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Rice response to cadmium bioremediation using sorghum and mycorrhiza

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Abstract. *Harsono P, Hasanah NAU, Purwanto E, Samanhudi. 2025. Rice response to cadmium bioremediation using sorghum and mycorrhiza. Asian J Agric 9: 522-532.* Cadmium (Cd) contamination at 2.9 mg kg^{-1} in paddy soils reduces rice productivity and threatens food safety. This study aimed to evaluate the effectiveness of sorghum (*Sorghum bicolor*) phytoremediation combined with Arbuscular Mycorrhizal Fungi (AMF) in improving rice growth under mild Cd contamination. A factorial randomized complete block design with two cropping systems (seed vs. ratoon, where the same crop is re-grown from the stubble or roots of the first crop) and seven remediation treatments (control; three sorghum varieties (Super 1, Samurai 1, Kawali) combine with/without 10 g AMF plot^{-1}) was applied in 42 plots (3×4 m). Rice cv. IR 64 was transplanted 30 days after remediation. Significant interactions ($p<0.05$) between cropping system and remediation treatment occurred for plant height and leaf area. The Kawali + AMF treatment produced the most significant gains relative to the control: plant height +7.7%, leaf area +207%, chlorophyll +236% and total dry biomass +152%, while extending the vegetative phase by 23%. Improvements were consistent across sorghum seed and sorghum ratoon systems, indicating that remediation benefits persist beyond the first harvest. Ratoon sorghum-maintained remediation benefits, with 7-10% taller plants and 8% greater leaf area than the control, despite slightly reduced canopy size compared with seed sorghum; all sorghum treatments delayed flowering by ~11 days, indicating reduced Cd stress. High-biomass sorghum, especially Kawali, combined with AMF, enhances rice growth and physiology while stabilizing Cd, providing a scalable remediation strategy for sustainable rice production in Cd-contaminated tropical agroecosystems.

Keywords: Cadmium contamination, heavy metals, phytoremediation, ratoon cropping system, remediating plants

INTRODUCTION

Heavy metal contamination has emerged as a significant global challenge in agricultural lands. Recent assessments indicate that nearly 242 million hectares, representing 14-17% of global farmland, are contaminated by toxic metals, with cadmium (Cd) among the most dangerous due to its high mobility and persistence. Cd readily accumulates in rice (*Oryza sativa* L.), a staple food for more than half of the world's population, threatening both productivity and human health (Hou et al. 2025). The overuse of phosphate fertilizers, which naturally contain high Cd concentrations, is a leading cause of contamination (Grant 2015; Karimi et al. 2022). Long-term application results in Cd buildup in soils, with adverse impacts on crop physiology, soil health, and microbial communities (Singh et al. 2017; Suciu et al. 2022; Cai 2025). However, with the right measures, such as the judicious use of fertilizers and the adoption of soil remediation techniques, we can mitigate these adverse effects and restore soil health and crop productivity.

Regional case studies further highlight the severity of the issue. In China, soil Cd levels of 5.42 mg kg^{-1} have been recorded in the Minjiang River basin, while rice grains in Hunan Province contained up to 0.69 mg kg^{-1}, surpassing national limits (Liu et al. 2015; Huang et al. 2022). In India, Cd contamination in Coimbatore soils reached 8.2 mg kg^{-1} (Radha et al. 2014), while Pakistan reported ground-water concentrations exceeding WHO thresholds by more than thirtyfold (Murtaza et al. 2022). Across Europe, 5.5% of topsoil samples exceed safe Cd levels (Ballabio et al. 2023). Chronic Cd exposure is linked to kidney damage, osteoporosis, and cancer, underscoring the urgency for effective mitigation strategies.

Conventional remediation approaches, including soil washing and chemical immobilization, have been widely tested but remain constrained by cost and side effects. Soil washing can deplete essential nutrients, degrade organic matter, and inhibit microbial activity (Han 2019; Zhang et al. 2022; Resmi et al. 2023; Mindari et al. 2025). Chemical immobilization stabilizes Cd but does not remove it, leaving risks of remobilization under acidic or organic-rich conditions (Hamid et al. 2019; Liu et al. 2022). Both methods are costly, with estimated expenses of $8,000-12,000 per hectare and repeated applications required every few years (Tang and Ni 2021; Wang et al. 2021). These limitations render such approaches impractical for smallholder farmers in developing regions. Consequently, low-cost and sustainable alternatives are urgently needed.

Phytoremediation, which involves using plants to extract, stabilize, or transform heavy metals, is a promising solution. It is environmentally friendly, cost-effective, and causes minimal disruption to soil structure (Li et al. 2022;

Musa et al. 2024). Sorghum (*Sorghum bicolor* (L.) Moench) is especially attractive because of its rapid growth, deep root system, and high biomass production. Some varieties can accumulate 200-500 mg Cd kg^{-1} dry matter while still growing well (Hassan et al. 2020). Sorghum is also highly tolerant of drought and heat stress, making it suitable for tropical agroecosystems (Behera et al. 2022). These characteristics make sorghum a strong candidate for large-scale phytoremediation.

Yet, sorghum alone may not optimize remediation efficiency. Arbuscular Mycorrhizal Fungi (AMF) can enhance sorghum performance by improving nutrient uptake, reducing Cd translocation, and immobilizing metals in fungal structures (Zhang et al. 2020; Bhantana et al. 2021). AMF symbiosis strengthens root systems, reduces Cd accumulation in edible tissues, and improves plant tolerance to stress (Dharma et al. 2024; Zhao et al. 2024). Integrating sorghum with AMF, therefore, holds strong potential for sustainable Cd remediation. Moreover, applying a ratoon system—harvesting sorghum while allowing regrowth from stubble—offers a cost-saving strategy that extends remediation capacity without repeated planting (Zhou et al. 2022; Pratama et al. 2024).

Previous research has studied sorghum or AMF separately, but their combined use in a ratoon-based remediation system remains unexplored. This gap is especially important in Asian rice-dominated agroecosystems, where remediation efficiency, economic feasibility, and sustainability are critical. Therefore, this study aimed to evaluate the effectiveness of integrating sorghum varieties, AMF inoculation, and ratoon cropping in improving the growth and physiological performance of rice (cv. IR 64) cultivated on Cd-contaminated paddy soils.

MATERIALS AND METHODS

Site Description and Soil Analysis

The field experiment was conducted in a cadmium-contaminated paddy field at Balecatur Village, Gamping Sub-district, Sleman District, Yogyakarta, Indonesia (7°45′ S; 110°18′ E; 110 masl). Before the treatments were applied, composite soil samples were collected from a depth of 0–20 cm for initial chemical analysis. The soil was characterized by low organic C (1.02%) and low total N (0.21%). The initial soil Cd concentration was 2.91 mg kg^{-1}, significantly above the critical limit of 0.50 mg kg^{-1}. The bioavailable Cd concentration was determined using Atomic Absorption Spectrometry (AAS) following a Diethylenetriaminepentaacetic acid (DTPA) extraction method. The complete chemical profile of the initial soil is presented in Table 1.

Experimental design and treatments

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three blocks (replications). A factorial treatment structure was applied with two factors: (i) cropping system (seeded sorghum vs. ratooned sorghum) and (ii) biological remediation (seven levels). This resulted in 14 treatment combinations, replicated across the three blocks to yield 42 experimental units. Each experimental plot measured 3×4 m and was separated from neighboring plots by 0.5 m earthen bunds. The seven biological remediation treatments were: an unremediated control (T0, no sorghum), and six combinations of three sorghum varieties (Super 1, Samurai 1, Kawali) with or without Arbuscular Mycorrhizal Fungi (AMF) inoculation. The AMF inoculum was made from *Rhizophagus intraradices* sourced from the Indonesian Soil Research Institute in Bogor, with about 150 spores per gram of zeolite-based carrier material. For treatments with inoculation, 10 g of AMF per plot was applied by mixing the inoculum into the top 10 cm of soil along the planting rows just before sowing sorghum. The specific treatment codes and descriptions are listed in Table 2.

Crop management and remediation procedure

Sorghum was first established according to the assigned treatments. For the ratooned sorghum plots, the crop was harvested by cutting the stems at approximately 15 cm above the ground, and the remaining stubble was allowed to re-grow to produce a second cycle. After the sorghum remediation phase (one cycle for seeded plots, two cycles for ratooned plots), all sorghum residue was chopped and incorporated into the soil. The plots were then puddled in preparation for rice planting; thirty-day-old seedlings of rice cv. IR 64 were transplanted at a spacing of 20×20 cm, with two to three seedlings per hill. A standard fertilizer regime was applied uniformly to all plots, consisting of Petro-granule organic fertilizer (5 t ha^{-1}) and compound NPK (15-15-15) fertilizer (200 kg ha^{-1}). Standard practices for irrigation, manual weed management, and integrated pest and disease control were conducted as needed for the duration of the rice cultivation period.

Data collection and statistical analysis

Agronomic and physiological data were collected throughout the rice growing season. Measurements included plant height, total leaf area per hill, SPAD chlorophyll index, stem and root dry weights, total dry biomass, flowering time, root-to-shoot ratio, plant growth rate, and net assimilation rate. All collected data were subjected to Analysis of Variance (ANOVA) appropriate for a factorial RCBD using the widely-recognized SAS software (Version 6.12, SAS Institute Inc., Cary, NC). Any variables with a coefficient of variation greater than 30% were square-root transformed prior to analysis to ensure homogeneity of variances. When the F-test indicated a significant treatment effect ($p<0.05$), means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Table 1. Soil characteristics before remediation

C-organic (%)	Organic materials (%)	Nitrogen (%)	P_2O_5 (%)	K_2O (ppm)	Cr (ppm)	Cu (ppm)	Cd (ppm)	Pb (ppm)	Zn (ppm)
1.016	1.751	0.207	0.084	253.5	2.758	0.162	**2.914**	0.167	0.233

Source: Result of soil sample analyzed before the application of any remediation treatments

Table 2. Codes and descriptions of biological-remediation treatments applied before rice planting

Code	Sorghum variety	AMF inoculum (g plot^{-1})	Description
T0	–	0	Unremediated control (continuous rice, no sorghum)
C1M0	Super 1	0	Super 1 without AMF
C1M1	Super 1	10	Super 1 + AMF
C2M0	Samurai 1	0	Samurai 1 without AMF
C2M1	Samurai 1	10	Samurai 1 + AMF
C3M0	Kawali	0	Kawali without AMF
C3M1	Kawali	10	Kawali + AMF

Note: AMF: Arbuscular Mycorrhizal Fungi

RESULTS AND DISCUSSION

Sorghum bioremediation enhances rice vegetative growth: Plant height and leaf area

Analysis of variance detected a significant interaction between the planting system and the remediation treatment for rice height 8 weeks after transplanting (F System × Treatment, $P<0.05$). Within the sorghum-seed system, rice that followed Samurai 1 without AMF (C2M0) reached ≈ 86 cm, about 36% taller than the non-remediated control (≈ 63 cm). Every sorghum treatment, whether inoculated or not, produced plants taller than the control. In the sorghum-ratoon system, the absolute range narrowed (≈ 67-71 cm), yet all sorghum treatments still exceeded the control. Across systems, canopy stature ranked C2M0 > Kawali + AMF (C3M1) ≈ Super 1 + AMF (C1M1) ≫ T0 (Figure 1).

The stature gains confirm that pre-planting phytoremediation by sorghum alleviates Cd toxicity and improves rhizosphere conditions. Sorghum's deep roots sequester Cd and release citrate, malate, and oxalate that either precipitate Cd-phosphate complexes or promote microbial immobilization (Bai et al. 2021). A two-season field study in Hunan, China, recorded a 52% drop in extractable Cd after successive sorghum crops (Liu et al. 2024), mirroring the height response observed here. The supremacy of Samurai 1 without AMF suggests that its larger root system (Sagimbayeva 2023) captured more Cd, while the absence of AMF avoided early competition for available phosphorus (an element already marginal in many Andisols).

AMF nonetheless raised plant height in more moderate-rooted cultivars such as Kawali (C3M1). Hyphal networks extend the depletion zone for PO_4^{3-} and Mg^{2+}, adsorb Cd onto chitosan-rich walls, and downregulate the transporter *OsNRAMP5,* thereby lowering Cd entry into rice tissues (Etesami et al. 2021; Kaur et al. 2022). Such variety-specific synergy is consistent with the 28% height increase reported for rice, AMF combinations in Pb-Cd soils when root colonization exceeded 60% (Guigard et al. 2023).

The rice behind the sorghum-ratoon phase remained 7-10% shorter than behind seed sorghum, a pattern attributable to depleted carbohydrate reserves in older stubble and slower N-P replenishment after the first sorghum harvest (Farooq et al. 2022). However, the success of every ratoon treatment in outperforming the control demonstrates that re-grown sorghum roots continue to chelate Cd and modulate the soil environment. This resilience supports a multi-season remediation concept in which sorghum is sown once, ratooned, and then followed by rice (maintaining Cd extraction while reducing land preparation costs). This strategy not only maintains Cd extraction but also contributes to a healthier soil environment, aligning with our environmental goals.

Pairing high-biomass sorghum cultivars (e.g., Samurai 1) with targeted AMF inoculation, where responsive (e.g., Kawali + AMF), offers a flexible framework for rehabilitating Cd-contaminated paddy soils. Height responses in the present study corroborate earlier gains in leaf area and chlorophyll, indicating coordinated improvements in photosynthetic capacity and biomass accumulation. Future work should quantify Cd residues in roots, straw, and grain to ensure that the observed growth advantage is accompanied by compliance with Codex limits (<0.40 mg kg^{-1} polished rice).

The increase in phytoremediation capacity in sorghum inoculated with arbuscular mycorrhizal fungi can be attributed to several synergistic mechanisms. First, extensive external hyphal networks beyond the root depletion zone increase overall plant nutrient uptake (especially phosphorus), thereby enhancing its resistance to heavy metal stress (Cheng et al. 2021). Second, most cadmium is immobilized and stored in fungal structures (hyphae and spores) in the soil and roots, a process that effectively reduces metal translocation to the aerial parts of the plant (Chauhan et al. 2022). Third, this symbiosis stimulates the production of higher metal-binding compounds such as phytochelatins and metallothioneins in plant roots. These compounds function as a detoxification pathway by binding cadmium ions (Zhao et al. 2024).

Analysis of variance revealed a strong interaction between the planting system and remediation treatment for rice leaf area eight weeks after transplanting (F System × Treatment, $P<0.05$). Within the sorghum-seed system, plots pre-conditioned with Samurai 1 without AMF (C2M0) developed the most extensive canopies (≈ 4,460 cm^2 hill^{-1}, almost fourfold the untreated control). In contrast, every sorghum ± AMF combination gave significantly wider leaves than the control. In the sorghum-ratoon system, the numerical range narrowed (≈ 2,220-3,980 cm^2) yet the ranking persisted: C2M0 and Kawali + AMF (C3M1) remained in the top statistical group (DMRT 5%). Canopy means across systems, followed by the order C2M0 > C3M1 ≈ C1M1 ≫ T0 (Figure 2).

These results confirm that a single sorghum crop (especially the high-biomass cultivar Samurai 1) can cut Cd bioavailability and release low-molecular organic acids, thereby restoring rhizosphere pH and mobilising P, Zn, and Mg that drive leaf expansion in the succeeding rice crop (Eid et al. 2021; Sagimbayeva 2023). AMF magnified this benefit in the medium-vigor cultivar Kawali: hyphal networks enlarge the depletion zone for PO_4^{3-} and micronutrients, improve leaf water potential, and irreversibly bind Cd to chitin-rich cell walls (Yang et al. 2024; Zhao et al. 2024). A similar cultivar-by-AMF specificity was reported by Etesami et al. (2021), who showed that rice leaf area increased 34% only when AMF colonisation exceeded 60% root length in metal-enriched soils.

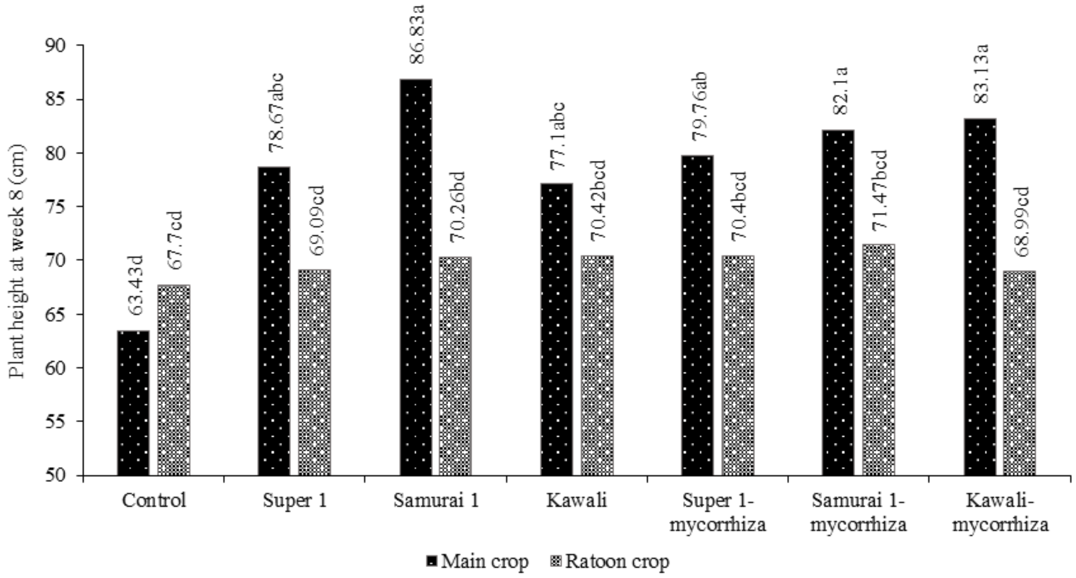

Figure 1. The effect of sorghum-based bioremediation and cropping system on rice plant height at eight weeks after transplanting. Bars represent the mean plant height (cm) of three replicates. Different letters above the bars indicate a significant difference between treatment combinations according to Duncan's Multiple Range Test (DMRT) at $p<0.05$. A significant interaction was observed between the cropping system and remediation factors

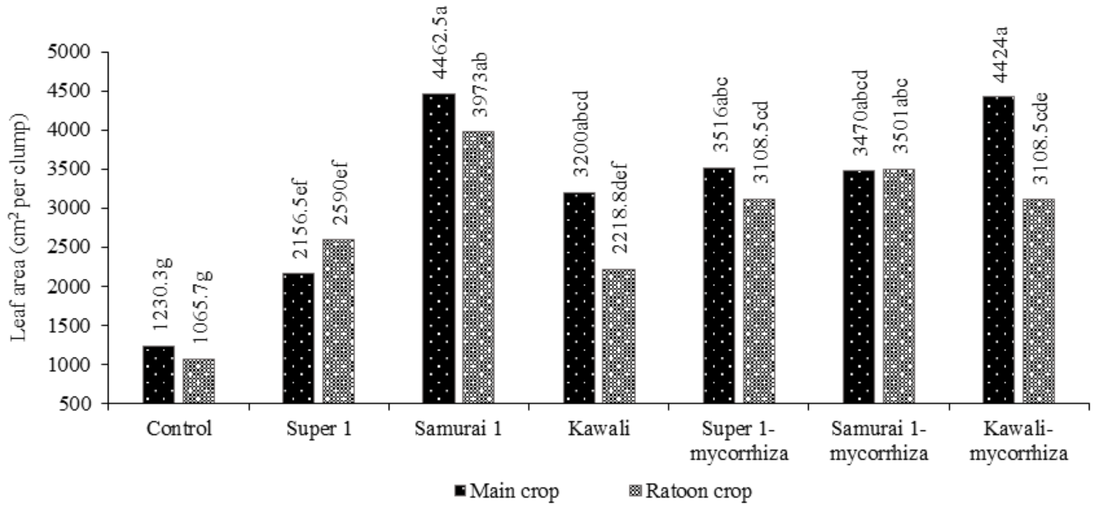

Figure 2. The effect of sorghum-based bioremediation and cropping system on rice leaf area at eight weeks after transplanting. Bars represent the mean total leaf area per hill (cm^2) of three replicates. Different letters above the bars indicate a significant difference between treatment combinations according to Duncan's Multiple Range Test (DMRT) at $p<0.05$. A significant interaction was observed between the cropping system and remediation factors

On average, the ratoon phase produced canopies 8% smaller than the seed phase, consistent with lower post-harvest N and P and depleted carbohydrate pools in the stubble (Farooq et al. 2022), yet still far outperformed the non-remediated control. This demonstrates that even regrowth sorghum roots continue to chelate Cd and condition the soil. Because leaf area index is a primary determinant of radiation capture and yield potential, enlarging it through deep-rooted sorghum cultivars (and, where responsive, AMF inoculation) offers a physiologically sound route to rehabilitate Cd-contaminated paddies. Confirmation of residual Cd in soil and grain, however, remains essential for consumer safety before widescale adoption of multi-season sorghum-rice rotations.

Impact on photosynthetic capacity and biomass accumulation

Leaf-chlorophyll analysis by the 80% acetone extraction method (Wintermans and De Mots 1965) revealed that only the remediation treatment significantly influenced pigment concentration ($P<0.05$); the cropping system (sorghum seed vs. ratoon) and its interaction were non-significant. The highest value (0.37 mg kg^{-1} DM) occurred in the Kawali + AMF (C3M1) treatment, whereas all other sorghum ± AMF combinations ranged from 0.10 to 0.15 mg kg^{-1} and did not differ from one another (Figure 3). This pronounced superiority of C3M1 underscores the dual role of a deep-rooted sorghum variety and AMF in enhancing leaf greenness under Cd stress. External hyphae of *R. intraradices* enlarge the root absorption zone, boost P and Mg uptake (both essential for chlorophyll biosynthesis), and chelate Cd within hyphal walls, thereby suppressing chlorophyll degradation (Zhang et al. 2018; Wahab et al. 2023). The strong response of plots preceded by Kawali aligns with Babadi et al. (2019), who reported that higher root biomass of the remediator crop fosters AMF colonization and elevates chlorophyll content in subsequent rice.

The absence of chlorophyll differences between seed and ratoon sorghum systems indicates that phytoremediation benefits persist even when the second sorghum cycle derives solely from regrowth stubble, supporting earlier findings on leaf-area recovery. Field evidence shows that ratoon sorghum roots remain active in Cd sorption and organic-acid exudation, sustaining soil enzymatic activity well beyond a single season (Wang et al. 2022). Improved chlorophyll concentration is physiologically important because it correlates with the higher net-assimilation rate observed in this study and, consequently, with greater shoot biomass and yield potential (Huang et al. 2024). Taken together, these results demonstrate that pairing high-biomass sorghum varieties with targeted AMF inoculation not only lowers Cd bioavailability but also preserves photosynthetic capacity, thereby supporting sustainable rice production on contaminated paddy soils. Verification of residual Cd in both soil and grain remains essential to confirm food-safety compliance.

The nearly fivefold increase in Root Dry Weight (RDW) in the Samurai 1 + AMF (C2M1) treatment confirms the synergy between deep-rooted sorghum varieties and the colonization of AMF in expanding the rhizosphere zone (Figure 4). External AMF hyphae function as "root extensions" that enhance P, Zn, and water uptake while binding Cd to the hyphal walls (Ullah et al. 2024). These findings are consistent with Gao et al. (2024), who reported a 68% increase in rice RDW and a 55% decrease in leaf Cd after pre-planting sorghum + AMF on Cd-2.5 mg kg^{-1} soil.

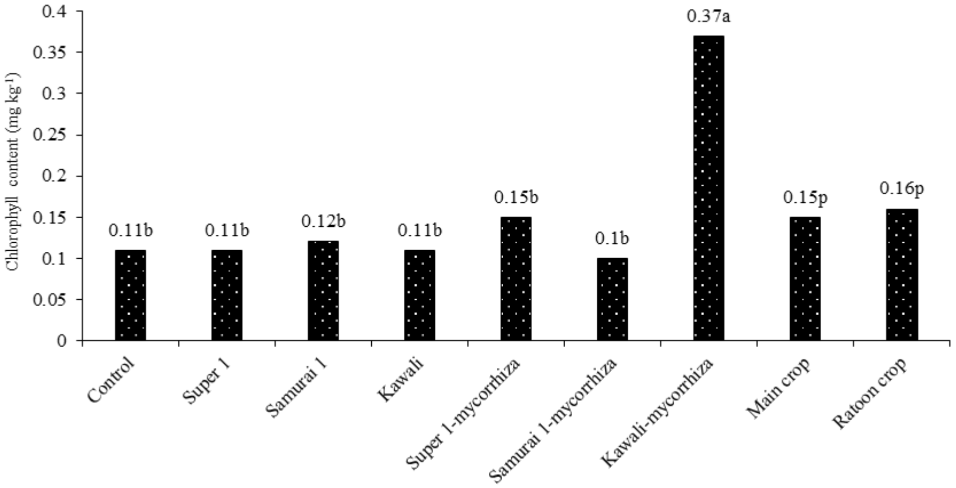

Figure 3. The main effect of biological remediation treatments on rice leaf chlorophyll content. Bars represent the mean chlorophyll content (mg kg^{-1} DM) of three replicates, averaged across both cropping systems, as there was no interaction. Different letters above the bars indicate a significant difference between remediation treatments according to Duncan's Multiple Range Test (DMRT) at $p<0.05$

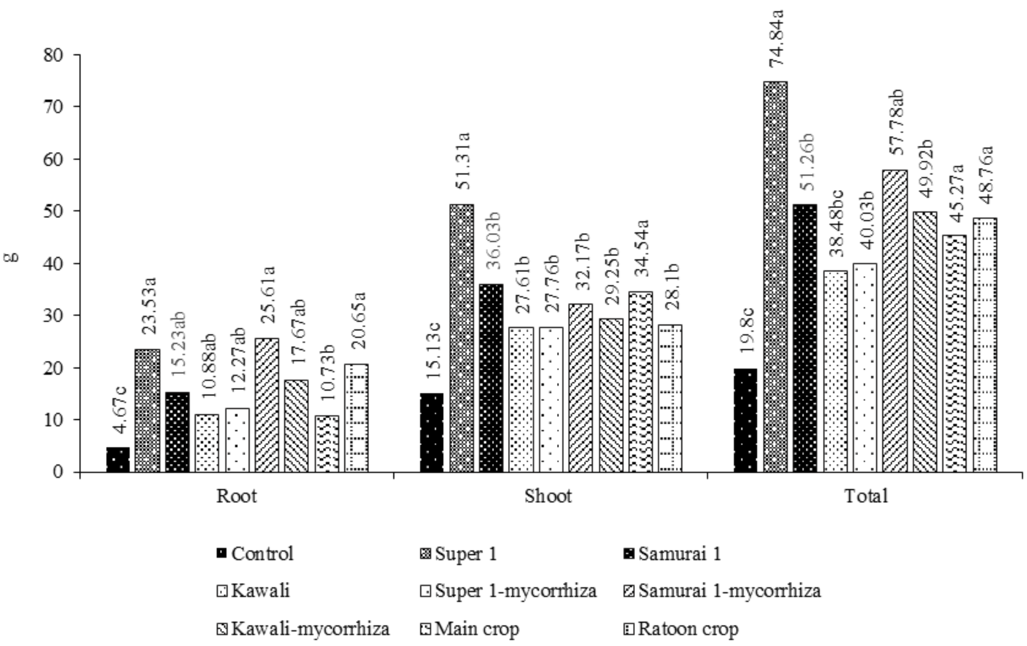

Figure 4. The main effect of biological remediation treatments and cropping systems on the dry weight of rice plant components. Bars represent the mean dry weight (g) of three replicates for shoot, root, and total biomass. Within each component, bars with different letters indicate a significant difference between treatments according to Duncan's Multiple Range Test (DMRT) at $p<0.05$. No significant interaction was observed between the factors

Conversely, the dominance of canopy biomass (SDW ≈ 51 g) and total biomass (TBW ≈ 75 g) in Super 1 without AMF (C1M0) indicates that the high-canopy-mass sorghum variety leaves behind organic acid-rich residues that improve N-P-K availability, thereby allowing rice to allocate more carbon to the shoot when Cd toxicity had decreased. Dai et al. (2024) found a similar pattern: sunflower rotation without AMF increased rice SDW by 34% in Zn–Cd soil because sunflower exudates stabilized metals and provided nutrients.

Although the sorghum ratoon system produced heavier roots than sorghum seed (20.7 vs. 10.7 g), its shoot growth was slightly inhibited (28.1 vs. 34.5 g). Highly lignified ratoon roots act as a "bank" for Cd and carbohydrate reserves (Jiang et al. 2023), but photosynthate flow to the shoot tip decreases with increasing tissue age. As a result, TBW in both systems was comparable (≈ 45–49 g). Wang et al. (2023) reported that Cd extraction from soil by ratoon sorghum was 17% higher than replanted sorghum, supporting our findings that phytoremediation benefits persist in regrowth stubble.

Differences in responses between sorghum varieties indicate the need for variety-specific strategies. High-shoot-biomass varieties (Super 1) are effective for enhancing photosynthesis and grain yield when Cd is sufficiently stabilized; AMF is not essential as P is adequately available. Deep-rooted varieties (Samurai 1, Kawali) benefit most from AMF; high NAT and RDW help trap Cd in the rhizosphere and reduce translocation to the canopy. A meta-analysis by Al-Huqail et al. (2024) showed that the combination of deep-rooted cultivars + AMF on average increased RDW by 52% and reduced grain Cd by 48%, reinforcing the relevance of our findings.

Mitigation of cadmium stress: Delayed flowering and physiological responses

Pre-planting remediation using sorghum consistently delayed the generative transition of rice by approximately 11 days compared to the control without remediation (47.5 vs. 58.5 DAT), while the difference of less than 1 day between the sorghum seed and sorghum ratoon systems indicates that the benefits of phytoremediation are maintained even though the second phase originated solely from the regrowth of sorghum stumps (Figure 5).

Mechanism of flowering delay

Research by Soma et al. (2023) showed that internal Cd accumulation triggers the expression of the gene *OsFD1* and accelerates flowering as an "escape strategy". In this experiment, the reduction in Cd bioavailability (through absorption by deep-rooted sorghum biomass and chelation in the hyphae of *R. intraradices*) inhibited the activation of *Hd3a/RFT1*, thereby prolonging the vegetative stage. These findings are consistent with those of Li et al. (2022), who reported a 9–12-day delay in rice after sunflower-AMF phytoremediation in Pb-Cd-contaminated soil, as well as a 46% reduction in tissue Cd levels. In addition to the florigen pathway, AMF also reduced the ABA/GA_3 ratio (stress hormone vs. growth hormone), thereby suppressing flowering acceleration signals (Xing et al. 2022).

Agronomic implications

Although the generative phase was delayed, the more extended vegetative period increased leaf area and photosynthetic index, as indicated by increased shoot-root biomass in the AMF-treated sorghum (Figure 2). Huang et al. (2024) reported a positive correlation (r=0.72) between

8-15 days of vegetative elongation and an 18% increase in grain weight in rice grown on Ni-Cd-remediated corn soil. Increased canopy area provides more time for assimilate accumulation, while reduced internal Cd stabilizes chloroplasts and reduces ROS (reactive oxygen species) that damage PSII (Zhou et al. 2024).

Role of sorghum varieties and AMF

High-biomass sorghum varieties (Super 1, Kawali) produce roots and straw rich in organic acids (malic, citric) that complex Cd and increase soluble C for soil microbes (Chen et al. 2022). Further AMF inoculation enhances the availability of P and Mg (key chlorophyll cofactors) and reduces the expression of Cd transporters (*OsNramp5*) in rice roots (Bao et al. 2025). These two synergistic processes extend the vegetative phase without disrupting flowering synchronization between plots, crucial for simultaneous harvest.

Advantages of the ratoon system

Old sorghum roots left behind after seed harvest act as an additional "metal buffer"; a study by Wang et al. (2023) showed that the ratoon phase of sorghum extracted 17% more Cd from the soil than replanting. This explains the high NAT (Figure 6) and minimal flowering delay in ratoon plots, despite slightly lower photosynthate conduction to the canopy compared to seed crops.

Thus, the integrated sorghum rotation (seed → ratoon) + AMF → IR 64 rice system offers two simultaneous benefits: (i) reduction of Cd stress through metal immobilization/extraction, and (ii) extended vegetative duration enhancing photosynthetic capacity. However, measurements of Cd residues in grains are still needed to confirm that delayed flowering is indeed followed by reduced Cd levels in the harvest, a critical requirement for food safety according to Codex (<0.4 mg kg^{-1} rice).

Observations at 55 days after planting showed that land remediation and cropping system treatments had different effects on rice physiological parameters, namely Root-to-Shoot Ratio (RTS), Plant Growth Rate (PGR), and Net Assimilation Rate (NAR), although there were no significant interactions between these factors (Figure 6). The highest RAT values were obtained in the Super 1-mycorrhiza (0.78) and Kawali-mycorrhiza (0.70) treatments, which were significantly different from the control (0.34) and other treatments. This indicates that the combination of the superior varieties Super 1 and Kawali with mycorrhiza and the ratoon cropping system significantly increased root growth relative to the canopy. This increase is likely due to the role of mycorrhiza in expanding the root absorption area and improving nutrient availability in cadmium-contaminated soil (Chen et al. 2025). Additionally, the ratoon system, which utilizes energy reserves from residual roots, demonstrates better efficiency in supporting root growth.

The highest increase in NAT in the combinations Super 1 + AMF and Kawali + AMF indicates that phytoremediation by deep-rooted sorghum, coupled with colonization of *R. intraradices*, increases carbon allocation to rice roots. Research by Xu et al. (2024) found that rice root biomass increased by 71% and leaf Cd decreased by 52% when crops were preceded by high-biomass AMF-colonized sorghum varieties, supporting the "external trap" mechanism of Cd in the hyphal walls and root tissues of rice. The NAT value of the ratoon system was nearly 2.5× seed, providing additional evidence: old, highly lignified roots act as a second Cd sink after sorghum.

Conversely, the Super 1 without AMF treatment yielded the highest LPT and LAB. The Super 1 variety is known for its broad canopy and high soluble sugar content; its root residues release exudates that mobilize P and Zn, reducing the Cd and Fe, thereby improving rice photosynthesis (Hasanah et al. 2023). Our results (LAB 0.22 g m^{-2} day^{-1}) are close to the range of rice in non-contaminated fields and consistent with the report by Chen et al. (2019) found that "biomass"-type sorghum residues increase rice PGR by 35-40%. The slight difference in LPT-LAB between sorghum seed and sorghum ratoon confirms that sorghum–rice rotation maintains uniform physiological conditions of rice after two remediation cycles. The ratoon phase leaves behind an older and more extensive sorghum root system, prolonging root–Cd contact and increasing rice NAT. In contrast, the seed phase contributes rapidly decomposable fresh green biomass rich in organic acids, supporting rice photosynthesis (LAB).

Sorghum variety selection determines the pathway for post-remediation physiological improvement in rice. Super 1 (without AMF) maximizes photosynthesis and rice canopy growth, suitable when the primary target is to increase grain yield. Super 1 or Kawali + AMF directs carbon to rice roots (high NAT), effectively retaining Cd in the rhizosphere and reducing metal translocation. A recent meta-analysis (Al-Huqail et al. 2024) shows that the combination of deep-rooted sorghum varieties + AMF increases average NAT in rice by 44% and reduces Cd in grain by 48%. These findings confirm the potential of the sorghum (seed → ratoon) – IR 64 rice rotation as a sustainable phytoremediation strategy; however, measurements of Cd residues in roots, straw, and grain are still required to ensure food safety and confirm the correlation between high NAT and reduced Cd in tissues.

The selection of a land remediation strategy using sorghum and mycorrhiza is a crucial decision that balances the urgency of cleanup with long-term ecological restoration goals. Conventional methods, such as excavation, focus primarily on eliminating contaminants, often sacrificing the physical and biological integrity of the soil (Koushal et al. 2025). In contrast, the phytoremediation approach in this study serves the purpose of sustainable remediation. In addition to reducing cadmium toxicity, treatment with sorghum and mycorrhiza actively rebuilds soil fertility (Babadi et al. 2019). This improved soil quality provides the foundation for the positive physiological response observed in rice plants. The positive response of rice demonstrates that this method not only removes contaminants but also revives the land for future agronomic productivity. This restorative approach makes phytoremediation a superior ecological investment for sustainable agriculture.

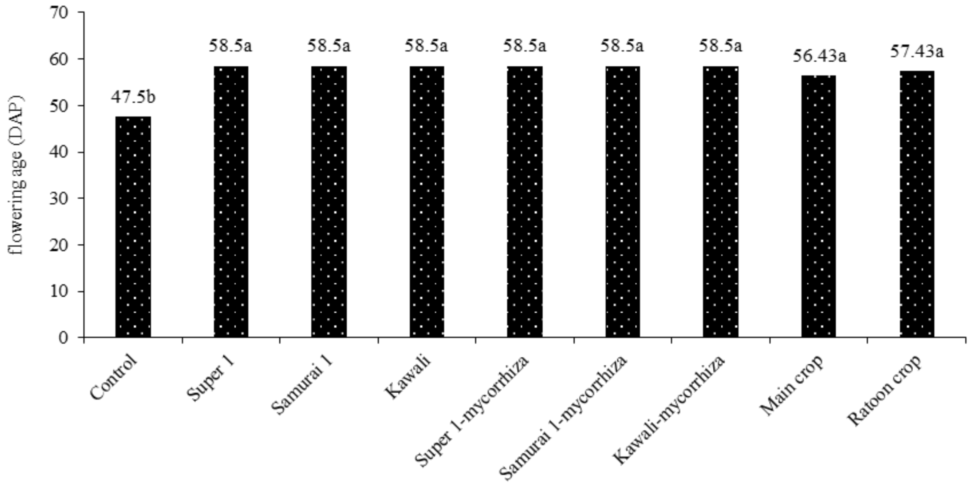

Figure 5. The main effect of biological remediation treatments and cropping systems on the flowering age of rice. Bars represent the mean days to 50% flowering after transplanting (DAP) of three replicates. Within each factor (remediation or cropping system), bars with different letters indicate a significant difference according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$. No significant interaction was observed between the factors

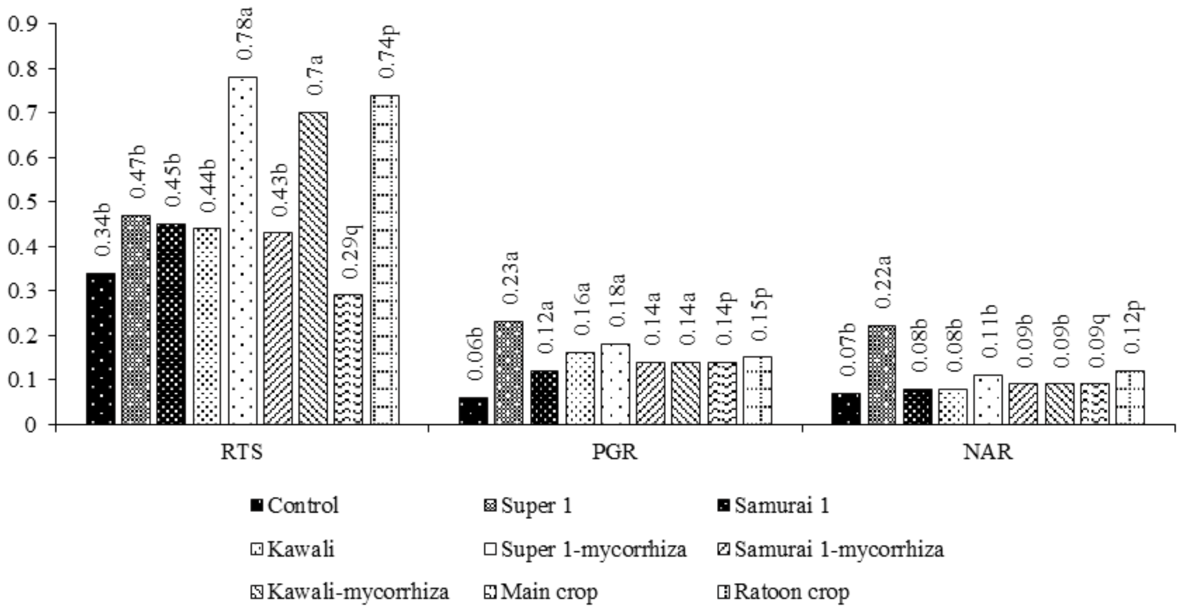

Figure 6. The main effect of biological remediation treatments and cropping systems on rice physiological parameters at 55 DAP. Parameters include Root-to-Shoot Ratio (RTS), Plant Growth Rate (PGR), and Net Assimilation Rate (NAR). Bars represent the mean values of three replicates. Within each parameter, bars with different letters indicate a significant difference between treatments according to Duncan's Multiple Range Test (DMRT) at $p<0.05$. No significant interaction was observed between the factors

However, it is crucial to acknowledge that the effectiveness of this phytoremediation approach is inherently linked to specific local conditions. A range of edaphic factors, including soil pH, moisture content, and the indigenous microbial community, highly influence the efficacy of the process (Miranda et al. 2022). The site-specific nature of this remediation process is governed by several key abiotic factors that influence both cadmium bioavailability and the efficacy of the sorghum-mycorrhiza symbiosis. Soil pH is a primary determinant, as lower pH levels (acidic conditions) typically increase the solubility and mobility of cadmium, making it more available for plant uptake (Lian et al. 2022). Conversely, a higher soil organic matter content can reduce cadmium bioavailability through chelation and adsorption (Gutiérrez et al. 2022; Karimah et al. 2024), while soil moisture levels are critical for both host plant and mycorrhizal activity (Zhang et al. 2024).

Beyond these site-specific and technical considerations, the practical adoption of this remediation method also hinges on socio-economic factors (da Silva et al. 2023). The potential resistance from farmers to change conventional practices is a significant barrier to implementation, despite the method's long-term cost-effectiveness (Wang and Delavar 2023). Furthermore, a critical consideration for long-term sustainability is the repeated application on the same land. Continuous monoculture cultivation of high-biomass crops such as sorghum has the potential to cause depletion of essential soil nutrients over time (de Resende et al. 2022).

Despite these considerations, this study demonstrates that pre-planting phytoremediation using sorghum, from both seed and ratoon systems, substantially improves the growth physiology of rice planted in Cd-contaminated soil. Our main quantitative findings indicate that the Samurai 1 treatment without AMF produced the highest increase in plant height (~36%) and leaf area (3.9-fold) compared to the control, while Kawali with AMF recorded the highest chlorophyll concentration (~0.37 mg kg^{-1} DM). These findings can be translated into clear practical recommendations. For farmers, a sorghum rotation (seed → ratoon)–rice, reinforced with AMF on responsive cultivars, offers a low-input option for rehabilitating lightly to moderately contaminated rice fields. Variety selection should be tailored to objectives: Samurai 1 is highly suitable for maximizing biomass and yield potential, while Kawali with AMF should be prioritized to maintain plant health and immobilize Cd. For agricultural policymakers, this study highlights the potential for promoting sorghum-based bioremediation as a subsidizable alternative for regional land rehabilitation programs. To validate and scale up this technology, future research is essential. Key priorities should include: (i) measuring Cd residues in polished rice to ensure food safety; (ii) conducting multi-season trials to evaluate long-term sustainability, ideally incorporating practices like crop rotation with legumes (Rajput et al. 2024) and organic amendments (Ghimire et al. 2024) to maintain soil health; (iii) conducting economic analyses to confirm its feasibility for smallholder farmers; and (iv) investigating synergistic effects with other amendments to accelerate metal stabilization.

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