Vol. 15, No. 4 (2026): 1471 - 1482
http://dx.doi.org/10.23960/jtepl.v15i4.1471-1482

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Mitigation of Cadmium Stress in Lettuce (*Lactuca sativa* L.) Using Rice Husk Biochar and Silica Fertilizer

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Article History:

Received : 25 April 2026
Revised : 31 July 2026
Accepted : 01 August 2026

Keywords:

Cadmium Stress,
Lettuce Growth,
Phytoremediation,
Rice Husk Biochar,
Silica Fertilizer.

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ABSTRACT

Heavy metal contamination, particularly cadmium (Cd), can arise from the use of organic fertilizers derived from urban waste. Cd is highly toxic and mobile, posing serious health risks when accumulated in edible crops such as lettuce. This study aimed to evaluate the potential of rice husk biochar (RHB) and silica fertilizer to mitigate Cd stress on lettuce growth. The research employed a factorial Completely Randomized Design with two factors: biochar dose (0, 15, 30, 45 tons/ha) and silica dose (0, 3, 6 g per 3 kg soil per polybag). Soil was spiked with 15 ppm Cd. Parameters measured included plant height, leaf number, fresh weight, soil Cd, and plant Cd. Data were analyzed using ANOVA and LSD test at 5%. Results showed that biochar significantly affected plant height and leaf number ($P<0.01$), with 45 tons/ha producing the tallest plants (19.81 cm) and most leaves (18.56 leaves), while reducing plant Cd to 0.47 ppm. The combination of P3S1 (45 tons/ha biochar + 3 g/3 kg silica) resulted in the lowest soil Cd (1.16 ppm) and plant Cd (0.32 ppm). Regression analysis showed a weak correlation (R^2 = 22.74%) between soil and plant Cd. Although the interaction between biochar and silica was not statistically significant ($P > 0.05$), the main effect of biochar significantly reduced plant Cd concentration ($P < 0.05$), with the highest dose (45 tons/ha) producing the lowest plant Cd (0.47 ppm). The treatment combination P3S1 (45 tons/ha biochar + 3 g/3 kg silica) numerically produced the lowest soil Cd (1.16 ppm) and plant Cd (0.32 ppm), but did not show synergistic interaction.

1. INTRODUCTION

Cadmium (Cd) poses a serious threat to food safety when it accumulates in edible crops, and its entry into agricultural systems is often exacerbated by the use of urban waste-based organic fertilizers (Das *et al*., 2023). Cadmium (Cd) is a highly toxic heavy metal contaminating agricultural soils through mining, phosphate fertilizers, and wastewater irrigation, threatening crop productivity and food safety (Alhaj *et al*., 2024). Cd contamination disrupts nutrient uptake, damages soil properties, and induces chlorosis, stomatal closure, and photosynthetic inhibition (Alkharabsheh *et al*., 2021; Krishnani *et al*., 2023). Through the food chain, Cd causes kidney failure, bone demineralization, and cancer (Rasin *et al*., 2025).

Lettuce (*Lactuca sativa* L.) has high Cd absorption capacity and is often consumed raw, making it a significant health concern. Cd exposure in lettuce induces growth inhibition, oxidative stress, and reduced nutritional quality (Chaubey *et al*., 2024; Zhang *et al*., 2023).

Rice husk biochar (RHB) is a promising, low-cost amendment for mitigating heavy metal mobility in soils. RHB immobilizes Cd, modifies soil physicochemical properties, and supports crop growth through its high silica content and functional groups (Li *et al*., 2022; Li *et al*., 2023). Studies show RHB reduces Cd bioavailability and enhances plant performance under stress (Alkharabsheh *et al*., 2021; Chaubey *et al*., 2024; Krishnani *et al*., 2023).

Silica (SiO_2) from rice husk similarly strengthens plant defenses, improves mechanical properties, and increases tolerance to heavy metal exposure (Hamidu *et al*., 2025; Rodriguez-Otero *et al*., 2023). Biochar amendments have been shown to decrease Cd concentrations in lettuce shoots and enhance biomass under Cd stress (Zhang *et al*., 2023). Silica-based amendments improve stress tolerance through structural reinforcement and reduced metal translocation (Hamidu *et al*., 2025). The integration of RHB and silica offers complementary mechanisms: RHB immobilizes Cd while silica bolsters plant defense, supporting growth under adverse conditions. Co-application strategies can enhance contaminant immobilization and crop performance more effectively than either input alone (Ndoung *et al*., 2021). Nevertheless, previous studies have primarily tested RHB or silica separately, and their combined effects on lettuce a crop with high Cd accumulation potential have not been examined. Furthermore, no research has determined the optimal dose combination of RHB (0–45 tons/ha) and silica (0–6 g/3 kg soil) for mitigating Cd stress at a contamination level of 15 ppm. Therefore, this study aimed to evaluate the potential of rice husk biochar, silica fertilizer, and their combination on lettuce growth and Cd accumulation under Cd stress.

Based on this background, the objectives of this research were to evaluate the potential of rice husk biochar, silica fertilizer, and their combination on lettuce growth under cadmium stress.

2. MATERIALS AND METHODS

2.1. Time and Place of Research

This research was conducted over three months from October 2023 to January 2024. The research took place at Green House Mas Obama, Jalan Srikoyo 16, Krajan Village, Patrang District, Jember Regency.

2.2. Tools and Materials

The tools used in this research included writing instruments, a ruler, a camera, a bucket, a measuring cup, a hoe, an analytical balance, a chlorophyll meter (Soil Plant Analysis Development, SPAD), a soil analyze tester, a Graphite Furnace AAS, a walkway plank, an observation table, a thermometer, a pH meter, a 100 ml measuring cylinder, and a 1000 ml measuring cylinder. The materials used in this research included lettuce seeds, silica fertilizer, rice husk biochar, Cadmium ($CdSO_4$), water, 30 cm x 30 cm polybags, humus soil, sand, and NPK 16:16:16 fertilizer.

2.3. Research Design

The experiment was arranged factorially using a completely randomized design (CRD) with soil spiked with cadmium at 15 ppm (Hoshmand, 2006). The first factor was rice husk biochar (RHB) dose consisting of four levels: 0 (control), 15, 30, and 45 tons/ha. The second factor was silica fertilizer dose consisting of three levels: 0, 1, and 2 g per kg soil, equivalent to 0, 3, and 6 g per 3 kg soil per polybag, respectively. Biochar doses were selected based on previous studies showing effective Cd immobilization within the range of 10–45 tons/ha (Li *et al*., 2022; Zhang *et al*., 2023). Silica doses were derived from field application rates of 100–200 kg SiO_2/ha, converted to pot experiments assuming 1 ha of soil (15 cm depth) weighs approximately 2.25×10^6 kg (Hamidu *et al*., 2025). Each treatment combination was replicated three times. Each experimental unit (polybag) contained four lettuce plants, resulting in 36 experimental units and 144 plant samples in total.

2.4. Research Implementation

2.4.1. Lettuce Seed Germination

Preparation of lettuce plants began with the seed germination stage. A total of 350 lettuce seeds were sown in seedling trays and watered every morning to maintain water availability until the age of 14 days. The criteria for lettuce ready for transplanting were having 3-4 leaves, an upright stem, fresh green color, and no pest or disease infestation. If any seeds had not germinated by day 3 after sowing, reseeding was performed.

2.4.2. Planting Media Preparation

The planting media used was humus soil derived from decomposed plant material, giving it high mineral and nutrient content; it is classified as a very fertile soil type. Due to its high mineral and nutrient content, this soil is blackish in color. The soil media was mixed evenly and placed into 30 cm × 30 cm polythene bags with a media weight of 3 kg per bag. The filled polythene bags were labeled using type X according to the treatment to be given and arranged according to the research layout. Cadmium contamination was applied as cadmium sulfate ($CdSO_4 \cdot 8/3H_2O$, ≥99% purity, Sigma-Aldrich, USA) to achieve a final soil concentration of 15 mg Cd per kg dry soil (15 ppm). The required amount of $CdSO_4$ was calculated based on the dry weight of soil per polybag (3 kg). The molecular weight of $CdSO_4 \cdot 8/3H_2O$ is approximately 256.52 g/mol, containing 112.41 g/mol of Cd (43.8% Cd by mass). To obtain 45 mg Cd per polybag (15 mg/kg × 3 kg), the amount of $CdSO_4$ required was calculated as:

$$\text{CdSO}_4 \text{ mass} = \frac{45 \text{ mg Cd}}{0.438} = 102.74 \text{ mg per polybag} \quad (1)$$

The $CdSO_4$ was dissolved in distilled water to prepare a stock solution, and 20 mL of the solution was uniformly applied to the soil in each polybag. After application, the soil was thoroughly mixed and incubated for 7 days to allow Cd equilibration. Soil samples were taken before and after Cd application to verify the actual Cd concentration using atomic absorption spectrophotometry (AAS).

2.4.3. Application of Rice Husk Biochar and Silica Fertilizer

Application of rice husk biochar was carried out one week before transplanting. Biochar was applied by spreading it according to the treatment dose indicated on the polythene bag and mixing it evenly with the soil inside the bag. Subsequently, silica fertilizer was applied to the planting media three days before transplanting according to the treatment dose by sprinkling it evenly over the entire soil surface.

2.4.4. Planting

Soil loosening was done by stirring the soil to aerate it before transplanting. Next, all polythene bags were given planting holes approximately 5 cm deep. Seedlings that had been germinated for 14 days and met the criteria were then planted in each polythene bag. Transplanting was carried out in the morning. Plants were watered and provided with shade from dried leaves to prevent the lettuce seedlings from being exposed to direct sunlight after transplanting. After one week, the shade could be removed.

2.4.5. Plant Maintenance

Lettuce plant maintenance included watering, fertilizing, weed removal, and pest and disease control. Watering was done sufficiently every two days in the morning; if the soil was still wet, watering was not performed. Weeds were removed by pulling them from the polythene bags and the planting area. Pest and disease control was carried out as needed based on the level of infestation. Fertilization was performed by applying NPK fertilizer at 3 grams per polythene bag at 14 days after transplanting.

2.4.6. Harvesting

Harvesting was conducted when the lettuce was 35 days after planting by dismantling the planting media to prevent root breakage. Roots were cleaned using water until no soil remained attached.

2.5. Observation Parameters

2.5.1. Planting Media

Observation of the planting media was conducted to determine: (1) Initial soil condition before treatment (baseline characteristics), (2) Soil condition after Cadmium application at 15 ppm (to verify uniform Cd spiking), and (3) Soil condition after planting (to measure treatment effects). The observed parameters included plant height (cm), number of leaves, fresh weight (g per plant), soil Cd concentration (mg/kg), and plant Cd concentration (mg/kg). In addition, soil pH (H_2O and 1 M KCl, 1:2.5 ratio) and cation exchange capacity (CEC, ammonium acetate method) were measured to support the interpretation of Cd immobilization mechanisms. Soil and plant Cd concentrations were determined by atomic absorption spectrophotometry (AAS) after wet digestion.

2.5.2. Plant Morphology

Morphological parameters included plant height, number of leaves, and plant fresh weight.

a. Plant Height (cm): Plant height measurement was conducted by measuring from the base of the stem to the tip of the longest leaf. Measurements were taken once a week starting from when the plants were 15 days after planting until 35 days after planting.
b. Number of Leaves (strands): Counting the number of leaves was done by counting all leaves that had fully opened. Leaf counting was performed once a week when the plants were 7 days after planting until 35 days after planting.
c. Plant Fresh Weight (grams): Weighing of fresh weight per plant was conducted at 35 days after planting. Calculation of plant fresh weight was performed on harvested lettuce plants that had been cleaned of residual soil by weighing the entire plant using a digital balance.

2.5.3. Initial Soil Media Characteristics

The initial soil used in this study was humus soil collected from Jember, Indonesia. Physicochemical analysis (Table 1) showed that the soil had a loam texture with pH 6.2, organic carbon 1.85%, total nitrogen 0.16%, C/N ratio 11.6, and CEC 19.4 cmol$^+$/kg. No detectable Cd (<0.05 mg/kg) was found in the initial soil. After spiking with $CdSO_4$ solution at a target concentration of 15 ppm, the measured total Cd was 14.8 mg/kg, confirming uniform contamination. These baseline characteristics indicate that the soil was suitable for lettuce cultivation and Cd stress experiments

Table 1. Characteristics of initial soil media before treatment

Parameter	Value	Unit	Function / Interpretation
pH (H_2O)	6.2	-	Slightly acidic to neutral (optimal for lettuce: 6.0–7.0)
Organic Carbon (C-organic)	1.85	%	Moderate organic matter (typical for humus soil in Indonesia)
Total Nitrogen (N-total)	0.16	%	Moderate N content
C/N Ratio	11.6	-	Moderate decomposition rate (optimal for plant growth)
CEC	19.4	cmol$^+$/kg	Moderate to high cation exchange capacity (good for Cd adsorption)
Texture	Loam (42.5% sand, 35.2% silt, 22.3% clay)	-	Balanced texture (good drainage + nutrient retention)
Initial Total Cd	< 0.05 (not detected)	mg/kg	No prior Cd contamination – safe control
Total Cd after spiking (15 ppm)	14.8	mg/kg	Successful uniform Cd application (target 15 ppm achieved)

2.6. Analytical Methods

2.6.1. Cd Content in Plants

Cadmium concentrations in soil and plant samples were determined using a Graphite Furnace Atomic Absorption Spectrophotometer (AAS) at the Testing Laboratory of Jember State Polytechnic.

a. Plant sample preparation: At harvest, lettuce plants were separated into roots and shoots. Each sample (2.5 g) was placed in a digestion tube, mixed with 5 mL of concentrated HNO_3, and left overnight. The mixture was heated at 100 °C for 90 min, cooled, and an additional 1 mL of HNO_3 was added. The mixture was then heated sequentially at 130 °C for 60 min and at 150 °C for 150 min. After cooling, the digestate was filtered and diluted with deionized water to 25 mL. The solution was stored overnight before AAS analysis.
b. Soil sample preparation: A similar digestion procedure was applied to 2.5 g of air-dried soil, followed by AAS quantification.

2.6.2. Cd Content in Soil

Testing of Cadmium content in soil was conducted before treatment, after treatment, and after harvest. Cadmium content testing using a Graphite Furnace Atomic Absorption Spectrophotometer (AAS) involved storing 2.5 grams of sample in a digestion tube, adding 5 ml of nitric acid, and leaving it overnight. The mixture was then heated at 100 °C for one and a half hours, allowed to cool, and another 1 ml of nitric acid was added. It was then heated again at 130 °C for one hour, and the temperature was increased to 150 °C for two and a half hours. Subsequently, a 1 ml white precipitate formed. The precipitate was extracted and diluted with deionized water to 25 ml, shaken until homogeneous, and stored overnight. Cadmium content testing was carried out at the Testing Laboratory of Jember State Polytechnic.

2.7. Data Analysis

All data were analyzed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). Prior to ANOVA, normality of residuals was tested using the Shapiro-Wilk test, and homogeneity of variances was tested using Levene's test at a 5% significance level. When both assumptions were met, data were subjected to a two-way Analysis of Variance (ANOVA) at a 5% significance level to determine the main effects of biochar, silica, and their interaction. When the F-test showed a significant effect, means were compared using the Least Significant Difference (LSD) test at a 5% significance level (Darma, 2021).

2.7.1. Statistical Model

The two-way ANOVA model used was as follows:

$$Y_{ijk} = \mu + P_i + S_j + (P \times S)_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is observed value of the k-th replication in the i-th biochar dose and j-th silica dose, μ is overall mean, P_i is effect of biochar dose at the i-th level (i = 0, 15, 30, 45 tons/ha), S_j is effect of silica dose at the j-th level (j = 0, 1, 2 g/kg soil), $(P \times S)_{ij}$ is interaction effect between biochar and silica, and $\varepsilon_{ijk}$is random error.

The analysis tested three hypotheses: (1) Main effect of biochar dose (P), (2) Main effect of silica dose (S), and (3) Interaction effect between biochar and silica (P × S). When the F-test showed a significant effect ($P < 0.05$), means were compared using the Least Significant Difference (LSD) test at a 5% significance level (Darma, 2021).

3. RESULTS AND DISCUSSION

3.1. Effect of Biochar Treatment on Plant Height, Number of Leaves, and Fresh Weight

Plant growth is characterized by an increase in the volume of constituent cells (Birhanu *et al*., 2025). Plant height is one observational variable used to determine growth that has occurred. Analysis of variance (ANOVA) revealed that biochar treatment had a highly significant effect on plant height ($F = 8.34$, $p < 0.01$) and number of leaves ($F = 12.67$, $p < 0.01$), but no significant effect on fresh plant weight ($F = 0.42$, $p > 0.05$).

Table 2 shows that biochar treatment significantly affected plant height of lettuce grown under 15 ppm cadmium stress, whereas silica treatment and the interaction between biochar and silica showed no significant effect. The highest plant height was observed in P3 (19.81±1.25 cm), followed by P2 (19.55±1.30 cm), and both treatments were different significantly from P0 and P1 according to the LSD test at $p < 0.05$. In contrast, the average values among silica treatments

Table 2. Effect of biochar and silica treatments on plant height (cm) of lettuce under Cd stress

Treatment	S0	S1	S2	Average
P0	18.20 ± 1.15	17.85 ± 1.18	17.98 ± 1.27	18.01 ± 1.20^{c}
P1	17.70 ± 1.10	17.45 ± 1.20	17.62 ± 1.15	17.59 ± 1.15^{d}
P2	19.60 ± 1.28	19.42 ± 1.35	19.63 ± 1.27	19.55 ± 1.30^{b}
P3	19.95 ± 1.20	19.70 ± 1.30	19.78 ± 1.25	19.81 ± 1.25^{a}
Average	18.86 ± 1.18^{A}	18.61 ± 1.26^{A}	18.75 ± 1.24^{A}	

Note: Values are mean ± standard deviation of three replication ($n = 3$). Different letters following values indicate significant differences according to LSD test at α = 0.05. Lowercase for biochar treatment (row), uppercase for silica (column).

Table 3. Effect of biochar and silica treatments on number of leaves of lettuce under Cd stress

Treatment	S0	S1	S2	Average
P0	13.90 ± 1.00	13.55 ± 1.08	13.88 ± 1.07	13.78 ± 1.05[c]
P1	14.35 ± 1.12	14.05 ± 1.05	14.27 ± 1.13	14.22 ± 1.10[bc]
P2	18.95 ± 1.20	18.75 ± 1.30	18.97 ± 1.25	18.89 ± 1.25[a]
P3	18.70 ± 1.15	18.40 ± 1.25	18.58 ± 1.20	18.56 ± 1.20[a]
Average	16.48 ± 1.12[A]	16.19 ± 1.17[A]	16.43 ± 1.16[A]	

Note: Values are mean ± standard deviation of three replication (n = 3). Different letters following values indicate significant differences according to LSD test at α = 0.05. Lowercase for biochar treatment (row), uppercase for silica (column).

(S0, S1, and S2) were statistically similar, as indicated by the same superscript letter. These results indicate that higher biochar dosages were able to improve plant height under cadmium-contaminated conditions, while silica application did not significantly influence plant growth.

Table 3 indicates that biochar application significantly increased the number of lettuce leaves under cadmium stress, whereas silica treatment and the interaction between both factors did not show significant effects. The highest number of leaves was obtained in P2 (18.89 ± 1.25) and P3 (18.56 ± 1.20), which were significantly higher than P0 (13.78 ± 1.05) and P1 (14.22 ± 1.10) based on the LSD test at $p < 0.05$. Meanwhile, the average values among silica levels were not significantly different because all treatments shared the same superscript letter. This finding demonstrates that higher biochar dosages effectively promoted leaf formation in lettuce grown in cadmium-contaminated media, while silica addition did not contribute significantly to leaf production.

Table 4 shows that biochar treatment, silica treatment, and their interaction had no significant effect on fresh weight of lettuce grown under 15 ppm cadmium stress. The average fresh weight values ranged from 28.93 ± 2.10 g to 29.31 ± 2.12 g, and all treatments were statistically similar according to the LSD test at $p < 0.05$. Although slight numerical differences were observed among treatments, these variations were not significant statistically. Therefore, under Cd stress conditions, neither biochar nor silica application was able to significantly increase fresh weight of lettuce plants.

Biochar releases nutrients gradually, so soil improvement and plant growth effects require more time (Okalia *et al*., 2021). Higher biochar doses can increase soil's ability to adsorb Cd, reducing heavy metal availability to plants. Biochar also binds soil cations that plants can utilize for growth processes (Ibrahim *et al*., 2022).

Regarding leaf number, P0 produced 13.78 ± 1.05 leaves, P1 produced 14.22 ± 1.10 leaves, P2 produced 18.89 ± 1.25 leaves, and P3 produced 18.56 ± 1.20 leaves. The LSD test showed that P2 and P3 produced significantly higher leaf numbers than P0 and P1 ($P < 0.05$), with the highest value recorded at P2 (18.89 leaves). Biochar treatment is related to its ability to improve soil physical, chemical, and biological properties, supporting optimal plant growth. Biochar increases macro-nutrient availability (N, P, K) essential for cell division and leaf tissue formation. Rice husk biochar at 40 g/pot required specific doses to produce optimal leaf number in amaranth (Ebido *et al*., 2021).

Fresh weight data showed non-significant differences among biochar treatments ($P > 0.05$). P0 produced 28.93 ± 2.10 g, while other treatments ranged from 29.03–29.30 g. Although biochar significantly increased plant height and leaf number, this did not translate into a significant increase in fresh weight under the 15 ppm Cd stress conditions. This suggests that Cd toxicity may primarily affect biomass accumulation through mechanisms other than plant height and leaf count, or that the observed morphological improvements were insufficient to offset Cd-induced growth suppression in terms of fresh weight (He *et al*., 2024).

Table 4. Effect of biochar and silica treatments on fresh weight (g) of lettuce under Cd stress

Treatment	S0	S1	S2	Average
P0	28.95 ± 2.05	28.80 ± 2.15	29.05 ± 2.10	28.93 ± 2.10
P1	29.20 ± 2.00	29.05 ± 2.08	29.15 ± 2.07	29.13 ± 2.05
P2	29.10 ± 2.05	29.00 ± 2.10	29.12 ± 2.08	29.07 ± 2.08
P3	29.35 ± 2.10	29.22 ± 2.15	29.36 ± 2.12	29.31 ± 2.12
Average	29.15 ± 2.05	29.02 ± 2.12	29.17 ± 2.09	29.11 ± 2.09

Note: Values are mean ± standard deviation of three replication (n = 3).

3.2. Effect of Silica Treatment on Plant Height, Number of Leaves, and Fresh Weight

Variance analysis showed that silica treatment on plant height produced LSD of 0.87 (significant), on leaf number produced LSD of 1.12 (highly significant), and on fresh weight showed non-significant differences. Silica treatment did not improve plant height. The highest plant height was observed in the control without silica (S0 = 19.15 ± 1.10 cm), while the lowest was recorded at S1 (3 g/3 kg = 17.98 ± 1.05 cm). S2 (6 g/3 kg = 19.09 ± 1.08 cm) was not significantly different from S0. These results indicate that silica application, particularly at 3 g/3 kg, had no beneficial effect on plant height under 15 ppm Cd stress (El Rasafi *et al*., 2022).

Table 5. Mean ± SD of silica treatment on plant height, number of leaves, and fresh weight

Treatment	Plant Height (cm)	Number of Leaves	Fresh Plant Weight (g)
S0	19.15 ± 1.10[a]	16.58 ± 1.15[a]	29.02 ± 2.00
S1	17.98 ± 1.05[b]	15.25 ± 1.10[b]	29.10 ± 2.05
S2	19.09 ± 1.12[a]	17.25 ± 1.20[a]	29.20 ± 2.08
LSD	0.87*	1.12**	not significant

Note: Values followed by different superscript letters within the same column indicate significant differences based on LSD test ($p < 0.05$). Values after ± are standard deviations ($n = 3$)

Silica application through soil proved more optimal than spraying (Shafeeq-ur-Rahman *et al*., 2020). Soil-applied silica is more easily absorbed by roots, facilitating plant access to nutrients and improving growth. Silica forms complexes with other nutrient ions, increasing availability and uptake of essential nutrients. Regarding leaf number, highly significant differences were shown between S1 (15.25 ± 1.10 leaves) and S2 (17.25 ± 1.20 leaves). S0 showed non-significant results. This indicates that silica application can increase leaf number, though its effect is not as large as rice husk biochar.

Fresh weight showed non-significant differences across silica treatments. S0 produced 29.02 ± 2.00 g, S1 produced 29.10 ± 2.05 g, and S2 produced 29.20 ± 2.08 g, with an increase range of only 0.18 g. Although not significantly different, a positive increase trend was observed. This is thought to occur due to volatile organic compounds and underdeveloped pore structure of biochar during pyrolysis, causing plant stress (Thomas *et al*., 2025). Biochar and silica use not only reduces Cd bioavailability but also improves soil health and fertility (Chen *et al*., 2025).

3.3. Effect of Biochar and Silica on Cadmium Accumulation

Analysis of variance showed that biochar treatment on soil Cd was non-significant, while plant Cd showed significant differences. Table 6 shows that biochar treatment significantly affected plant Cd concentration ($F = 3.17$, $p < 0.05$) but not soil Cd ($F = 2.71$, $p > 0.05$) (Mansoor *et al*., 2021; Sun *et al*., 2020). The lowest plant Cd (0.47 ppm) was recorded at P3 (45 tons/ha), which was significantly lower than P0 (0.78 ppm) and P1 (1.02 ppm) based on the LSD test (Table 4). However, all treatments produced plant Cd concentrations above the WHO/FAO safety limit of 0.2 ppm.

Table 7 presents the effect of silica application (S0 = without silica, S1 and S2 = different silica doses) on cadmium (Cd) concentration in soil and plant tissues, presented in mean ± standard deviation format. The LSD test results showed that silica treatment did not have a significant effect on either soil Cd or plant Cd ($P > 0.05$), which is indicated by the statement "not significant" in the LSD row and the absence of different superscript letters. Descriptively, the highest soil Cd was found in S0 (2.38 ± 0.12 ppm) and slightly decreased in S1 (2.16 ± 0.11 ppm) and S2 (2.17 ± 0.10 ppm), while plant Cadmium showed a decreasing trend from S0 (0.91 ± 0.08 ppm) to S2 (0.55 ± 0.06 ppm), but this difference was not

Table 6. Mean ± SD of biochar treatment on soil Cd and plant Cd

Treatment	Soil Cd (ppm)	Plant Cd (ppm)
P0	1.88 ± 0.15	0.78 ± 0.08[b]
P1	2.39 ± 0.20	1.02 ± 0.10[a]
P2	2.70 ± 0.22	0.65 ± 0.07[b]
P3	1.96 ± 0.18	0.47 ± 0.05[c]
LSD	not significant	0.39*

Table 7. Mean ± SD of silica treatment on soil Cd and plant Cd (ppm)

Treatment	Soil Cd (ppm)	Plant Cd (ppm)
S0	2.38 ± 0.12	0.91 ± 0.08
S1	2.16 ± 0.11	0.74 ± 0.07
S2	2.17 ± 0.10	0.55 ± 0.06
LSD	not significant	not significant

Note: Silica treatment showed non-significant differences for both soil Cd and plant Cd (p> 0.05). Values are mean ± SD (n=3). No superscript letters are presented because the LSD test did not detect significant differences.

statistically significant. Thus, silica application up to the highest dose was not effective enough to significantly reduce Cd accumulation under these experimental conditions.

The less visible effect of silica fertilizer on Cd stress may be due to NPK fertilizer use in the planting media. NPK addition positively affects plant growth but also increases cadmium accumulation through soil enzyme activity under Cd stress. Additionally, Cd accumulation in plant tissues varies due to factors including cultivar, soil reaction, soil texture, and bioavailable Cd concentration (Goswami *et al*., 2022).

3.4. Initial Soil Properties

Table 8 shows the plant height (cm) for each combination of biochar (P0–P3) and silica (S0–S2) treatments along with the LSD value for the interaction effect, with all data presented as mean ± standard deviation. The analysis results show that the LSD value for the interaction between biochar and silica (P × S) is 1.74 with the caption "ns" (non-significant) at the α = 0.05 level, which means there is no statistically significant interaction effect between the two factors. In other words, the effect of biochar on plant height does not depend on the dose of silica given, and vice versa, so there is no synergistic or antagonistic effect between biochar and silica. Although descriptively some combinations such as P2S0 (20.09 ± 1.10 cm) and P3S1 (19.78 ± 1.02 cm) show higher values, this difference is not statistically significant due to the non-significant interaction.

Table 8. Mean ± SD of Plant Height (cm) for Each Biochar × Silica Combination

Biochar	S0	S1	S2
P0	18.44 ± 0.95	16.29 ± 0.88	19.30 ± 1.02
P1	18.25 ± 0.90	17.06 ± 0.85	17.45 ± 0.92
P2	20.09 ± 1.10	18.79 ± 0.98	19.78 ± 1.05
P3	19.82 ± 1.00	19.78 ± 1.02	19.83 ± 1.03
LSD (P × S)	**1.74 (ns)**	**1.74 (ns)**	**1.74 (ns)**

Note: The interaction between biochar and silica (P × S) was not significant for all parameters (P > 0.05). Values are mean ± SD (n=3). Different superscript letters are not shown because the interaction effect is non-significant.

3.5. Relationship between Heavy Metal Parameters

The results of the regression graph analysis above show a regression coefficient of 0.2985 (positive), meaning that every one unit increase in Cd in the soil tends to be followed by an increase in Cd levels in plants by approximately 0.985 units. This value indicates a positive number with a unidirectional relationship. The coefficient of determination R2 value shows that variations in Cd levels in the soil only explain approximately 22.74% of variations in Cd levels in plants, with the remaining 77.26% influenced by other factors. At the data points, they are quite spread far from the regression line/trend, which is confirmed by the low R^2 value.

A linear regression analysis was conducted to evaluate the relationship between soil cadmium (Cd) concentration and plant Cd accumulation in lettuce. The results showed a weak positive correlation (r = 0.4769) between the two variables. The regression equation was Plant Cd = 0.0209 × (Soil Cd) + 0.4426, with a coefficient of determination (R^2) of 0.2274. This indicates that only 22.74% of the variation in plant Cd concentration could be explained by soil Cd levels, meaning the predictive relationship is poor. The F-statistic was 1.818 with 1 and 6 degrees of freedom, producing a p-value of 0.2261. Similarly, the t-test for the slope yielded a p-value of 0.2261. Since the p-value exceeds the 0.05 of

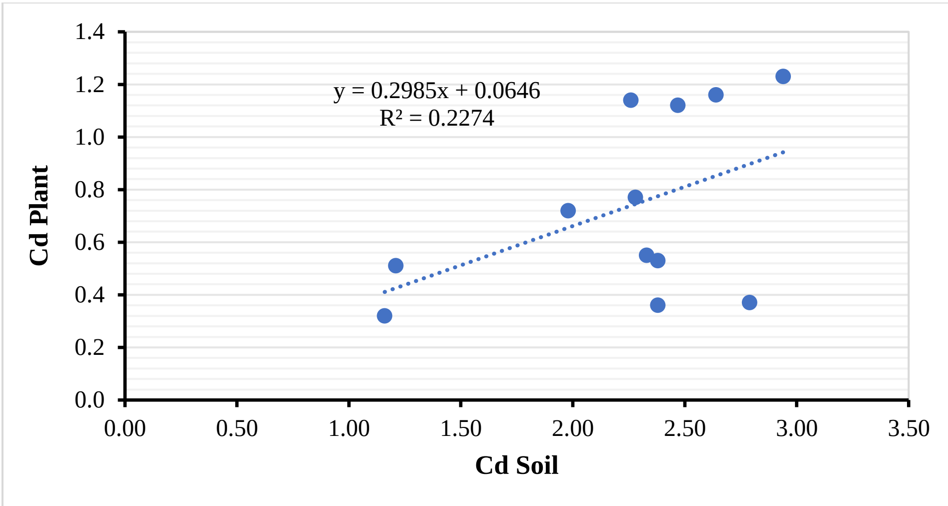

Figure 1. Relationship between heavy metal Cd in the soil and plant

Table 9. Linear regression analysis between soil Cd and plant Cd concentration in lettuce

Parameter	Value
Sample size (n)	8
Regression equation	Plant Cd = 0.0209 × (Soil Cd) + 0.4426
Coefficient of determination (R^2)	0.2274 (22.74%)
Adjusted R^2	0.0986 (9.86%)
Correlation coefficient (r)	0.4769
Standard error of the estimate	0.1345
Sum of squares (regression)	0.0329
Sum of squares (residual)	0.1085
Mean square (regression)	0.0329
Mean square (residual)	0.0181
F-statistic	1.818
Degrees of freedom (regression)	1
Degrees of freedom (residual)	6
p-value (model)	**0.2261**
t-statistic (slope)	1.348
Standard error of slope	0.0155
p-value (slope)	**0.2261**
95% confidence interval for slope	-0.0178 to 0.0596

Note: $p > 0.05$ indicates that the linear relationship between soil Cd and plant Cd is not statistically significant.

significance threshold of 0.05, the relationship between soil Cd and plant Cd is not statistically significant. Therefore, other factors such as biochar application, silica treatment, soil pH, organic matter content, or Cd immobilization mechanisms likely play a more dominant role in determining Cd accumulation in lettuce under the conditions of this study.

The regression analysis revealed a weak and statistically non-significant relationship between soil Cd and plant Cd ($p = 0.2261$; $R^2 = 22.74\%$). The low R^2 value is particularly noteworthy, as it indicates that more than 77% of the variation in plant Cd remains unexplained by soil Cd levels alone. This finding aligns with previous studies showing that under amended conditions such as with biochar or silica the bioavailability of Cd in soil is altered, weakening the direct relationship between total soil Cd and plant uptake (Li *et al.*, 2022; Zhang *et al.*, 2023). The non-significant p-value further supports the conclusion that the treatments applied in this study (biochar and silica) likely interfered with Cd mobility and root uptake, thereby decoupling the simple linear relationship between soil and plant Cd concentrations. From a practical perspective, a predictive model based solely on soil Cd would be unreliable under the tested conditions, given the poor R^2 value and lack of statistical significance.

Table 10. Mean ± SD of Biochar and Silica Treatment Effects on Soil Cd and Plant Cd (ppm)

Treatment	Soil Cd (ppm)	Plant Cd (ppm)	Status vs. Safety Limit (0.2 ppm)
P0S0	1.98 ± 0.15	0.72 ± 0.06	Exceeds limit (260% above)
P0S1	2.47 ± 0.18	1.12 ± 0.09	Exceeds limit (460% above)
P0S2	1.21 ± 0.10	0.51 ± 0.04	Exceeds limit (155% above)
P1S0	2.26 ± 0.16	1.14 ± 0.08	Exceeds limit (470% above)
P1S1	2.64 ± 0.20	1.16 ± 0.09	Exceeds limit (480% above)
P1S2	2.28 ± 0.17	0.77 ± 0.06	Exceeds limit (285% above)
P2S0	2.94 ± 0.22	1.23 ± 0.10	Exceeds limit (515% above)
P2S1	2.38 ± 0.18	0.36 ± 0.03	Exceeds limit (80% above)
P2S2	2.79 ± 0.20	0.37 ± 0.03	Exceeds limit (85% above)
P3S0	2.33 ± 0.17	0.55 ± 0.04	Exceeds limit (175% above)
P3S1	1.16 ± 0.09	0.32 ± 0.03	Exceeds limit (60% above)
P3S2	2.38 ± 0.18	0.53 ± 0.04	Exceeds limit (165% above)

Note: Lowest plant Cd concentration was observed in P3S1 (0.32 ppm), which is still 60% above the safety limit of 0.2 ppm. Values are mean ± SD (n=3). No superscript letters are presented because multiple comparison tests were not performed or were non-significant.

Table 10 presents the concentration of cadmium (Cd) in soil and plant tissue for each combination of biochar (P0–P3) and silica (S0–S2) in the format of mean ± standard deviation, and their comparison to the safe limit of Cd in food crops of 0.2 ppm. The results show that all treatment combinations produced plant Cd concentrations that exceeded the safe limit, with the excess ranging from 60% to 515% above the limit. The best combination that produced the lowest plant Cd was P3S1 (highest dose of biochar + medium dose of silica) at 0.32 ± 0.03 ppm, but this value was still 60% above the safe limit, while the worst combination was P2S0 with a plant Cd of 1.23 ± 0.10 ppm (515% above the safe limit). Because the statistical tests for the main effects and previous interactions showed non-significant results, no further LSD test was conducted between the combinations and therefore there are no superscript letters in this table. In conclusion, although biochar and silica can improve growth parameters such as plant height and number of leaves, both are not effective enough to reduce plant Cd accumulation to below the safe limit of consumption at a Cd stress level of 15 ppm.

The novelty of this study lies in the evaluation of the combined application of rice husk biochar and silica fertilizer across a defined dose range under controlled cadmium stress (15 ppm) in lettuce, a leafy vegetable known for its high Cd accumulation potential. Unlike previous studies that mainly investigated biochar or silica separately, this research demonstrates that although rice husk biochar significantly improved lettuce growth and reduced Cd accumulation, its combination with silica did not produce a statistically significant synergistic effect. These findings provide an important scientific contribution by identifying the practical limitations of this remediation strategy under severe Cd contamination and establishing a baseline for future optimization of integrated soil amendment approaches aimed at improving food safety and sustainable vegetable production.

4. CONCLUSIONS

Under 15 ppm cadmium stress, the application of rice husk biochar at 45 tons/ha significantly increased lettuce plant height (19.81 cm) and number of leaves (18.56 leaves) compared to lower biochar doses. However, no significant synergistic interaction was observed between biochar and silica ($p > 0.05$), and silica application alone did not significantly affect plant growth or Cd accumulation. Most importantly, despite the improvements in morphological parameters, all treatments produced lettuce Cd concentrations above the WHO/FAO food safety limit of 0.2 ppm. The lowest Cd concentration achieved (0.32 ppm) still exceeded the safety limit by 60%. This means that none of the tested treatments biochar alone, silica alone, or their combination succeeded in producing lettuce that is safe for human consumption under the conditions of this study (15 ppm Cd, 45 days growth period). Therefore, from a food safety perspective, neither rice husk biochar nor silica fertilizer, nor their combination, can be recommended as an effective remediation strategy for lettuce grown on highly Cd-contaminated soil (15 ppm). Further research using higher biochar doses, longer soil equilibration periods, different biochar feedstocks, or integrated remediation approaches (e.g., biochar combined with Cd-immobilizing microbes or chelating agents) is necessary before any practical recommendation for safe lettuce production can be made.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE MANUSCRIPT PREPARATION

Not applicable. The authors did not use any generative AI tools or services in the preparation of this manuscript. All content is entirely the intellectual work of the authors.

AUTHOR CONTRIBUTION

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
DE	✓	✓	✓			✓	✓	✓	✓	✓	✓			
MA				✓	✓							✓		
M				✓	✓							✓		

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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