Jurnal Biodjati 11(1): 150–165, May 2026

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

# Effects of Corn Cob Biochar and Plant Density on Zinc Uptake and Growth of Vetiver Grass (*Chrysopogon zizanioides*) in Leachate-Contaminated Landfill Soil

**Muhammad Luthfi Fachrezi, Endah Dwi Hastuti*, Yulita Nurchayati**

Received: January 5, 2026
Revise from: January 17, 2026
Accepted: May 22, 2026

DOI: 10.15575/biodjati.v11i1.54277

*Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Soedarto No.13, Tembalang, Semarang, Central Java,50275, Indonesia.*

*Corresponding author*
**endah_pdil@yahoo.com*

***Abstract***. *Leachate contamination from landfill waste can increase zinc (Zn) concentrations in soil beyond permissible limits. This study evaluated the effects of corn cob biochar and plant density on Zn uptake and growth of vetiver grass (Chrysopogon zizanioides) grown in leachate-contaminated landfill soil. The experiment was arranged in a completely randomized design with a 2 × 2 factorial scheme and three replications. The first factor was biochar application (without biochar and with biochar), and the second factor was plant density (1 plant and 3 plants per planting bag). The results showed that neither biochar nor plant density, nor their interaction, had a significant effect on soil Zn concentration, root bioconcentration factor (BCF), shoot BCF, and translocation factor (TF). Biochar treatment was associated with lower residual soil Zn concentrations (48.33 mg/kg), higher root bioconcentration factor (BCF) (1.21), and lower shoot BCF (0.78) and translocation factor (TF) (0.70) compared to the treatment without biochar. Similarly, higher plant density showed comparable trends, with lower residual soil Zn concentrations (47.92 mg/kg), higher root BCF (1.33), and lower shoot BCF (0.85) and TF (0.85) compared to lower plant density. However, biochar significantly increased root length, while higher plant density significantly increased shoot dry weight, root biomass, leaf number, and tiller number per planting bag, but decreased plant height per planting bag. Fresh shoot weight per planting bag did not differ significantly among treatments. There was an interaction between biochar and low plant density that increased plant height. The absence of an unplanted control limits the interpretation of Zn reduction. Overall, biochar application and higher plant density were associated with improved growth performance of vetiver grass and may contribute to enhanced Zn uptake characteristics under leachate-contaminated conditions*

***Keywords***: *biochar, leachate, phytoremediation, plant density, vetiver, zinc*

## INTRODUCTION

Population growth and urbanization can increase global solid waste volumes (Dagwar & Dutta, 2024). The city of Semarang has the Jatibarang landfill, which receives around 800 tonnes of waste per day (Hasthi et al., 2023). This leads to leachate pollution, a liquid formed when water percolates through waste and contains high levels of heavy metals, which can migrate into surrounding soil and groundwater, thereby spreading contamination (Jagasri et al., 2024).

**Citation**
Fachrezi, M.L., Hastuti, E.D., & Nurchayati, Y. (2026). Effects of Corn Cob Biochar and Plant Density on Zinc Uptake and Growth of Vetiver Grass (*Chrysopogon zizanioides*) in Leachate-Contaminated Landfill Soil. *Jurnal Biodjati, 11*(1), 150-165.

Copyright © 2026 Jurnal Biodjati
This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Jurnal Biodjati 11(1): 150–165, May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Preliminary tests conducted on 2 May 2025 at the Jatibarang landfill showed soil Zn levels of 174.41 mg/kg, determined as pseudo-total Zn using acid digestion followed by AAS analysis. This value exceeds the ecological soil screening level (Eco-SSL) for Zn of 160 mg/kg established by USEPA (2007), which is a conservative benchmark used to identify potential ecological risk rather than a regulatory cleanup standard. Exceedance of this threshold indicates the need for further site-specific evaluation of Zn bioavailability and ecological impact. In contrast, Cd, Pb, Cu, and Fe concentrations were below their respective threshold values. These findings are consistent with the study by Amelinda et al. (2017), which reported a soil Zn concentration of 234 mg/kg at the Jatibarang landfill.

Phytoremediation is an inexpensive and environmentally friendly alternative; however, the process is relatively slow and poses a risk of contaminant transfer into the food chain through the accumulation of metals in edible plant parts (Khan et al., 2023). Vetiver grass (*Chrysopogon zizanioides*), an aromatic non-food plant, minimizes this risk because its biomass is primarily utilized for essential oil and bioenergy production rather than direct consumption. However, biomass harvested from contaminated sites still requires proper management to prevent secondary contamination. In addition, grasses exhibit rapid growth and high biomass production, which are advantageous for enhancing metal uptake and overall remediation efficiency (Sulistiyani et al., 2020; Mishra & Chandra, 2022; Rabêlo et al., 2021).

Vetiver grass shows a bioaccumulation factor (BCF) of >1 and a translocation factor (TF) of <1 for Zn, indicating its potential as a Zn phytostabilizer (Melato et al., 2016). This suggests that Zn is predominantly retained in the roots rather than translocated to the shoots. Consistent with this, vetiver grass accumulates higher concentrations of Zn in the root system, as evidenced by greater expression of genes involved in heavy metal chelation in roots compared to shoots (Wu et al., 2022).

Optimizing phytoremediation may involve increasing plant density to enhance total biomass and Zn uptake per unit soil volume (Khalid et al., 2019). Higher plant density can increase total root biomass within the same planting medium, thereby improving overall Zn uptake. However, it may reduce individual plant growth due to competition for nutrients, water, and space (Li et al., 2016).

Biochar is a carbon-rich material produced through the pyrolysis of organic feedstocks at temperatures ranging from 300 to 800 °C under anaerobic conditions (Boorboori & Lackóová, 2023; He et al., 2024). Corn cob-derived biochar has demonstrated a higher Zn immobilization capacity than peanut shell biochar, attributed to its higher pH, organic carbon (OC) content, and electrical conductivity (EC) (Vuong et al., 2023). However, the effectiveness of biochar in immobilizing heavy metals can vary depending on feedstock type, pyrolysis temperature, application rate, pH, surface functional groups, and pore structure.

Corn cob biochar has also shown a higher Cd adsorption capacity than rice husk and wheat straw biochars, likely due to its more developed pore structure, larger specific surface area, and greater abundance of active adsorption sites (Amen et al., 2020). In addition, corn cobs are an abundant agricultural by-product in Indonesia, with large quantities generated annually, making them a cost-effective and sustainable feedstock for biochar production and waste valorization (Sugiantara et al., 2025). Biochar can support Zn phytoremediation by immobilizing heavy metals and stimulating root growth and plant biomass (Boorboori & Lackóová, 2023; Zheng et al., 2023).

The interaction between biochar amendment and plant density remains poorly understood in phytostabilization systems, particularly in landfill soils contaminated with leachate. Although biochar can immobilize heavy metals and improve soil properties, and higher plant density can increase total root biomass, the combined effects of these factors on Zn stabilization and plant growth dynamics have not been clearly elucidated. Therefore, this study investigates the combined effects of corn cob biochar and plant density on Zn uptake and vetiver grass growth in leachate-contaminated soil from the Jatibarang landfill, Semarang.

## MATERIALS AND METHODS

This study was conducted from March to June 2025. Contaminated soil samples were collected from the Jatibarang municipal landfill, Semarang, Indonesia. Experimental activities, including planting, maintenance, and growth observations, were conducted in a greenhouse at the Faculty of Science and Mathematics (FSM), Diponegoro University (UNDIP). The average greenhouse conditions were 25.6 °C temperature, 64.35% relative humidity, and light intensity of approximately 42,857.68 lux. Plants were watered once daily using approximately 250 mL of water per planting bag.

Sample digestion for Zn analysis was conducted at the Laboratory of Plant Structure and Function Biology, FSM UNDIP. Analysis of Zn concentration using Atomic Absorption Spectrophotometry (AAS) was performed at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang (UNNES). Preliminary analysis of heavy metal concentrations in landfill soil was conducted at the ICBB Laboratory, PT Biodiversity Biotechnology Indonesia, Bogor.

The experiment was arranged in a completely randomized design (CRD) with a 2 × 2 factorial arrangement and three replicates per treatment. The first factor was biochar application, consisting of biochar-amended soil (B) and soil without biochar (T). The second factor was plant density, consisting of low density (D1) and high density (D2). The experiment consisted of four treatment combinations, each with three replicates, for a total of 12 experimental units.

### Soil Sampling

Soil sampling followed the method described by Thomas et al. (2024) using a purposive sampling approach. Soil samples were collected from four different sampling points located along the main leachate irrigation channel, which was identified as the area with the highest leachate exposure. The four sampling points were divided into two active-zone sites and two passive-zone sites, representing the spatial variability of the landfill area. Sampling was conducted along the leachate flow gradient, from the upstream to the downstream sections of the channel (Figure 1).

Soil samples were collected using a shovel at a depth of 0–15 cm at each location. The samples were then combined and homogenized to obtain a composite landfill soil. The homogenized soil was air-dried and sieved through a 5 mm mesh prior to use as the planting medium. The initial physicochemical properties of the composite soil showed a pH of 6.00 and a total Zn concentration of 174.41 mg/kg, exceeding the ecological screening level for Zn. Other metal concentrations included Cu (92.34 mg/kg), Pb (21.55 mg/kg), and Fe (20,723.57 mg/kg), while Cd was not detected.

Jurnal Biodjati 11(1): 150–165, May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

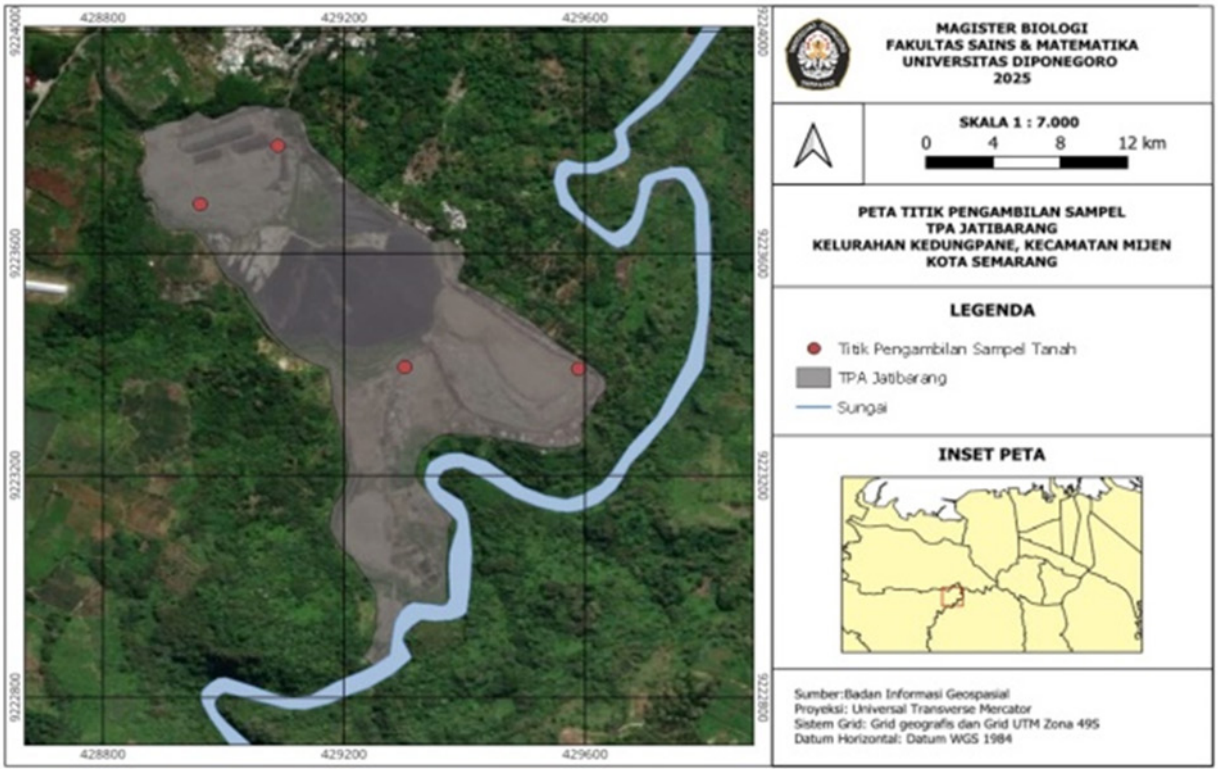


**Figure 1:** Soil sampling locations at the Jatibarang Landfill, Semarang, Indonesia

## Planting Bag Experiment

Plastic planting bags (35 cm × 35 cm) were used as experimental containers. Corn cob biochar was produced at a pyrolysis temperature of 400 °C at the Agricultural Environmental Instrument Testing and Standardization Center (BRMP Lingkungan), Pati Regency, Indonesia. The pyrolysis process was conducted under limited oxygen conditions for approximately 24 h. The resulting biochar was air-dried and ground into a fine powder prior to application.

For the biochar treatment, the soil medium was prepared by thoroughly mixing 2.85 kg of landfill soil with 150 g of corn cob biochar, corresponding to 5% (w/w, dry weight basis) in each planting bag, for a total of 3 kg per bag. For the control treatment, each planting bag was filled with 3 kg of landfill soil without biochar, following the procedure described by Thomas et al. (2024). The application rate of 5% (w/w) was selected based on previous studies showing that this dosage effectively reduces heavy metal concentrations in soil and promotes plant biomass production, while higher application rates may inhibit plant growth (Himaya et al., 2023; Liu et al., 2020).

One-month-old vetiver grass (*Chrysopogon zizanioides* (L.) Roberty) slips were obtained from the Indonesian Medicinal and Aromatic Crops Research Institute (B2P2TOOT), Karanganyar. Healthy vetiver slips with uniform morphology, approximately 20 cm in height and bearing five leaves, were selected before planting. Plants were planted at densities of 1 and 3 slips per planting bag. Plants were irrigated daily with 250 mL of water.

## Harvesting and Sample Preparation

After a two-month growth period, plants were harvested, and soil samples were collected for further analysis. Harvesting and sampling procedures followed Thomas et al. (2024). Plants were separated into aboveground (shoot) and belowground (root) parts. Roots were gently washed under

running tap water to remove adhering soil particles and carefully wiped with a clean cloth to remove residual surface moisture before measurement. Fresh weight was measured immediately using a digital balance to minimize water loss due to evaporation. Total fresh biomass per planting bag was recorded. For dry biomass determination, shoot and root samples were oven-dried at 60 °C for 4 days until a constant weight was achieved. Soil samples were air-dried, ground, and passed through a 0.5 mm sieve before analysis of soil Zn concentration.

Growth parameters were assessed at the population level within each planting bag, which was considered as a single experimental unit. Shoot and root biomass, leaf number, and tiller number were recorded as total values per planting bag. Plant height was measured as the height of the tallest plant within each planting bag to represent the maximum growth of the population.

**Determination of Zn Concentration**

Sample preparation for Zn analysis followed the method described by Nugroho et al. (2012) with minor modifications. Air-dried soil samples that had passed through a 0.5 mm sieve were weighed to 0.5 g. Root and leaf samples were oven-dried, finely ground using a blender, sieved, and weighed to 0.3 g. Each sample was placed in a 25 mL Erlenmeyer flask, and 15 mL of an acid mixture containing 75% $HNO_3$ and 25% HCl (v/v) was added.

The samples were digested on a hot plate at 160 °C for approximately 3 h, or until the solution became clear and the remaining volume reached about 5 mL. After digestion, the samples were allowed to cool for 15 min, then filtered through fine filter paper into a 10 mL volumetric flask and diluted to volume with distilled water. The resulting solutions were thoroughly mixed and transferred into 15 mL centrifuge tubes, sealed tightly, and subsequently analyzed for Zn concentration.

The Zn concentration was determined using a flame Atomic Absorption Spectrophotometer (AAS) (PinAAcle 900F, PerkinElmer) at a wavelength of 213.86 nm. Calibration was performed using Zn standard solutions over the range 0.0–1.0 mg $L^{-1}$, with a linear calibration model based on calculated intercepts. The calibration curves showed good linearity, with correlation coefficients ranging from 0.998272 to 0.999464. Blank correction was applied using reagent blanks and instrument auto-zero procedures during analysis. All blanks, calibration standards, and samples were analyzed in triplicate to ensure analytical consistency.

Zinc concentrations measured by atomic absorption spectrophotometry (AAS) (mg $L^{-1}$) were converted to actual heavy metal concentrations (mg $kg^{-1}$) using the equation described by Desalegn et al. (2024):

$$C\ actual\ \left(\frac{mg}{kg}\right) = \frac{C\ \ AAS\ \ \left(\frac{mg}{L}\right) \times V(L)}{W(Kg)}$$

where $C_{actual}$ is the actual heavy metal concentration in the sample (mg $kg^{-1}$), $C\ AAS$ is the concentration obtained from AAS analysis (mg $L^{-1}$), $V$ is the volume of the extracting solution (L), and $W$ is the sample dry weight (kg).

The bioconcentration factor (BCF) and translocation factor (TF) were determined at the end of the experimental period. The capacity of plants to accumulate Zn in their tissues was evaluated using the bioconcentration factor (BCF), calculated according to Rigoletto et al. (2020) as:

$$BCF\ \ Root = \frac{Root\left(\frac{mg}{kg}\right)}{Soil\left(\frac{mg}{kg}\right)} \qquad BCF\ \ Shoot = \frac{Shoot\left(\frac{mg}{kg}\right)}{Soil\left(\frac{mg}{kg}\right)}$$

where the soil Zn concentration used in the calculation refers to the final residual Zn concentration measured at the end of the experimental period.

Jurnal Biodjati 11(1): 150–165, May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

The ability of plants to translocate Zn from roots to shoots was assessed using the translocation factor (TF), calculated as:

$$TF = \frac{Shoot\left(\frac{mg}{kg}\right)}{Root\left(\frac{mg}{kg}\right)}$$

**Data Analysis**

Experimental data were analyzed using two-way analysis of variance (ANOVA) to evaluate the effects of biochar application, plant density, and their interaction. Prior to ANOVA, the assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk test and Levene's test, respectively. When significant differences among treatment combinations were detected, Duncan's Multiple Range Test (DMRT) was applied at a 95% confidence level ($p < 0.05$) for post hoc comparison among treatments. All statistical analyses were performed using SPSS software (version 25).

## RESULTS AND DISCUSSION

**Soil Zn Concentration and Plant Zn Uptake**

Based on the initial analysis, the contaminated soil contained 174.41 mg $kg^{-1}$ Zn, exceeding the U.S. Environmental Protection Agency (2007) permissible limit of 160 mg $kg^{1}$. As shown in Table 1, residual soil Zn concentrations measured after the experimental period were below the reference threshold in all planted treatments.

Results of the two-way ANOVA indicated that biochar application (B1), high plant density (D2), and their interaction did not significantly affect ($p > 0.05$) soil Zn concentration, the bioconcentration factor of shoots (BCF Shoot), bioconcentration factor of roots (BCF Root), and the translocation factor (TF) of Zn.

**Table 1.** Soil Zn concentration, BCF, and TF of *Chrysopogon zizanioides* under biochar and plant density treatments

| Parameter | Treatment | D1 | D2 | Biochar mean |
|---|---|---|---|---|
| Soil Zn concentration (mg/kg) | B0 | 52.17 ± 1.79 | 49.34 ± 5.89 | 50.76 ± 4.19 |
| | B1 | 50.17 ± 1.89 | 46.49 ± 3.94 | 48.33 ± 3.42 |
| | Plant density mean | 51.17 ± 1.98 | 47.92 ± 4.75 | |
| BCF Root | B0 | 0.91 ± 0.29 | 1.37 ± 0.25 | 1.14 ± 0.35 |
| | B1 | 1.13 ± 0.36 | 1.29 ± 0.30 | 1.21 ± 0.31 |
| | Plant density mean | 1.02 ± 0.31 | 1.33 ± 0.25 | |
| BCF Shoot | B0 | 1.03 ± 0.39 | 0.93 ± 0.07 | 0.98 ± 0.26 |
| | B1 | 0.79 ± 0.15 | 0.78 ± 0.20 | 0.78 ± 0.16 |
| | Plant density mean | 0.91 ± 0.30 | 0.85 ± 0.16 | |
| TF | B0 | 1.32 ± 0.96 | 0.69 ± 0.09 | 1.01 ± 0.70 |
| | B1 | 0.76 ± 0.32 | 0.64 ± 0.26 | 0.70 ± 0.27 |
| | Plant density mean | 1.04 ± 0.71 | 0.66 ± 0.18 | |

**Remarks :** Data are expressed as mean ± SE (n = 3). Different lowercase letters within a column indicate significant differences among means at $p \leq 0.05$ based on DMRT. B0 = without biochar; B1 = biochar application at 5% (w/w); D1 = low plant density; D2 = high plant density.

The absence of a significant biochar effect may be related to the relatively low pyrolysis temperature (400 °C) of the corn cob biochar used in this study. Low-temperature biochar has been reported to exhibit lower pore volume and BET surface area, potentially limiting heavy metal adsorption capacity (Fahmi et al., 2018). Nevertheless, this interpretation remains speculative because biochar's physicochemical properties were not directly characterized in the present study.

A tendency toward reduced soil Zn concentration was observed under the biochar treatment (B1) (Table 1). Previous studies have suggested that low-temperature biochar may retain oxygen-containing functional groups, such as carboxyl (–COOH) and hydroxyl (–OH), which could contribute to Zn immobilization through ion exchange, electrostatic interaction, complexation, and precipitation mechanisms (Alkharabsheh et al., 2021). In addition, biochar-derived organic components, including phenolic compounds, cellulose, and hemicellulose, have been reported to enhance metal complexation within the biochar matrix (Ghosh & Maiti; Deka et al., 2024). However, because no FTIR or other surface chemistry characterization was conducted in the present study, these mechanisms should be considered possible explanations rather than confirmed effects.

Biochar-treated soils (B1) showed a numerical tendency toward higher Zn accumulation in roots and lower Zn translocation to shoots, although these differences were not statistically significant ($p > 0.05$). This trend may be associated with reduced Zn toxicity, enhanced root growth, and microbial stimulation, which could promote Zn retention in root tissues (Deka et al., 2024; Zhang et al., 2019). Similar observations were reported by Radziemska et al., (2022). in *Dactylis glomerata* L., where biochar application tended to increase Zn accumulation in roots while limiting its translocation to shoots.

In the absence of biochar (B0), the TF value approached 1, whereas biochar-treated soil (B1) consistently exhibited TF < 1. The landfill soil, characterized by high total organic carbon (TOC), likely stimulates Zn-mobilizing microorganisms, such as Sphingomonas, thereby increasing Zn bioavailability. Excess Zn absorbed by roots triggers Zn translocation to the shoot (Clemens & Ma, 2016). The roots of vetiver grass are dominated by transmembrane transport genes such as Zinc/Iron-regulated Transporter-like Proteins (ZIP) and Heavy-metal-transporting ATPase (HMA), which regulate Zn absorption and translocation. If Zn is excessive in the roots, unchelated Zn can reach the xylem through HMA, then cross the xylem and pass through ZIP proteins to enter leaf cells (Wu et al., 2022; Asare et al., 2023; Balafrej et al., 2020). This is consistent with the research by Yashim Zakka (2015), which showed that landfill soil increases Zn translocation to the shoot of vetiver grass.

High plant density (D2) did not substantially increase root length capable of accessing deeper Zn pools (Postma et al., 2020), whereas low density (D1) appeared sufficient to generate organic acid exudates that stimulate Zn-mobilizing microorganisms (Wu et al., 2022). However, high plant density (D2) tended to reduce soil Zn concentration and increase BCF Root while decreasing BCF Shoot and TF (Table 1). Higher plant density increases the total root population within each planting bag, thereby expanding the collective root surface area and potentially enhancing rhizosphere interactions at the community level rather than the individual plant level. This condition allows roots to retain Zn more effectively and reduces its translocation to aboveground tissues (Jacobs et al., 2018; Cheng et al., 2020). Similar results were reported by Masinire et al. (2021), where increased planting density of vetiver grass expanded root surface area and reduced Cr translocation to shoots due to greater accumulation in roots. These findings indicate that higher plant density enhances phytostabilization processes.

Jurnal Biodjati 11(1): 150–165, May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Overall, most treatments, particularly the biochar-amended and high-density treatments, exhibited characteristics associated with Zn phytostabilization, including greater Zn retention in roots and lower shoot translocation. In contrast, the B0D1 treatment showed relatively higher Zn translocation to shoots. indicating that *Chrysopogon zizanioides* exhibits strong potential as a Zn phytostabilizer. This phytostabilization capacity is likely mediated by glutathione–glutathione S-transferase (GSH–GST) pathways, HMA/ZIP transporters localized in roots, and Zn binding to root cell walls, thereby restricting Zn translocation to aerial parts (Rigoletto et al., 2020; Wu et al., 2022).

**Shoot Biomass**

Two-way ANOVA indicated that neither biochar application nor plant density significantly affected shoot fresh weight per planting bag. In contrast, high plant density (D2) significantly increased total shoot dry biomass per planting bag, whereas biochar had no significant effect on this parameter (Table 2).

**Table 2.** Shoot fresh and dry weight per planting bag of *Chrysopogon zizanioides* under biochar and plant density treatments

| Parameter | Treatment | D1 | D2 | Biochar mean |
|---|---|---|---|---|
| Shoot fresh weight per planting bag (g) | B0 | 119.00 ± 11.27 | 120.33 ± 12.50 | 119.67 ± 10.67 |
| | B1 | 112.67 ± 15.50 | 144.67 ± 18.34 | 128.67 ± 23.19 |
| | Plant density mean | 115.83 ± 12.61 | 132.50 ± 19.36 | |
| Shoot dry weight per planting bag (g) | B0 | 29.46 ± 4.69 | 40.41 ± 3.94 | 34.94 ± 7.14 |
| | B1 | 27.39 ± 2.82 | 40.57 ± 3.49 | 33.98 ± 7.76 |
| | Plant density mean | 28.43$^{b}$ ± 3.65 | 40.49$^{a}$ ± 3.33 | |

p-values:
Shoot fresh weight = B: 0.32 ns; D: 0.09 ns; B×D: 0.11 ns
Shoot dry weight = B: 0.67 ns; D: 0.01 *; B×D: 0.62 ns
ns = not significant ($p > 0.05$); * = significant at $p \leq 0.05$

**Remarks :** Data are expressed as mean ± SE (n = 3). Different lowercase letters within a column indicate significant differences among means at $p \leq 0.05$ based on DMRT. B0 = without biochar; B1 = biochar application at 5% (w/w); D1 = low plant density; D2 = high plant density.

Higher shoot biomass under the no-biochar treatment (B0) may be attributable to the relatively high nutrient availability in landfill soil, particularly nitrogen (N) and phosphorus (P), which can promote cell division, cell expansion, and carbohydrate synthesis through enhanced photosynthetic activity (Zhan et al., 2025; Luo et al., 2020; Ludang et al., 2022). However, since soil N and P were not measured in this study, this explanation should be considered as a potential mechanism rather than a confirmed cause.

Low-temperature pyrolysis biochar may exhibit lower BET surface area and total pore volume due to limited micropore development, potentially reducing its capacity to retain nitrogen and phosphorus (Deng et al., 2022; Alkharabsheh et al., 2021). However, as biochar's physicochemical properties were not directly measured in this study, this explanation should be considered a possible mechanism rather than a confirmed effect. In addition, applying biochar at low rates limits its overall effectiveness, as higher rates have been shown to more effectively reduce nutrient leaching and enhance plant biomass production (Joseph et al., 2021; Rubin et al., 2020; Daulay & Sitepu, 2023).

High plant density (D2) did not increase shoot fresh weight per planting bag but resulted in a significant increase in shoot dry weight (Table 2), indicating a greater accumulation of structural biomass accompanied by reduced tissue water content due to crowding stress (Assefa & Debella, 2020). Reduced turgor pressure under limited water availability constrains cell expansion, particularly in leaves, thereby limiting increases in fresh biomass (Salsinha et al., 2021). The absence of a significant difference in shoot fresh biomass per planting bag is consistent with the non-significant differences observed in shoot BCF and TF values. Although biochar application (B1) and high plant density (D2) tended to increase shoot fresh biomass, both treatments also showed a decreasing trend in shoot BCF and TF (Table 1). This suggests that Zn accumulation in shoots was diluted by increased biomass production, indicating a biomass dilution effect rather than enhanced Zn translocation (Zheng et al., 2023).

**Plant Height, Tiller Number, and Leaf Number**

Plant height per planting bag was highest under the B1D1 treatment (biochar application combined with low plant density) (Table 3). A significant interaction between biochar and plant density was observed ($p = 0.03$), indicating that the effect of biochar on plant height depended on plant density. Under low-density conditions, biochar application may have increased plant height, possibly through improved soil water retention and enhanced cellular turgor, thereby promoting cell elongation. However, since soil moisture was not directly measured in this study, this explanation should be considered as a potential mechanism rather than a confirmed effect. In contrast, biochar did not significantly affect tiller or leaf number (Table 3), as these traits are more closely associated with cell division than with cell elongation (Thompson & Islam, 2021; Ghorbani et al., 2022; Igalavithana et al., 2017). This finding is supported by Chrysargyris et al. (2024), who reported that biochar application increased plant height in *Antirrhinum majus* but did not significantly increase biomass production.

**Table 3.** Plant height, tiller number, and leaf number per planting bag of *Chrysopogon zizanioides* under biochar and plant density treatments

| Parameter | Treatment | D1 | D2 | Biochar mean |
|---|---|---|---|---|
| Plant height per planting bag (cm) | B0 | 114.67b ± 5.86 | 113.67b ± 2.31 | 114.17 ± 4.02 |
| | B1 | 122.67a ± 4.04 | 108.67b ± 3.06 | 115.67 ± 8.31 |
| | Plant density mean | 118.67a ± 6.28 | 111.17b ± 3.66 | |
| Tiller number per planting bag | B0 | 15.67 ± 1.53 | 18.67 ± 2.89 | 17.17 ± 2.64 |
| | B1 | 14.00 ± 1.73 | 19.67 ± 2.52 | 16.83 ± 3.66 |
| | Plant density mean | 14.83b ± 1.72 | 19.17a ± 2.48 | |
| Leaf number per planting bag | B0 | 96 ± 5.00 | 108 ± 13.75 | 102.00 ± 11.35 |
| | B1 | 84.33 ± 9.29 | 116.33 ± 19.14 | 100.33 ± 22.10 |
| | Plant density mean | 90.17b ± 9.24 | 112.17a ± 15.59 | |

p-values:
Plant height = B: 0.54 ns; D: 0.02 *; B×D: 0.03 *
Tiller number = B: 0.80 ns; D: 0.01 *; B×D: 0.33 ns
Leaf number = B: 0.83 ns; D: 0.02 *; B×D: 0.22 ns
ns = not significant ($p > 0.05$); * = significant at $p \leq 0.05$

**Remarks :** Data are expressed as mean ± SE (n = 3). Different lowercase letters within a column indicate significant differences among means at $p \leq 0.05$ based on DMRT. B0 = without biochar; B1 = biochar application at 5% (w/w); D1 = low plant density; D2 = high plant density.

High plant density reduced plant height both with and without biochar, indicating that crowding stress effects outweighed the benefits of biochar application. Competition for water and nitrogen under high density may reduce turgor pressure and limit cell elongation, thereby suppressing vertical growth (Li et al., 2016; Seleiman et al., 2021; Yan et al., 2017). Plant height per planting bag was defined as the height of the tallest plant in each polybag, representing the maximum growth response of the plant population.

Biochar application (B1) had no significant effect on tiller or leaf number (Table 3). This may be attributed to the relatively high nutrient availability in the landfill soil even without biochar (B0), particularly nitrogen, which is known to stimulate tillering and leaf formation via cytokinin-mediated regulation of shoot meristems (Zhan et al., 2025; Bauer et al., 2020). However, since soil nitrogen content was not measured in this study, this explanation should be considered as a potential mechanism rather than a confirmed cause. The limited biochar effect may also be related to its low application rate and low pyrolysis temperature, which can reduce nitrogen retention capacity, consistent with previous findings in rice and sorghum systems showing that biochar responses are highly dose- and type-dependent (Chen et al., 2021; Mercyana et al., 2023).

In contrast, high plant density (D2) increased total tiller and leaf numbers per planting bag. This increase is partly attributable to the greater number of individual plants per unit area. However, the increase was not necessarily proportional to plant number, suggesting the presence of intraspecific competition that may reduce tiller production per plant under crowded conditions (Yang et al., 2019). Increased canopy leaf number under high density may reflect a community-level strategy to maximize light interception under competitive conditions (Postma et al., 2020). Thus, these results should be interpreted as population-level responses rather than improvements in individual plant performance.

**Table 4.** Fresh root weight, dry root weight, root length per planting bag of *Chrysopogon zizanioides* under biochar and plant density treatments

| Parameter | Treatment | D1 | D2 | Biochar mean |
|---|---|---|---|---|
| Fresh root weight per planting bag (g) | B0 | 38.00± 8.54 | 59.33 ± 3.94 | 48.67 ± 16.00 |
| | B1 | 42.00 ± 10.00 | 74.33 ± 19.14 | 58.17 ± 22.36 |
| | Plant density mean | 40.00b ± 8.60 | 66.83a ± 17.44 | |
| Dry root weight per planting bag (g) | B0 | 6.22 ± 1.73 | 16.22 ± 6.03 | 11.22 ± 6.76 |
| | B1 | 6.77 ± 1.40 | 16.95 ± 3.42 | 11.86 ± 6.05 |
| | Plant density mean | 6.50b ± 1.44 | 16.59a ± 4.40 | |
| Root length per planting bag (cm) | B0 | 40.00 ± 0.00 | 37.67 ± 3.21 | 38.83b ± 2.40 |
| | B1 | 51.33 ± 7.02 | 49.33 ± 6.66 | 50.33a ± 6.22 |
| | Plant density mean | 45.67 ± 7.63 | 43.50 ± 7.92 | |

p-values:
Fresh root weight = B: 0.27 ns; D: 0.01 *; B×D: 0.51 ns
Dry root weight = B: 0.77 ns; D: 0.001 *; B×D: 0.97 ns
Root length = B: 0.005 *; D: 0.02 ns; B×D: 0.22 ns
ns = not significant ($p > 0.05$); * = significant at $p \leq 0.05$

**Remarks :** Data are expressed as mean ± SE (n = 3). Different lowercase letters within a column indicate significant differences among means at $p \leq 0.05$ based on DMRT. B0 = without biochar, B1 = biochar application at 5% (w/w), D1 = Low plant density, D2 = High plant density.

**Root Growth**

Two-way ANOVA revealed that plant density significantly affected root fresh weight and root dry weight per planting bag, whereas biochar application had no significant effect on these parameters. Conversely, biochar application significantly increased root length, while plant density had no significant effect on this trait (Table 4).

Biochar application did not increase root biomass but promoted root length (Table 4). Biochar-induced increases in root tip number and root length were proportionally greater than increases in root biomass (Xiang et al., 2017). Biochar application can reduce soil bulk density and increase water-holding capacity, thereby lowering mechanical resistance and facilitating root penetration into deeper soil layers. These changes create a looser, more porous, and better-aerated soil environment, allowing roots to elongate without substantially increasing total root mass (Chang et al., 2021). However, since soil physical properties were not directly measured in this study, this explanation should be considered as a potential mechanism rather than a confirmed effect. Root elongation is driven by cell division in the meristematic zone and cell elongation in the elongation zone, processes regulated by plant hormones and acid growth mechanisms (Bizet et al., 2015; Pacifici et al., 2018). Li et al. (2024) reported that gramineous species exhibited a stronger growth response to biochar application, with root length increasing by approximately 47–90%, particularly in *Pennisetum alopecuroides*.

Higher plant density increased total root biomass per planting bag due to the greater number of plants, but it did not enhance root length (Table 4). However, this increase in root biomass reflects a population-level response rather than improved root growth per individual plant, as root biomass was not evaluated on a per-plant basis. Root penetration depth is more strongly influenced by soil physical conditions than by belowground competition. Postma et al. (2020) reported that individual plants tend to explore a similar soil volume even as plant density increases, indicating that roots are more plastic in response to soil moisture, aeration, and nutrient availability than to the presence of neighboring roots. High plant density does not promote root elongation per plant but increases root biomass per unit soil volume by accumulating roots from multiple plants. Therefore, root responses to plant density are collective, reflecting adaptive strategies that optimize soil space exploration rather than individual root elongation.

This pattern is consistent with Zn accumulation results, where increased root length under biochar treatment (B1) and higher root biomass under high plant density (D2) tended to reduce residual soil Zn concentrations and increase root BCF values, although these effects were not statistically significant (Table 1). These trends suggest that enhanced root system development may improve Zn retention in the root zone, supporting a phytostabilization mechanism rather than increased translocation to shoots.

## CONCLUSION

Corn cob biochar and plant density did not significantly affect residual soil Zn concentration, BCF Root, BCF Shoot, or TF under the present experimental conditions. However, biochar application significantly increased root length, while high plant density significantly increased shoot dry weight, root fresh weight, and root dry weight per planting bag. Although Zn-related parameters were not statistically significant, most biochar-amended and high-density treatments showed a tendency toward increased root Zn accumulation and reduced translocation to shoots, indicating a root-based Zn retention pattern. These findings suggest the potential role of vetiver grass in Zn phytostabilization under landfill conditions. However, further research, including unplanted controls and Zn mass balance analysis, is required to confirm the relative contributions of plant uptake and biochar-mediated immobilization.

## AUTHOR CONTRIBUTION

**M.L.F.**, as the lead author, conceived the research idea, designed the methodology, performed data analysis, interpreted the results, and prepared the initial draft of the manuscript. **E.D.H.** contributed to data collection, provided input on the experimental design, assisted with the literature review, supported statistical analysis and data modeling, offered technical guidance in data processing, and contributed to writing the discussion and conclusion sections. **Y.N.** supervised field data collection and research documentation management, prepared the tables and figures included in the manuscript, and revised the manuscript to improve clarity, coherence, and consistency of the presented arguments..

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Universitas Diponegoro for providing financial support through the International Publication Research Funded scheme, supported by the State Revenue and Expenditure Budget (APBN) for the 2025 fiscal year. Number 21B/UN7.F8/PP/II/2025.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this study

## REFERENCES

Alkharabsheh, H. M., Seleiman, M. F., Battaglia, M. L., Shami, A., Jalal, R. S., Alhammad, B. A., Almutairi, K. F., & Al-Saif, A. M. (2021). Biochar and Its Broad Impacts in Soil Quality and Fertility, Nutrient Leaching and Crop Productivity: A Review. *Agronomy, 11*(5), 993. DOI: https://doi.org/10.3390/agronomy11050993

Amen, R., Yaseen, M., Mukhtar, A., Klemeš, J. J., Saqib, S., Ullah, S., Al-Sehemi, A. G., Rafiq, S., Babar, M., Fatt, C. L., Ibrahim, M., Asif, S., Qureshi, K. S., Akbar, M. M., & Bokhari, A. (2020). Lead and cadmium removal from wastewater using eco-friendly biochar adsorbent derived from rice husk, wheat straw, and corncob. *Cleaner Engineering and Technology*, 1, 100006. DOI: https://doi.org/10.1016/j.clet.2020.100006

Asare, M.O., Száková, J., Tlustoš, P. et al. Zinc contamination in soils and its implications on plant phytoalexins. *International Journal of Environmental Science and Technology*. 22, 8581–8600 (2025). DOI: https://doi.org/10.1007/s13762-025-06437-x

Assefa, A., & Debella, A. (2020). Review on dry matter production and partitioning as affected by different environmental conditions. *International Journal of Advanced Research in Biological Sciences*, *7*(3), 37–46. DOI: https://doi.org/10.22192/ijarbs

Balafrej, H., Bogusz, D., Triqui, Z.-E. A., Guedira, A., Bendaou, N., Smouni, A., & Fahr, M. (2020). Zinc hyperaccumulation in plants: A review. *Plants*, *9*(5), 562. DOI: https://doi.org/10.3390/plants9050562

Bauer, B., & von Wirén, N. (2020). Modulating tiller formation in cereal crops by the signalling function of fertilizer nitrogen forms. *Scientific Reports*, 10, 20504. DOI: https://doi.org/10.1038/s41598-020-77467-3

Bizet, F., Hummel, I., & Bogeat-Triboulot, M.-B. (2015). Length and activity of the root apical meristem revealed in vivo by infrared imaging. *Journal of Experimental Botany*, *66*(5), 1387–1395. DOI: https://doi.org/10.1093/jxb/eru488

Boorboori, M. R., & Lackóová, L. (2023). Biochar; an effective factor in improving phytoremediation of metal(iod)s in polluted sites. *Frontiers in Environmental Science*, 11. DOI: https://doi.org/10.3389/fenvs.2023.1253144

Chang, Y., Rossi, L., Zotarelli, L. et al. (2021). Biochar improves soil physical characteristics and strengthens root architecture in Muscadine grape (*Vitis rotundifolia* L.). *Chemical and Biological Technologies in Agriculturec*, 8, 7 DOI: https://doi.org/10.1186/s40538-020-00204-5

Chen, X., Yang, S., Ding, J., Jiang, Z., & Sun, X. (2021). Effects of Biochar Addition on Rice Growth and Yield under Water-Saving Irrigation. *Water*, *13*(2), 209. DOI: https://doi.org/10.3390/w13020209

Cheng, Y., Peng, J., Gu, Y., Guo, H., Jiang, T., & Yang, H. (2023). The Early Effect of Plant Density on Soil Physicochemical Attributes and Bacterial and Understory Plant Diversity in Phoebe zhennan Plantations. *Forests*, *14*(8), 1612. DOI: https://doi.org/10.3390/f14081612

Chrysargyris, A., Prasad, M., & Tzortzakis, N. (2024). Wood-Based Biochar Ratio Used for Partial Peat Replacement in Growing Media for Antirrhinum majus Pot Production. *Agriculture*, *14*(11), 1860. DOI: https://doi.org/10.3390/agriculture14111860

Clemens, S., & Ma, J. F. (2016). Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annual Review of Plant Biology*, 67, 489–512. DOI: https://doi.org/10.1146/annurev-arplant-043015-112301

Dagwar, P. P., & Dutta, D. (2024). Landfill leachate: A potential challenge towards sustainable environmental management. *Science of the Total Environment*, 926, 171668. DOI: https://doi.org/10.1016/j.scitotenv.2024.171668

Deka, D., Patwa, D., Nair, A. M., et al. (2024). Influence of biochar amendment on removal of heavy metals from soils using phytoremediation by C. roseus and *Chrysopogon zizanioides*. *Environmental Science and Pollution Research*, 1–35. DOI: https://doi.org/10.21203/rs.3.rs-3785156/v1

Desalegn, E., Getachew, N., Tesfaye, G. et al., (2024) Determination of the levels of selected essential and toxic metals in soil collected from the flower farm in Roshara Roses PLC (Bishoftu) of Ethiopia and assessment of associated human health risks. *Discover Applied Sciences*. 6, 362. DOI: https://doi.org/10.1007/s42452-024-06028-4

Ekperusi, O. A., & Aigbodion, F. I. (2015). Bioremediation of petroleum hydrocarbons from crude oil-contaminated soil with the earthworm: Hyperiodrilus africanus. *3 Biotech*, *5*(6), 957–965. DOI: https://doi.org/10.1007/s13205-015-0298-1

Fahmi, A. H., Samsuri, A. W., Jol, H., & Singh, D. (2018). Physical modification of biochar to expose the inner pores and their functional groups to enhance lead adsorption. *RSC Advances*, 8(67), 38270–38280. DOI: https://doi.org/10.1039/C8RA06867D

Ghorbani, M., Konvalina, P., Neugschwandtner, R. W., Kopecký, M., Amirahmadi, E., Bucur, D., & Walkiewicz, A. (2022). Interaction of biochar with chemical, green and biological nitrogen fertilizers on nitrogen use efficiency indices. *Agronomy*, *12*(9), 2106. DOI: https://doi.org/10.3390/agronomy12092106

Ghosh, D., & Maiti, S. K. (2021). Biochar assisted phytoremediation and biomass disposal in heavy metal contaminated mine soils: a review. *International Journal of Phytoremediation*, *23*(6), 559–576. DOI: https://doi.org/10.1080/15226514.2020.1840510

He, D., Luo, Y., & Zhu, B. (2024). Feedstock and pyrolysis temperature influence biochar properties and its interactions with soil substances: Insights from a DFT calculation. *Science of the Total Environment*, 922, 171259. DOI: https://doi.org/10.1016/j.scitotenv.2024.171259

Himaya, S. M. M. S., Kumara, A. D. N. T., Premanandarajah, P., & Thariq, M. G. M. (2023). Effective mitigation of Cadmium contamination in soil through Sawdust Biochar application. AGRIEAST: Journal of Agricultural Sciences, 17(2), 38-47. DOI: https://doi.org/10.4038/agrieast.v17i2.126

Igalavithana, A. D., Ok, Y. S., Niazi, N. K., Rizwan, M., Al-Wabel, M. I., Usman, A. R. A., Moon, D. H., & Lee, S. S. (2017). Effect of Corn Residue Biochar on the Hydraulic Properties of Sandy Loam Soil. *Sustainability*, *9*(2), 266. DOI: https://doi.org/10.3390/su9020266

Jacobs, Arnaud & De Brabandere, Léna & Drouet, Thomas & Sterckeman, Thibault & Noret, Nausicaa. (2018). Phytoextraction of Cd and Zn with Noccaea caerulescens for urban soil remediation: influence of nitrogen fertilization and planting density. *Ecological Engineering*. 116. DOI: https://doi.org/10.1016/j.ecoleng.2018.03.007

Jagasri, S., Kannan, J., Jayashree, R., Sheeba, S., Prabakaran, K., Jayabalakrishnan, R. M., Murugaragavan, R., Poornachandra, C., & Madhusree, S. (2024). Effect of landfill leachates on urban soil: A review. *Plant Science Today*, *11*(4), 485–493. DOI: https://doi.org/10.14719/pst.4767

Joseph, S., A. L. Cowie, L. Van Zwieten, N. Bolan, A. Budai, W. Buss, M. L. Cayuela, et al. (2021). "How Biochar Works, and When It Doesn't: A Review of Mechanisms Controlling Soil and Plant Responses to Biochar." *GCB Bioenergy 13* (11): 1731–64. DOI: https://doi.org/10.1111/gcbb.12885.

Khalid, H., Zia-ur-Rehman, M., Naeem, A., Khalid, M. U., Rizwan, M., Ali, S., Umair, M., & Sohail, M. I. (2019). *Solanum nigrum* L.: A novel hyperaccumulator for the phyto-management of cadmium contaminated soils. In M. Hasanuzzaman, M. N. V. Prasad, & M. Fujita (Eds.), *Cadmium toxicity and tolerance in plants* (pp. 451-477). Academic Press. DOI: https://doi.org/10.1016/B978-0-12-814864-8.00018-8

Khan, S., Masoodi, T. H., Pala, N. A., Murtaza, S., Mugloo, J. A., Sofi, P. A., Zaman, M. U., Kumar, R., & Kumar, A. (2023). Phytoremediation prospects for restoration of contamination in the natural ecosystems. *Water*, *15*(8), 1498. DOI: https://doi.org/10.3390/w15081498

Li, L., Weiner, J., Wang, Y., Wang, S., Zhou, D.-W. (2016). Yield-density relationships of above- and belowground organs in Allium cepa var. aggregatum populations. *Plant Ecology*. 217, 913–922. DOI: https://doi.org/10.1007/s11258-016-0616-7

Li, X., Zhang, X., Wang, J., Liu, Z., Song, H., & An, J. (2024). Biochar Organic Fertilizer Combined with Indigenous Microorganisms Enhances the Growth of Landscape Grass Cultivated in a Substrate Mixed with Iron Tailings and Mining Topsoil. *Plants*, *13*(21), 3042. DOI: https://doi.org/10.3390/plants13213042

Liu, J., Huang, W., Mo, A. L., Ni, J., Xie, H. Y., Hu, J. S., et al. (2020). Effect of lychee biochar on the remediation of heavy metal-contaminated soil using sunflower: A field experiment. Environmental Research, 188, 109886. DOI: https://doi.org/10.1016/j.envres.2020.109886

Ludang, Y., Supriyati, W., & Alpian, A. (2022). Distribusi biomassa dan karbon tingkat semai jenis manggis, lengkeng, sengon dan jelutung (Seed-level biomass and carbon distribution types of mangosteen, longan, sengon and jelutung). *Jurnal Hutan Tropika*, *17*(1), 61–67. DOI: https://doi.org/10.36873/jht.v17i1.4363

Luo, L., Zhang, Y., & Xu, G. (2020). How does nitrogen shape plant architecture?. *Journal of experimental botany*, *71*(15), 4415–4427. DOI: https://doi.org/10.1093/jxb/eraa187

Masinire, F., Adenuga, D. O., Tichapondwa, S. M., & Chirwa, E. M. N. (2021). Phytoremediation of Cr(VI) in wastewater using the vetiver grass (*Chrysopogon zizanioides*). *Minerals Engineering*, 172, 107141. DOI: https://doi.org/10.1016/j.mineng.2021.107141

Melato, F. A., Mokgalaka, N. S., & McCrindle, R. I. (2016). Adaptation and detoxification mechanisms of vetiver grass (*Chrysopogon zizanioides*) growing on gold mine tailings. *International Journal of Phytoremediation*, 18(5), 509–520. DOI: https://doi.org/10.1080/15226514.2015.1115963

Mercyana, V., Samanhudi, S., & Harsono, P. (2023). Growth response and sugar accumulation in first ratoon sweet sorghum: Effects of biochar and shoot number manipulation. International Journal of Design & Nature and Ecodynamics, 18, 741–746. DOI: https://doi.org/10.18280/ijdne.180328

Mishra, S., Tiwary, D., Ohri, A., & Agnihotri, A. K. (2019). Impact of municipal solid waste landfill leachate on groundwater quality in Varanasi, India. *Groundwater for Sustainable Development*, 9, 100230. DOI: https://doi.org/10.1016/j.gsd.2019.100230

Nugroho, A.P. and Frank, H. 2012. Effect of copper exposure on calcium, carbohydrate, and protein levels in the freshwater mussel *Anodonta anatina*. *Toxicological & Environmental Chemistry 94* (1): 99-108. DOI: http://dx.doi.org/10.1080/02772248.2011.630910

Pacifici, E., Di Mambro, R., Dello Ioio, R. et al. (2018). Acidic cell elongation drives cell differentiation in the Arabidopsis root. *The EMBO Journal*, 37, DOI: https://doi.org/10.15252/embj.201899134

Postma, J. A., Hecht, V. L., Hikosaka, K., Nord, E. A., Pons, T. L., & Poorter, H. (2021). Dividing the pie: A quantitative review on plant density responses. *Plant*, *cell & environment*, *44*(4), 1072–1094. DOI: https://doi.org/10.1111/pce.13968

Rabêlo, F. H. S., Vangronsveld, J., Baker, A. J. M., van der Ent, A., & Alleoni, L. R. F. (2021). Are grasses really useful for the phytoremediation of potentially toxic trace elements? A review. *Frontiers in Plant Science*, 12. DOI: https://doi.org/10.3389/fpls.2021.778275

Radziemska, M., Gusiatin, Z. M., Mazur, Z., Hammerschmiedt, T., Bęś, A., Kintl, A., Galiova, M. V., Holatko, J., Blazejczyk, A., Kumar, V., & Brtnicky, M. (2022). Biochar-Assisted Phytostabilization for Potentially Toxic Element Immobilization. *Sustainability*, *14*(1), 445. DOI: https://doi.org/10.3390/su14010445

Rigoletto, M., Calza, P., Gaggero, E., Malandrino, M., & Fabbri, D. (2020). Bioremediation Methods for the Recovery of Lead-Contaminated Soils: A Review. *Applied Sciences*, *10*(10), 3528. DOI: https://doi.org/10.3390/app10103528

Salsinha Y.C.F., Maryani Indradewa D., Purwestr Y.A., Rachmawati D. (2021). Leaf physiological and anatomical characters contribute to drought tolerance of Nusa Tenggara Timur local rice cultivars. *Journal of Crop Science and Biotechnology*, 24, 337,

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H. H., & Battaglia, M. L. (2021). Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants*, *10*(2), 259. DOI: https://doi.org/10.3390/plants10020259

Sugiantara, I. G. B., Khairunnisa, B. A. F., Hayyi, N. A. M., Cahyadi, M. B., Rahmiati, R., Oktaviani, O., Darmayanti, C. I. W., Nabilah, A., Fauzan, M., Diraksa, M. S., & Wulan, R. S. T. (2025). Pemanfaatan limbah tongkol jagung untuk pembuatan produk pupuk biochar hybrid kaya nutrisi makro N, P, K, S. *Jurnal Wicara Desa*, 3(4). DOI: https://doi.org/10.29303/q4eenw44

Sulistiyani, A., Falah, S., & Triadiati, T. (2020). Determining phytocomponent of vetiver grass (Chrysopogon zizanioides) under drought stress. *Biogenesis: Jurnal Ilmiah Biologi*, *8*(1), DOI: https://doi.org/10.24252/bio.v8i1.11686

Thomas, G., Sheridan, C., & Holm, P. E. (2024). Co-cropping vetiver grass and legume for the phytoremediation of an acid mine drainage (AMD) impacted soil. *Environmental pollution* (Barking, Essex : 1987), 341, 122873. DOI: https://doi.org/10.1016/j.envpol.2023.122873

Thompson, D. S., & Islam, A. (2021). Plant Cell Wall Hydration and Plant Physiology: An Exploration of the Consequences of Direct Effects of Water Deficit on the Plant Cell Wall. *Plants*, *10*(7), 1263. DOI: https://doi.org/10.3390/plants10071263

U.S. Environmental Protection Agency. (2007). Ecological soil screening levels for zinc (Interim final, OSWER Directive 9285.7-73). Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency.

Vuong, T. X., Pham, T. T. H., Nguyen, T. T. T., & Pham, D. T. N. (2023). Effects of biochar and apatite on chemical forms of lead and zinc in multi-metal-contaminated soil after incubation: A comparison of peanut shell and corn cob biochar. *Sustainability*, 15(15), 11992. DOI: https://doi.org/10.3390/su151511992

Wu, B., Li, J., Peng, D., Wang, Z., & Xu, H. (2022). Cadmium exposure alters rhizospheric microbial community and transcriptional expression of vetiver grass. *Frontiers in Plant Science*, 13, 808844. DOI: https://doi.org/10.3389/fpls.2022.808844

Xiang Y, Deng Q, Duan H, Guo Y (2017) Effects of biochar application on root traits: a meta-analysis. *Gcb Bioenergy* 9:1563–1572. DOI: https://doi.org/10.1111/gcbb.12449

Yan, P., Pan, J., Zhang, W., Shi, J., Chen, X., et al. (2017). A high plant density reduces the ability of maize to use soil nitrogen. *PLOS ONE*, *12*(2), e0172717. DOI: https://doi.org/10.1371/journal.pone.0172717

Yang, D., Cai, T., Luo, Y., & Wang, Z. (2019). Optimizing plant density and nitrogen application to manipulate tiller growth and increase grain yield and nitrogen-use efficiency in winter wheat. *PeerJ*, 7, e6484. DOI: https://doi.org/10.7717/peerj.6484

Yashim, Z., & Zakka, U. (2015). The effect of application of EDTA on the phytoextraction of heavy metals by *Vetiveria zizanioides*, *Cymbopogon citratus*, and *Helianthus annuus*. *American Journal of Applied Chemistry*, 3(1). DOI: https://doi.org/10.11648/j.ajac.20150301.11

Zhan, M., Sun, Y., Lan, H., Zhou, T., Zhao, Y., & Yang, L. (2025). Spatio-temporal distribution of soil microbial communities and nutrient availability around a municipal solid waste landfill. *Frontiers in microbiology*, 16, 1583149. DOI: https://doi.org/10.3389/fmicb.2025.1583149

Zhang, M., Wang, J., Bai, S. H., Zhang, Y., Teng, Y., & Xu, Z. (2019). Assisted phytoremediation of a co-contaminated soil with biochar amendment: Contaminant removals and bacterial community properties. *Geoderma*, 348, 115–123. DOI: https://doi.org/10.1016/j.geoderma.2019.04.031

Zheng, W L, Wang Y F, Mo J, Zeng P, Chen J and Sun C. (2023). Effects of biochar application and nutrient fluctuation on the growth, and cadmium and nutrient uptake of *Trifolium repens* with different planting densities in Cd-contaminated soils. *Front. Plant Sci.* 14:1269082. DOI: https://doi.org/10.3389/fpls.2023.1269082