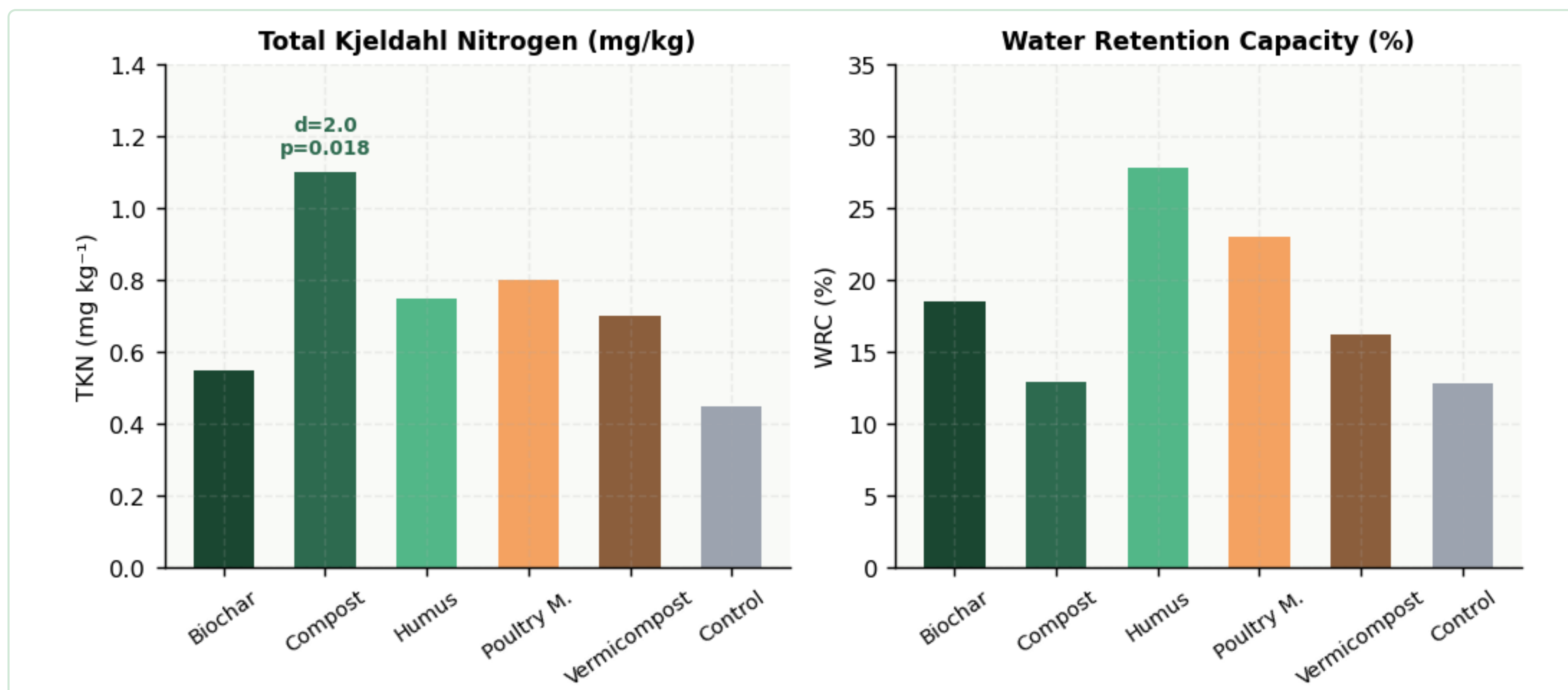

Fig. 2 — TKN and WRC by amendment. Compost delivered the highest TKN (d=2.0, p=0.018); humus and poultry manure improved WRC most. Biochar showed no TKN increase (d=0.1) — amendment choice must target the primary soil fertility bottleneck.

07 · METAL-SPECIFIC IMMOBILIZATION EFFICIENCY

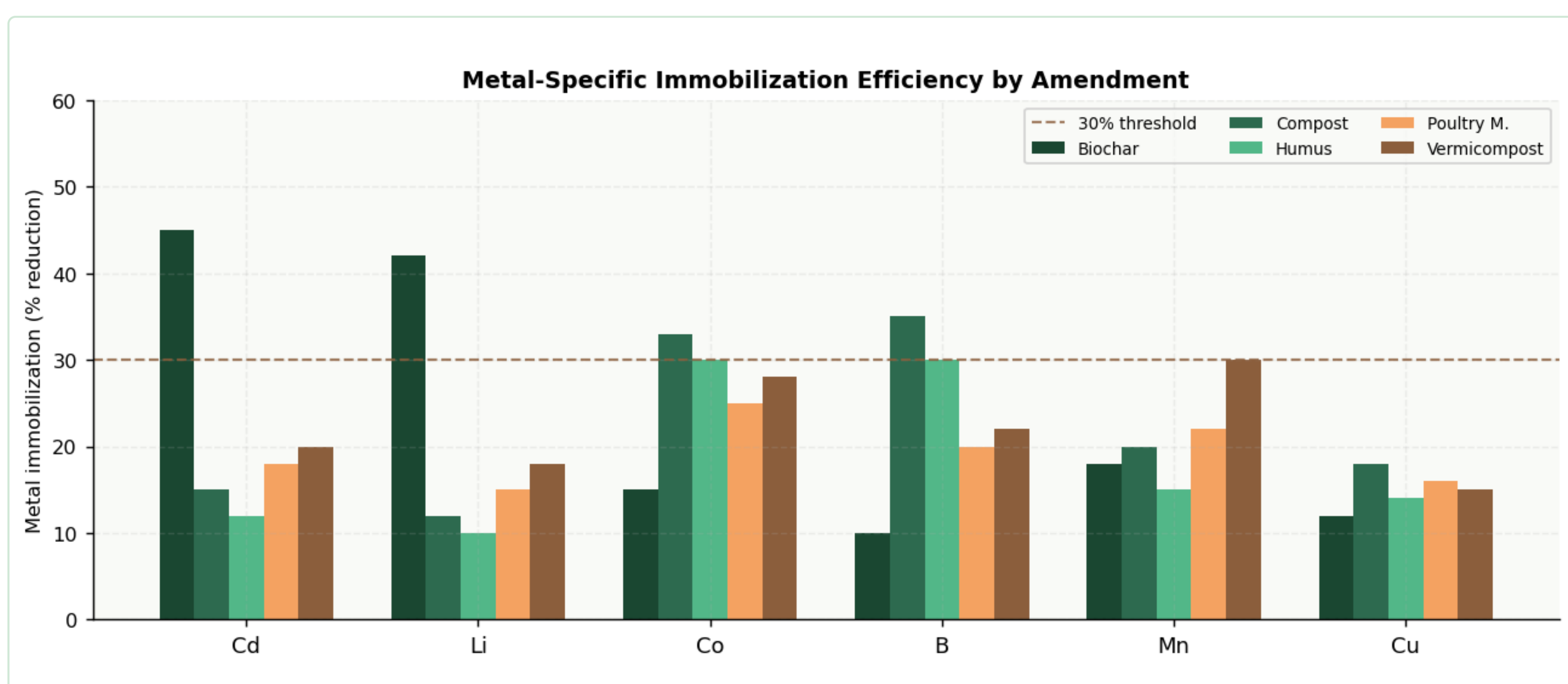

Fig. 3 — Immobilization efficiency (% reduction vs. control). Biochar exceeded the 30% threshold for Cd and Li (d≥1.3); compost and humus for Co and B (d≥1.0). Significant effects: B (p=0.016), Cd (p=0.005), Co (p=0.0001), Cu (p=0.028), Li (p=0.001), Mn (p=0.003).

08 · EFFECT SIZE DECISION MATRIX

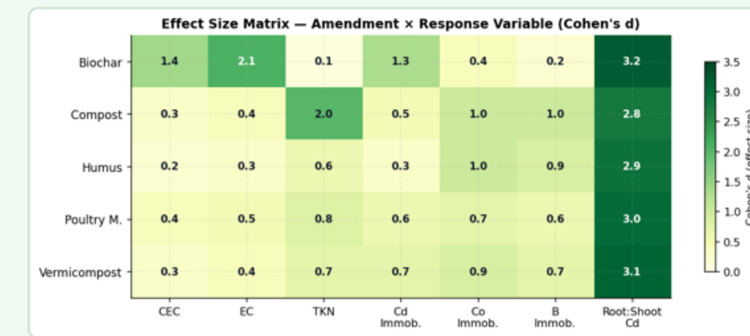

Fig. 4 — Cohen's d heatmap. Biochar surpasses agronomic thresholds for CEC and Cd/Li; compost leads for TKN and Co/B. Root:Shoot Cd ≥4 across all amendments confirms phytostabilization in every scenario evaluated.

09 · METAL DISTRIBUTION IN PLANT TISSUES

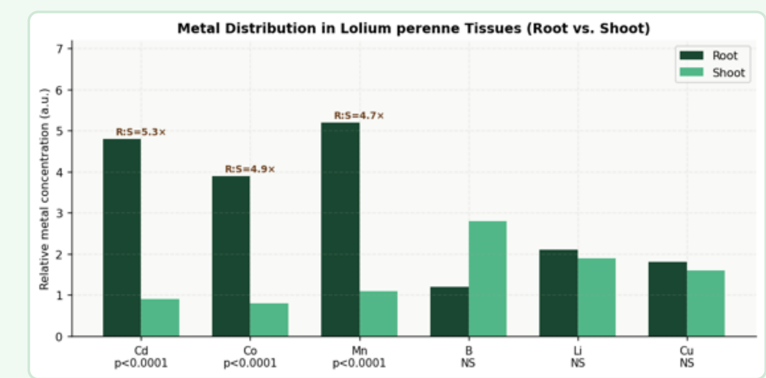

Fig. 5 — Root vs. shoot concentrations in L. perenne. Cd, Co, Mn: root:shoot ≥4 (d≥3.2, p<0.0001) — phytostabilization confirmed. B in shoots. Shoot-only surveys underestimate total stabilization by 60–75%.

10 · KEY QUANTITATIVE FINDINGS

+32% — CEC — Biochar — d=1.4, p=0.007

−45% — Cd/Li Immob. — d≥1.3, p<0.005

≥4× — Root:Shoot — d≥3.2, p<0.0001

PLANT GROWTH RESPONSE

No significant differences in root (p=0.754) or shoot (p=0.397) length, though **biochar & compost** produced the longest roots and **humus & biochar** the tallest shoots (>11 cm). With n=3, power was only 0.42 for a 15% biomass difference — at least 9 replicates needed to confirm these trends.

SIGNIFICANT METALS — $CaCl_2$ EXTRACTION

Treatment effects in 9 of 23 metals: **B, Cd, Co, Cu, Li, Mn** showed significant immobilization responses. Na, K, Mg accumulated in shoots (p<0.0001), reflecting physiological transport dynamics rather than contaminant behavior.

11 · CONCLUSIONS

1. **Amendment-metal pairing is essential** — Biochar for Cd/Li; compost and humus for Co/B. No universal amendment — selection must match the soil's metal contamination profile.
2. **Phytostabilization over phytoextraction** — Root:shoot ≥4 for Cd, Co, Mn confirms underground metal retention. Shoot-only analysis underestimates stabilization by 60–75%.
3. **Biochar raises salinity risk at 1% dose** — EC doubled (680 µS cm⁻¹) above the local limit. Dosage optimization critical before field-scale application in salt-sensitive soils.
4. **Compost delivers nitrogen — biochar does not** — TKN 2.5-fold with compost (d=2.0); no effect with biochar (d=0.1). Amendment choice must target the primary fertility bottleneck.
5. **A quantitative decision matrix for practitioners** — Cohen's d benchmarks paired with agronomic relevance thresholds provide a ready-to-use lookup table for amendment selection — compressing multi-year trials into a single 45-day crop cycle screening.

REMEDIATION STRATEGY

- Diffuse metal-contaminated agricultural soil
- Amendment-assisted phytoremediation
- In situ metal immobilization (↓ bioavailability)
- Root retention — limited translocation to shoots
- Restored soil fertility · Safer food crops

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