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# MITIGATION OF LEAD (PB) TOXICITY IN MAIZE BY USING HYDROXYETHYL CELLULOSE AND NITRILOTRIACETIC ACID CHELATED BIOCHAR

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**Abstract**

Lead (Pb) is a highly toxic heavy metal that severely impairs plant growth by disrupting key physiological and biochemical processes, including photosynthesis, respiration, and enzymatic activity. The application of soil amendments that enhance metal immobilization and improve plant resilience represents a promising strategy for mitigating Pb toxicity. In this context, hydroxyethyl cellulose (HEC), a hydrophilic polymer known for improving soil moisture retention and nutrient availability, and nitrilotriacetic acid–chelated biochar (NTA-BC), recognized for its metal-complexing capacity and nutrient retention, were evaluated individually and in combination for their effectiveness in alleviating Pb stress in maize. A pot experiment was conducted using a completely randomized design with six treatments: 0HEC+0NTA-BC, 5HEC+0NTA-BC, 10HEC+0NTA-BC, 0HEC+0.75NTA-BC, 5HEC+0.75NTA-BC, and 10HEC+0.75NTA-BC, applied under two Pb regimes (0 and 600 mg $kg^{-1}$ soil) with four replications. Lead stress markedly reduced maize growth, biomass accumulation, and photosynthetic performance. However, the combined application of HEC and NTA-BC significantly mitigated these adverse effects, with the 10HEC+0.75NTA-BC treatment exhibiting the most pronounced improvement under Pb stress. This treatment increased shoot length (26.73%), shoot fresh weight (13.26%), shoot dry weight (56.74%), root length (51.88%), root fresh weight (64.51%), and root dry weight (81.29%) relative to the Pb-stressed control. Moreover, chlorophyll a, chlorophyll b, and total chlorophyll contents were increased by 10.65%, 10.90%, and 12.60%, respectively, indicating enhanced photosynthetic efficiency. Enhanced leaf nitrogen, phosphorus, and potassium concentrations further confirmed improved nutrient uptake and metabolic functioning. Overall, the synergistic application of HEC and NTA-chelated biochar effectively alleviated Pb-induced toxicity, enhanced plant growth, and improved physiological performance in maize. The use of 10HEC+0.75NTA-BC is therefore recommended as a sustainable soil amendment for improving crop productivity in Pb-contaminated soils.

**Key words:** lead toxicity; hydroxyethyl cellulose; nitrilotriacetic acid; biochar; chlorophyll content; nutrient uptake

## Introduction

Lead (Pb) is a prominent environmental concern, accounting for a significant fraction of trace metal pollution and posing substantial risks to both plant and human health due to its toxic and non-biodegradable nature (Gupta *et al*., 2024;. Its ubiquitous presence in the ecosystem, stemming from both natural sources and anthropogenic activities like industrial waste, mining, and agricultural practices, exacerbates these concerns (Rani *et al.*, 2024). Once absorbed by plants, lead can severely disrupt cellular structures, impede critical metabolic functions, and induce oxidative stress, ultimately leading to impaired growth, underdeveloped root and shoot systems, chlorosis, and necrosis (Gupta *et al.*, 2024). These physiological disruptions are often accompanied by alterations in cell division, impaired seed germination, and compromised water balance, further diminishing plant vitality and crop yield (Shahzad *et al.*, 2024, Ur Rahman *et al.*, 2024). Furthermore, the bioaccumulation of lead in plants facilitates its entry into the food chain, posing a considerable health hazard to humans who consume contaminated produce (Aslam *et al.*, 2021). Given that lead contributes approximately 10% of total heavy metal pollution, understanding its uptake mechanisms and subsequent physiological impacts on plants is crucial for developing effective mitigation strategies (Collin *et al.*, 2022).

Hydroxyethyl cellulose, a non-ionic polysaccharide derivative of cellulose, is extensively utilized in various industrial and biomedical applications due to its unique physicochemical properties (Dey *et al.*, 2021). This versatility stems from its modifiable hydroxy groups, which enable the synthesis of various functional derivatives, thereby expanding its utility across diverse fields (Deng *et al.*, 2024). In agriculture, hydroxyethyl cellulose and other hydrogel formulations are emerging as transformative technologies that enhance crop resilience and water use efficiency, promoting sustainable farming practices, especially in drought-prone regions (Agbna & Zaidi, 2025). This is particularly relevant given the increasing global food demand coupled with the escalating threat of desertification and extreme weather events, such as prolonged droughts, exacerbated by climate change (Tursynova *et al.*, 2025). Hydrogels, characterized by their hydrophilic nature and porous structure, are adept at retaining substantial amounts of water, making them a palpable solution to mitigate water stress in crops (Iqbal *et al.*, 2024). This water-retention capability directly addresses the challenges posed by water scarcity, a critical issue for global food security exacerbated by climate change and unsustainable agricultural practices (Agbna & Zaidi, 2025). Specifically, bio-based superabsorbent hydrogels demonstrate potential not only to augment soil

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moisture but also to facilitate nutrient availability, thereby optimizing plant growth and biomass accumulation even under arid conditions (Ribeiro *et al.*, 2024). However, despite its widespread application, the specific role of hydroxyethyl cellulose as a foliar treatment in agricultural contexts, particularly regarding plant physiological responses to oxidative stress (i.e., induced by heavy metals), remains an area requiring further investigation.

Biochar, a stable form of carbon derived from the thermochemical conversion of biomass under oxygen-limited conditions, is a critical material in the discourse on sustainable environmental and agricultural practices (Mulabagal *et al*., 2024). This carbon-rich, porous material, generated through pyrolysis, offers multifaceted applications spanning carbon sequestration, soil amendment, and contaminant remediation, thereby contributing significantly to mitigating global environmental challenges (Mulabagal *et al.*, 2024, Rajput *et al.*, 2024). Its unique physicochemical properties, including a large surface area and complex pore structure, are directly influenced by the feedstock and pyrolysis conditions, rendering it a versatile tool for environmental remediation strategies (Masud *et al.*, 2023, Khan *et al.*, 2024). Specifically, biochar's efficacy in addressing heavy metal contamination, a pervasive environmental issue, stems from its capacity to immobilize these pollutants through various adsorption and precipitation mechanisms (Cho *et al.*, 2024). Specifically, the immobilization of Pb by biochar is attributed to its high cation exchange capacity, the presence of various active functional groups, and its highly microporous structure, which collectively enhance its complexation with metal cations (Rizwan *et al.*, 2021). This immobilization process reduces the bioavailability and mobilization of heavy metals, thereby minimizing their uptake by plants and subsequent entry into the food chain (Zafar-ul-Hye *et al.*, 2021).

Nitrilotriacetic acid (NTA) is a prominent amino polycarboxylate chelating agent widely employed across diverse industrial sectors due to its strong complexing capabilities with metal ions (Kołodyńska, 2013). Its efficacy is particularly notable in high-temperature environments, making it suitable for applications in the oil and gas industry (Almubarak *et al.*, 2022). Beyond industrial uses, nitrilotriacetic acid has also gained traction in agricultural applications, particularly for enhancing phytoremediation strategies in heavy metal-contaminated soils (Hosseinniaee *et al.*, 2023). Phytoremediation, a biologically driven process that uses plants to extract or stabilize pollutants, is considered a safe and effective technique for addressing heavy metal contamination (Yin *et al.*, 2024). However, its application, even as a seemingly environmentally friendly chelating agent, warrants careful consideration to prevent potential leaching of metal–chelate complexes and phytotoxicity (Licinio *et al.*, 2022). Additionally, bioremediation strategies utilizing soil microorganisms, such as *Aspergillus terreus* inoculation, have been proposed as promising alternatives for mitigating lead toxicity in cereal crops (Mustafa *et al.*, 2025).

The present study was designed to systematically evaluate the combined effectiveness of nitrilotriacetic acid–chelated biochar (NTA-BC) and hydroxyethyl cellulose in mitigating lead toxicity in maize plants. The specific objectives were to (i) assess the impact of NTA-chelated biochar on plant growth, (ii) investigate the role of hydroxyethyl cellulose in modulating plant physiological traits and oxidative stress responses under Pb stress, and (iii) elucidate the combined effects of these amendments on antioxidant defense systems. The novelty of this study lies in the integrated use of NTA-chelated biochar and hydroxyethyl cellulose as a dual soil–plant intervention strategy, which has not been previously reported for Pb stress mitigation in maize. It is hypothesized that the combined application of NTA-chelated biochar and hydroxyethyl cellulose will significantly reduce Pb uptake, regulate antioxidant defense mechanisms, and improve overall growth of maize plants under Pb stress.

## Material and Methods

**Preparation and characterization of Biochar:** Biochar was produced from discarded cotton sticks, which served as the lignocellulosic feedstock. The raw material underwent pyrolysis at 440°C under limited oxygen conditions, a process that thermochemically converts biomass into carbon-rich biochar while releasing volatile compounds and gases (Obia *et al.*, 2015). After pyrolysis, the biochar was allowed to cool, ground to a uniform particle size of 2 mm, and stored in airtight containers for subsequent experimental use. The total composition of biochar was evaluated by gravimetric analysis, following the method described previously (McLaughlin, 2010). To assess pH and electrical conductivity (EC), a 1:10 biochar-to-distilled water suspension was prepared, and measurements were performed according to the standard procedures outlined by (Page *et al.*, 1983) and (Rhoades, 1996), respectively. The total nitrogen (N) content of biochar was quantified using the Kjeldahl digestion and distillation method (Van Schouwenberg, & Walinge, 1973). For phosphorus (P) and potassium ($K^+$) analysis, the biochar samples were digested with a mixture of nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) (Miller, 1998). The phosphorus concentration was subsequently determined using a spectrophotometric method based on the yellow color reaction of ammonium vanadate–ammonium molybdate, while potassium levels were measured using a flame photometer (Pratt, 2016).

**NTA-BC preparation:** Nitrilotriacetic acid (NTA)-chelated biochar (BC) was prepared to enhance the metal-binding capacity of biochar and reduce Pb bioavailability in soil. The process began with finely ground biochar (2 mm), which was first suspended in distilled water at a 1:10 (w/v) ratio and stirred for 1 h to remove soluble impurities, followed by oven-drying at 60°C for 24 h. Subsequently, the dried biochar was mixed with a 0.1 M aqueous solution of NTA at a 1:5 (biochar:NTA solution, w/v) ratio and continuously stirred at 50°C for 12 h, allowing the NTA molecules to chelate with functional groups on the biochar surface. After chelation, the NTA-BC was separated by filtration and washed repeatedly with distilled water until neutral pH was achieved to remove unbound NTA. The washed NTA-BC was then oven-dried at 60°C for 24 h, ground to a uniform particle size (~2 mm), and stored in airtight containers for subsequent application. The functionalization of biochar with NTA was confirmed through Fourier Transform Infrared Spectroscopy (FTIR), which detected the introduction of carboxylate and amine groups.

**Seed sowing:** Prior to sowing, the selected seeds of maize (*Zea mays* L.) were subjected to surface sterilization to minimize microbial contamination. The sterilization protocol involved immersion of the seeds in a 5% sodium hypochlorite solution, followed by three successive rinses with 95% ethanol. Thereafter, the seeds were thoroughly washed three times with sterile deionized water to remove any residual sterilizing chemicals. Five seeds were initially sown in each pot, and after successful germination, thinning was performed to retain two uniform and healthy plant per pot.

**Treatment plan:** The control treatment received no supplemental amendments (0 HEC + 0 NTA-BC). The experimental design included three levels of hydroxyethyl cellulose (HEC): 0HEC (no application), 5HEC (5 mg $L^{-1}$ HEC applied as three foliar sprays at 10-day intervals), and 10HEC (10 mg $L^{-1}$ HEC applied as four foliar sprays at 10-day intervals). For both 5HEC and 10HEC treatments, foliar sprays were initiated at the V4 vegetative stage, followed by subsequent applications at 10-day intervals corresponding approximately to the V6–V8 stage, V8–V10 stage, and for 10HEC, a final spray at the tasseling (VT) stage, ensuring nutrient availability during critical growth periods. In addition, two levels of nitrilotriacetic acid–chelated biochar (NTA-BC) were evaluated, namely 0% NTA-BC and 0.75% NTA-BC. Lead (Pb) stress was imposed at two levels: a non-stress control (0Pb) and a stressed treatment receiving 600 mg Pb $kg^{-1}$ soil. All treatment combinations were arranged in a completely randomized design (CRD) with 4 replicates.

**Fertilizer:** To meet the nutritional requirements of maize in a pot experiment, nitrogen (N), phosphorus (P), and potassium (K) were applied at rates equivalent to 10, 7.5, and 5 mg $kg^{-1}$ soil, respectively. Phosphorus, supplied as single superphosphate, and potassium, applied through a suitable commercial source, were incorporated into the soil as basal applications at sowing. Nitrogen, supplied as urea, was applied using a split application strategy, with one-third as basal at planting, one-third at the V4–V6 vegetative stage during the first irrigation, and the final third at the silking stage during the second irrigation. All fertilizers were thoroughly mixed with the soil to ensure uniform distribution and optimal nutrient availability during key growth stages of maize.

**Irrigation:** Soil moisture was carefully monitored and maintained at 75% of field capacity (FC) to ensure optimal growth conditions. The pots were irrigated every two days to maintain the desired moisture level, and the soil water content was adjusted as needed throughout the experiment to compensate for evapotranspiration losses.

**Weed management:** To minimize competition for nutrients, water, and light, weeding was performed manually at regular intervals throughout the experiment. Care was taken to remove weeds without disturbing the maize plants or their root systems.

**Harvesting and data collection:** Maize plants were harvested 60 days after sowing to assess growth parameters and biomass accumulation. At the time of harvest, the roots were carefully uprooted to avoid damage. Shoot and root lengths were measured using a standard ruler, and the fresh weights of shoots and roots were recorded immediately using an analytical balance to prevent moisture loss affecting the measurements. To determine the dry biomass, the collected plant samples were placed in a forced-air oven at 65°C for 72 hours, ensuring that samples reached a constant weight, indicating complete removal of moisture. After drying, the dry weights of shoots and roots were measured using an analytical balance, providing accurate estimates of biomass allocation and growth performance.

**Chlorophyll contents and carotenoids:** Chlorophyll a, chlorophyll b, and total chlorophyll contents in freshly harvested maize leaves were quantified following the method described by (Arnon, 1949). Leaf samples were homogenized and extracted using 80% acetone, and the chlorophyll concentrations were determined spectrophotometrically by measuring absorbance at 663 nm, 645 nm, and 470 nm. The measured absorbance values were then used to calculate chlorophyll a, chlorophyll b, and total chlorophyll content using standard equations.

**Gas exchange attributes:** Measurements were conducted during the morning, between 10:30 and 11:30 AM, under clear-sky conditions to ensure optimal light intensity for photosynthetic saturation, using an Infrared Gas Analyzer (CI-340 Photosynthesis System, CID, Inc., USA). The leaf cuvette of the analyzer was adjusted to maintain ambient temperature and relative humidity, while $CO_2$ concentration was stabilized at atmospheric levels (~400 µmol $mol^{-1}$). Observations were recorded on the uppermost fully expanded leaves, avoiding shaded or damaged tissue, and three readings per plant were recorded and averaged to minimize variability (Nazar *et al.*, 2014).

**Antioxidants:** Superoxide Dismutase (SOD; EC 1.15.1.1): Activity was measured by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin. The reaction mixture included 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 2 µM riboflavin, and enzyme extract. After illumination for 15 minutes, absorbance was recorded at 560 nm (Dhindsa *et al.*, 1982). Peroxidase (POD; EC 1.11.1.7) activity was determined by monitoring the oxidation of guaiacol in the presence of $H_2O_2$. The reaction mixture contained 50 mM phosphate buffer (pH 6.0), 20 mM guaiacol, 10 mM $H_2O_2$, and enzyme extract. The increase in absorbance at 470 nm indicated POD activity (Hori *et al.*, 1997). Catalase (CAT; EC 1.11.1.6) activity was quantified by monitoring the decomposition of $H_2O_2$. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 15 mM $H_2O_2$, and enzyme extract. The decline in absorbance at 240 nm over time was recorded (Packer, 1984). Ascorbate Peroxidase (APX; EC 1.11.1.11) activity was measured based on the oxidation of ascorbate in the presence of $H_2O_2$. The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM $H_2O_2$, and enzyme extract, and the decrease in absorbance at 290 nm was monitored over time (Nakano & Asada, 1981).

**Nutrients and lead concentration in leaves:** Leaf nitrogen (N) content was determined using a modified micro-Kjeldahl method, as described by (Steyermark & McGee, 1961). For potassium (K) analysis, the samples were digested appropriately and measured using a flame photometer. Phosphorus (P) content was quantified using the yellow colorimetric method, with absorbance readings taken at 420 nm (Olsen *et al.*, 1982). Lead (Pb) concentrations in leaf tissues were determined after acid digestion of dried samples. The digested extracts were analyzed using an atomic absorption spectrophotometer, allowing highly sensitive and precise quantification of Pb in plant tissues.

**Statistical analysis**

All experimental data were subjected to conventional statistical analysis to evaluate the effects of treatments. A two-way analysis of variance (ANOVA) was performed to determine the significance of main effects and interactions among treatments. Mean comparisons were conducted using the Tukey's honestly significant difference (HSD) test at a significance level of $p \leq 0.05$. Multivariate analyses, including convex hulls of cluster plots, hierarchical cluster analysis (HCA), and Pearson correlation matrices, were performed in OriginPro (Origin Lab Corporation, 2021) to explore relationships among physiological, biochemical, and nutrient parameters. All analyses were conducted with data expressed as mean ± standard deviation (SD) of six replicates, ensuring reliability and reproducibility.

**Results**

**Shoot length, root length, and root/shoot ratio:** The mean shoot length in the control group with 0HEC and 0NTA-BC was 43.79 cm without any stress. When 10HEC+0NTA-BC was introduced, there was a 19.18% increase in shoot length, and when 5HEC+0NTA-BC was introduced, there was a 9.59% increase with any stress contrasted to the control (0HEC+0NTA-BC). When 0.75NTA-BC was applied with 0HEC, the mean shoot length was 58.78 cm under 0Pb stress. Introducing 10HEC+0.75NTA-BC in 0Pb stress resulted in a substantial 34.24% increase in shoot length, while 5HEC +0.75NTA-BC caused a 41.40% increase more than the control (0HEC+0NTA-BC). When no treatment was applied (0HEC, 0NTA-BC) 60Pb stress, the mean shoot length was 25.87 cm. The introduction of 10HEC+0NTA-BC led to a 13.54% increase in shoot length, and 5HEC+0NTA-BC treatment showed a 5.88% increase under 60Pb stress in contrast to the control (0HEC+0NTA-BC). When 0.75NTA-BC treatment was applied with 0HEC under 60Pb stress, the mean shoot length was 32.78 cm over the control (0HEC+0NTA-BC). When 10HEC+0.75NTA-BC was introduced, there was a substantial 26.73% increase in shoot length, and 5HEC+0.75NTA-BC resulted in a remarkable 44.23% increase under Pb stress parallel to the control (0HEC+0NTA-BC) (Table 1).

In the control group, the mean root length was 35.735 cm without any treatment and stress (0HEC, 0NTA-BC, 0 Pb). In the 10HEC, 0NTA-BC, 0 Pb group, the root length was increased by 7.30%, while the 5HEC, 0NTA-BC, 0 Pb group showed a 3.54% increase in contrast to the control (0HEC+0NTA-BC). The root length exhibited a 10.40% increase in the 0HEC+0.75NTA-BC, a 19.94% increase in the 10HEC+0.75NTA-BC, and a 15.11% increase in the 5HEC+0.75NTA-BC without Pb stress in contrast to the control. When 60Pb was introduced, the group (0HEC, 0NTA-BC) had a mean root length of 22.775 cm, whereas, in the 10HEC+0NTA-BC, there was a 21.36% increase. The 5HEC+0NTA-BC showed an 11.34% increase over the control (0HEC, 0NTA-BC). The 0HEC+0.75NTA-BC treatment showed a 36.22% increase over the control (0HEC, 0NTA-BC), and the 10HEC+0.75NTA-BC, had a substantial 51.88% increase under 60Pb stress. The 5HEC+0.75NTA-BC demonstrated a 40.59% increase in root length over the control (0HEC, 0NTA-BC) under the Pb stress condition (Table 1).

The treatment with 0 HEC and 0 NTA-BC, the mean shoot/root ratio was 1.23 under 0Pb. When 10 HEC was added without NTA-BC, the mean shoot/root ratio was increased to 1.36, and with 5 HEC and 0 NTA-BC without Pb, the mean shoot/root ratio was increased to 1.30 more than the control under 0Pb stress. The introduction of 0.75 NTA-BC with 0 HEC increased a mean shoot/root ratio of 1.49, and the combination of 10 HEC and 0.75 NTA-BC without Pb the mean shoot/root ratio was increased to 1.48 from the control. In 5 HEC+0.75 NTA-BC treatment, the mean shoot/root ratio reached 1.51 without Pb stress parallel to the control. Under 60Pb stress, the control group, with 0 HEC and 0 NTA-BC, had a mean shoot/root ratio of 1.06. With the introduction of 10 HEC with 0 NTA-BC, the mean shoot/root ratio was 1.06, and with 5 HEC and 0 NTA-BC in the presence of 60 Pb, the mean shoot/root ratio slightly increased to 1.08 in contrast to the control. Adding 0.75 NTA-BC to 0 HEC showed an average shoot/root ratio of 1.08. The combination of 10 HEC+ 0.75 NTA-BC resulted in an average shoot/root ratio of 1.09 as opposed to the control, and with 5 HEC+0.75 NTA-BC the average shoot/root ratio was increased to 1.14 over the control under 60Pb stress (Table 1).

**Shoot fresh and dry weight, Root fresh and dry weight:** The mean shoot fresh weight in the control group (0HEC+0NTA-BC) was 188.30 g. The 10HEC+0NTA-BC treatment exhibited a 5.80% increase in shoot fresh weight, while the 5HEC+0NTA-BC treatment showed an 11.41% increase over the control under no 0Pb stress. In comparison to the control, 0HEC+0.75NTA-BC treatment under 0Pb showed a 17.19% increase in shoot fresh weight, and the 10HEC+0.75NTA-BC 0 treatment showed a 20.16% increase, with the 5HEC 0.75NTA-BC 0 group exhibiting a 23.58% increase in shoot weight. Compared to the control under 60Pb stress, the 10HEC+0NTA-BC resulted in a 4.15% increase, and the 5HEC+0NTA-BC led to a 6.77% increase in shoot weight. The shoot fresh weight increased by 9.79% in the 0HEC+0.75NTA-BC, and the 10HEC+0.75NTA-BC treatment exhibited a 13.26% increase over the control under 60Pb stress. The most significant increase was observed in the 5HEC+0.75NTA-BC treatment, with an 18.23% increase in shoot fresh weight under 60Pb stress (Table 2).

**Table 1. Effect of different treatments on shoot length, root length, and shoot/root of the maize plant.**

| HEC | NTA-BC | Shoot length (cm) | Root length (cm) | Shoot/Root |
|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 43.79 ± 2.58d | 35.74 ± 0.31d | 1.23 ± 0.06d |
| 10HEC | 0NTA-BC | 52.19 ± 1.57e | 38.34 ± 0.64f | 1.36 ± 0.03d |
| 5HEC | 0NTA-BC | 47.99 ± 1.13e | 37 ± 0.28e | 1.3 ± 0.02d |
| 0HEC | 0.75NTA-BC | 58.78 ± 1.29a | 39.45 ± 0.15a | 1.49 ± 0.03a |
| 10HEC | 0.75NTA-BC | 63.6 ± 0.99c | 42.86 ± 0.4c | 1.48 ± 0.01c |
| 5HEC | 0.75NTA-BC | 61.92 ± 0.62b | 41.14 ± 0.67b | 1.5 ± 0.01b |
| | | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 25.86 ± 0.48d | 22.77 ± 1.35d | 1.14 ± 0.05b |
| 10HEC | 0NTA-BC | 29.37 ± 1.59f | 27.64 ± 1.27e | 1.06 ± 0.04ab |
| 5HEC | 0NTA-BC | 27.39 ± 0.74e | 25.36 ± 0.45d | 1.08 ± 0.01ab |
| 0HEC | 0.75NTA-BC | 32.78 ± 0.83a | 31.02 ± 0.57a | 1.06 ± 0.01a |
| 10HEC | 0.75NTA-BC | 37.3 ± 1.54c | 34.59 ± 0.7c | 1.08 ± 0.04a |
| 5HEC | 0.75NTA-BC | 35.05 ± 0.72b | 32.02 ± 0.29b | 1.09 ± 0.01ab |

Values are the mean of 6 replicates ± SE. Different letters showed significant changes at p≤0.05; Tukey Test. 0Pb = 600Pb, Carboxymethyl cellulose (HEC), Nitrilotriacetic acid chelated biochar (NTA-BC). Same as below

**Table 2. Effect of different treatments on shoot fresh weight, shoot dry weight, root fresh, and dry weight of the maize plant.**

| HEC | NTA-BC | Shoot fresh weight (g) | Shoot dry weight (g) | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 188.3 ± 2.66d | 37.44 ± 0.59d | 42.95 ± 0.47d | 22.24 ± 0.82d |
| 10HEC | 0NTA-BC | 209.78 ± 3.73f | 41.96 ± 0.64f | 50.07 ± 1.18f | 26.47 ± 1.49f |
| 5HEC | 0NTA-BC | 199.22 ± 3.74 e | 39.37 ± 0.67e | 45.73 ± 1.14e | 23.66 ± 0.47e |
| 0HEC | 0.75NTA-BC | 220.67 ± 1.31a | 45.25 ± 0.8a | 52.2 ± 0.68a | 29.02 ± 0.37a |
| 10HEC | 0.75NTA-BC | 232.69 ± 1.49c | 48.35 ± 0.53c | 57.77 ± 0.81c | 31.5 ± 0.41c |
| 5HEC | 0.75NTA-BC | 226.26 ± 2.56b | 47.14 ± 0.37b | 55.77 ± 1.11b | 30.24 ± 0.44b |
| | | 600Pb | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 178.82 ± 2.9f | 22.55 ± 1.67c | 25.18 ± 1.33d | 11.2 ± 0.17d |
| 10HEC | 0NTA-BC | 166.05 ± 2.03d | 28.06 ± 0.41e | 32.56 ± 1.56f | 15.82 ± 0.65f |
| 5HEC | 0NTA-BC | 171.3 ± 1.42e | 25.94 ± 0.98d | 29.87 ± 1.24e | 13.45 ± 0.61e |
| 0HEC | 0.75NTA-BC | 161.48 ± 1.3c | 28.96 ± 0.22a | 36.02 ± 1.18a | 17.56 ± 0.4a |
| 10HEC | 0.75NTA-BC | 151.24 ± 4.24a | 35.34 ± 1.53c | 41.42 ± 1.15c | 20.3 ± 0.96c |
| 5HEC | 0.75NTA-BC | 157.52 ± 1.61b | 31.59 ± 2.08b | 38.41 ± 0.48b | 18.74 ± 0.34b |

**Table 3. Effect of different treatments on shoot length stress tolerance index (SL STI), root length stress tolerance index (RL STI), and shoot fresh weight stress tolerance index (SFW STI) of the maize plant.**

| HEC | NTA-BC | SL STI | SFW STI | SDW STI |
|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 10HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 5HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 0HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 10HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 5HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| | | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 59.18 ± 2.45b | 93.2 ± 8.52c | 94.99 ± 2.87f |
| 10HEC | 0NTA-BC | 56.25 ± 1.39ab | 78.08 ± 1.86ab | 79.18 ± 2.23d |
| 5HEC | 0NTA-BC | 57.06 ± 0.22ab | 83.29 ± 0.9b | 86.02 ± 2.3 e |
| 0HEC | 0.75NTA-BC | 55.76 ± 0.24a | 70.92 ± 1.03a | 73.18 ± 0.94 c |
| 10HEC | 0.75NTA-BC | 58.64 ± 1.61ab | 72.68 ± 2.27a | 65.01 ± 2.21a |
| 5HEC | 0.75NTA-BC | 56.6 ± 0.85ab | 72.72 ± 1.3a | 69.63 ± 1.49b |

The average shoot dry weight in the control group with 0Pb stress was 37.44 g without any treatment 0HEC+0NTA-BC. When 10HEC was applied with 0NTA-BC, the shoot dry weight was increased by 12.07%, and applying 5HEC+0NTA-BC resulted in a 5.13% increase under 0Pb stress. When 0.75NTA-BC was introduced without Pb (0HEC), there was a substantial increase of 20.86% in shoot dry weight, and the addition of 10HEC+0.75NTA-BC further boosted shoot dry weight by 29.13%, and 5HEC+0.75NTA-BC led to a 25.89% increase compared to the control treatment. Under 60Pb stress, the control (0HEC and 0NTA-BC) had a mean shoot dry weight of 22.55 g. In contrast, when 10HEC was combined with 0NTA-BC, the shoot dry weight was increased by 24.45%, and on applying 5HEC in the same conditions 15.02% increase was recorded over the control treatment under 60Pb stress. When 0.75NTA-BC+0HEC, the shoot dry weight was increased by 28.45%, and with 10HEC+0.75NTA-BC, it increased by 56.74% over the control treatment under 60Pb stress. A 40.09% increase in shoot dry weight was observed when 5HEC+0.75NTA-BC was applied under 60Pb stress in contrast to the control (Table 2).

Without any treatment (0HEC 0NTA-BC), the control group had a mean root fresh weight of 42.96 g. When 10HEC was introduced with 0NTA-BC and no Pb stress (10HEC+0NTA-BC), the root fresh weight was increased by 16.56%, and 5HEC+0NTA-BC treatment showed a 6.46% increase over the control. When 0.75NTA-BC treatment was applied in the absence of Pb and 0HEC, the root fresh weight was also increased by 21.52%, and 10HEC+0.75NTA-BC treatment showed a 34.50% increase over the control. The 5HEC+0.75NTA-BC treatment exhibited a 29.83% increase over the control under no 60Pb stress. In the 0HEC+0NTA-BC treatment, the root fresh weight was decreased substantially to 25.18 g under 60Pb stress. When 10HEC and 0.75NTA-BC were added in the presence of Pb (10HEC+0.75NTA-BC), the root fresh weight increased dramatically by 64.51%, and the 5HEC+0.75NTA-BC 60mg treatment exhibited a notable 52.58% increase over the control (Table 2).

In the absence of HEC (0HEC), NTA-BC (0NTA-BC), and Pb (0 mg) stress, the mean root dry weight was 22.24 g. Adding 10HEC to 0NTA-BC without Pb resulted in a 19.01% increase in root dry weight, and 5HEC with 0NTA-BC resulted in a 6.36% rise over the control. When 0.75NTA-BC was introduced without HEC and Pb, the root dry weight was significantly increased by 30.47%, and the addition of 10HEC to 0.75NTA-BC without Pb further increased the root dry weight by 41.61% over the control. Treatment 5HEC with 0.75NTA-BC and no Pb led to a 35.96% increase in root dry weight was recorded as opposed to the control. The introduction of 60Pb in the absence of HEC and with 0NTA-BC resulted in a substantial decrease in root dry weight, with a mean of 11.20 g. When 10HEC was added to 0NTA-BC with 60Pb stress, there was a remarkable 41.29% increase in root dry weight, and 5HEC+0NTA-BC treatment showed a 20.11% increase. The root dry weight was increased significantly in the presence of 60Pb and 0.75NTA-BC. Adding 0.75NTA-BC and 10HEC led to an impressive 81.29% increase, and 5HEC with 0.75NTA-BC led to a 67.30% increase over the control under 60Pb stress (Table 2).

**Shoot length stress tolerance index (STI), Shoot fresh weight stress tolerance index (STI), and Shoot dry weight stress tolerance index (STI):** In the absence of NTA-BC and Pb, all treatments with varying HEC concentrations showed no significant change in shoot length, resulting in a 0.00% change. When 0.75NTA-BC was introduced without Pb (60Pb), all treatments still exhibited no significant change in shoot length, with values remaining at 0.00%. When Pb at a concentration of 60Pb was added to the control group (0HEC+0NTA-BC), the shoot length STI was 59.18. The shoot length tolerance index showed a 5.22% decrease with 10HEC+0NTA-BC and 5HEC+0NTA-BC treatment, exhibiting a 3.71% decrease over the control under 60Pb stress. When 0HEC+0.75NTA-BC treatment was applied, it resulted in a 6.13% decrease, treatment 10HEC+0.75NTA-BC led to a 0.93% decrease, and 5HEC+0.75NTA-BC showed a 4.56% decrease, relative to the control group (Table 3).

In the control group, 0HEC 0NTA-BC, there was no significant change in the shoot fresh weight stress tolerance index, with a minimal 0.00% difference. This indicates that the shoot fresh weight stress tolerance index remains relatively stable in the absence of additional treatments or stressors. However, in the treatments, 10HEC 0NTA-BC and 5HEC 0NTA-BC 0Pb showed no change, remaining 0%. Under the 600Pb stress condition, when 0HEC+0NTA-BC treatment was introduced, there was a 19.37% decrease in shoot fresh weight STI related to the control group, indicating that Pb stress alone harmed plant growth. The treatments 10HEC+0.75NTA-BC and 5HEC+0.75NTA-BC demonstrated even more pronounced effects, showing a substantial 28.23% and 28.17% decrease in shoot fresh weight stress tolerance index over the control under 60Pb stress (Table 3).

Without any treatment (0HEC 0NTA-BC 0), there was no significant change in the shoot dry weight stress tolerance index (STI), indicated by a 0.00% difference. The same trend was observed when only HEC was applied (10HEC+0NTA-BC 0 and 5HEC+0NTA-BC), and the shoot dry weight stress tolerance index (STI) remained at 0% under 0Pb stress. When 60Pb stress was introduced (0HEC+0NTA-BC), there was a substantial 94.99% decrease in shoot dry weight stress tolerance index (STI) compared to the control. When 10HEC+0NTA-BC and 5HEC+0NTA-BC treatment were applied, it resulted in a 19.96% and 10.43% decrease in shoot dry weight stress tolerance index (STI) under 60Pb stress. With the addition of 0HEC+0.75NTA-BC treatment, the shoot dry weight stress tolerance index (STI) was decreased by 29.81% under 60Pb stress. The 10HEC+0.75NTA-BC and 5HEC+0.75NTA-BC 60Pb treatments exhibited significant percentage decreases of 46.13% and 36.42 in shoot dry weight stress tolerance index (STI) under 60Pb stress conditions (Table 3).

**Root length stress tolerance index (STI), Root fresh weight stress tolerance index (STI), and Root dry weight stress tolerance Index (STI):** Treatment 10HEC+0NTA-Bc and 5HEC+0NTA-BC showed 0% root length stress tolerance index (STI) under 0Pb stress. When 10HEC+0.75NTA-Bc and 5HEC+0.75NTA-BC treatment was applied, the root length stress tolerance index (STI)

remained 0% in 0Pb stress. In contrast, when 0HEC and 0.75NTA-BC were combined, the root length stress tolerance index (STI) showed a substantial increase of 63.72% under 60Pb over th control treatment. In the treatments with 10HEC and 0.75NTA-BC, and 5HEC and 0.75NTA-BC, the root length stress tolerance index (STI) was increased by 13.09% and 7.53%, respectively, over the control under 60Pb stress (Table 4).

Treatment with 0HEC, 10HEC, and 5HEC with 0NTA-BC showed 0.00% root fresh weight stress tolerance index (STI) under 0Pb stress. When 0.75NTA-BC treatment was added with 0HEC, 10HEC, and 5HEC, the root fresh weight stress tolerance index (STI) remained 0% under 0Pb stress. Under 60Pb stress, when 5HEC+0NTA-BC treatment was applied, there was a 9.46% decrease in root fresh weight stress tolerance index (STI). On the other hand, when 0.75NTA-BC was applied along with 0HEC under 60Pb stress, it showed a 6.36% decrease in root fresh weight stress tolerance index (STI).

A significant increase was measured when 10HEC was added to 0.75NTA-BC, resulting in a 21.44% decrease in root fresh weight stress tolerance index (STI) under 60Pb stress. The 5HEC+0.75NTA-BC treatment indicated an 11.32% decrease in root fresh weight (STI) evaluated to the control group under 60Pb stress (Table 4).

Without Pb stress, treatment HEC levels (0HEC, 10HEC, and 5HEC) with 0NTA-BC showed no significant change in root dry weight, with the value remaining at 0.00% and even when these treatments (0HEC, 10HEC, and 5HEC) combined with 0.75NTA-BC there was no significant change or remained 0%. However, the 0HEC+0NTA-BC treatment resulted in a significant 58.59% decrease in root dry weight (STI). When 0.75NTA-BC was added, there was a 69.00% decrease in root dry weight (STI) under 60Pb stress and the 10HEC+0.75NTA-BC treatment, resulting in a significant 71.68% drop (Table 4).

**Chlorophyll (a, b, and total) and carotenoid content:** In the control group with no HEC, NTA-BC, the chlorophyll a content remained relatively stable, with a mean value of 1.09 mg/g. When 10HEC+0NTA-BC was added, it resulted in a 3.69% increase in chlorophyll a content over the control treatment, and 5HEC+0NTA-BC treatment exhibited a 0.92% increase without Pb stress. In the presence of 0.75NTA-BC without Pb, with 0HEC and 0.75NTA-BC showed a 5.76% increase, and 10HEC + 0.75NTA-BC was introduced, there was an even more substantial 8.53% increase in chlorophyll a content over the control. The 5HEC+0.75NTA-BC treatment exhibited a 7.37% increase parallel to the control treatment without Pb stress. When 60Pb was introduced, without HEC and with 0NTA-BC, there was a 0.96% increase in chlorophyll a content, and when 10HEC+0NTA-BC was added, there was a 2.86% increase over the control. The 5HEC + 0.75NTA-BC treatment showed a 1.56% increase in chlorophyll a content under 60Pb stress. The control group with 0HEC+0.75NTA-BC displayed a 4.68% increase over the control under lead stress. The 10HEC+0.75NTA-BC treatment exhibited a substantial 10.65% increase in chlorophyll a content over the control, and the 5HEC+0.75NTA-BC treatment displayed a 7.79% increase under 60Pb stress (Table 5).

Without HEC and NTA-BC, the chlorophyll b content was measured at 0.78 mg/g. When 10HEC was introduced along with 0NTA-BC, the chlorophyll b was content increased to 4.82%, and 5HEC and 0NTA-BC treatment indicated a 2.57% increase over the control under no Pb stress. When 0.75NTA-BC was added without HEC, the chlorophyll b content was measured at 0.85 mg/g. The introduction of 10HEC and 0.75NTA-BC resulted in a significant 9.00% rise in chlorophyll b content; when 5HEC and 0.75NTA-BC were applied, the chlorophyll b content was increased to 12.86% over the control. Without HEC and NTA-BC, the chlorophyll b content was measured at 0.67 mg/g under 60Pb stress. With 10HEC+0NTA-BC treatment, the chlorophyll b content was increased to 7.89%, with 5HEC+0NTA-BC treatment resulting in a 4.51% increase in chlorophyll b content under lead stress. When 0.75NTA-BC was added without HEC, it showed a 10.90% rise in chlorophyll b content over the control under 60Pb stress. The introduction of 10HEC along with 0.75NTA-BC resulted in a 10.90% rise in chlorophyll b content; with 5HEC and 0.75NTA-BC, the content increased to a 12.41% rise over the control under 60Pb stress (Table 5).

When 10 HEC and 0 NTA-BC were applied, the total chlorophyll content was increased by 4.16%, and in the 5 HEC and 0 NTA-BC treatment, there was a 1.61% increase over the control. When 0.75 NTA-BC was introduced without Pb, the total chlorophyll content was significantly increased by 7.25% in the 0 HEC condition. Meanwhile, in the 10 HEC and 0.75 NTA-BC treatment, the total chlorophyll content was increased by 11.68% over the control, and in the 5 HEC and 0.75 NTA-BC treatment, it increased by 9.66% under no Pb stress. When 60Pb was added, the 0 HEC, 0 NTA-BC treatment experienced a 12.60% increase in total chlorophyll content. The 5 HEC+0 NTA-BC treatment with 60Pb stress showed a 2.76% increase. With the addition of 0.75 NTA-BC, the total chlorophyll content was increased by 7.07% in the 0 HEC condition, while in the 10 HEC+0.75 NTA-BC treatment, it was increased by 12.60% over the control, and in the 5 HEC+0.75 NTA-BC treatment, it was increased by 9.83% under 60Pb stress (Table 5).

When comparing the control group with 10HEC+0NTA-BC, there was a 9.35% increase in carotenoid levels and a 4.32% increase for the 5HEC+0NTA-BC treatment more than the control. The addition of 0.75NTA-BC to 0HEC (0.75NTA-BC) resulted in a substantial 19.42% increase in carotenoid levels and a significant 38.49% increase for the 10HEC+0.75NTA-BC treatment, while the 5HEC+0.75NTA-BC treatment exhibited a 31.65% increase under no Pb stress. Introducing 60Pb stress to the 0HEC treatment (0HEC+0NTA-BC) caused a significant decrease of 50% in carotenoid content. However, addition of 10HEC treatment (10HEC+0NTA-BC 60mg) led to a notable 28.99% increase, and the 5HEC treatment (5HEC+0NTA-BC) exhibited a 16.67% increase over the control under Pb stress. When 0.75NTA-BC treatments were applied with Pb stress, a significant 55.07% increase in carotenoid levels was observed for the 0HEC treatment over the control. In contrast, the 10HEC+0.75NTA-BC treatment showed a substantial 92.75% increase, and the 5HEC treatment combination with 0.75NTA-BC showed a significant 70.29% increase in carotenoid content over the control under lead stress (Table 5).

**Table 4. Effect of different treatments on root length stress tolerance index (SDW STI), root fresh weight stress tolerance index (RFW STI), and root dry weight stress tolerance index (RDW STI) of the maize plant.**

| HEC | NTA-BC | RL STI | RFW STI | RDW STI |
|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 10HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 5HEC | 0NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 0HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 10HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 5HEC | 0.75NTA-BC | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| | | 600Pb | 600Pb | 600Pb |
| | | 63.72 ± 3.27d | | |
| 0HEC | 0NTA-BC | 72.06 ± 2.17d | 60.18 ± 3.5ab | 58.59 ± 2.44c |
| 10HEC | 0NTA-BC | 68.52 ± 0.88d | 66.88 ± 0.28c | 65.04 ± 2.06c |
| 5HEC | 0NTA-BC | 78.65 ± 1.24a | 65.87 ± 1.38b | 65.3 ± 1.57c |
| 0HEC | 0.75NTA-BC | 80.72 ± 1.23c | 64.02 ± 0.64a | 69.05 ± 1.47a |
| 10HEC | 0.75NTA-BC | 77.84 ± 0.71b | 73.08 ± 2.59b | 71.68 ± 1.28b |
| 5HEC | 0.75NTA-BC | 63.72 ± 3.27d | 66.99 ± 3.93b | 68.89 ± 0.82b |

**Table 5. Effect of different treatments on Chlorophyll (a, b, and total) and Carotenoid content of the maize plant.**

| HEC | NTA-BC | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Total chlorophyll (mg/g FW) | Carotenoids (mg/g FW) |
|---|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 1.08 ± 0.01d | 0.78 ± 0.01d | 1.86 ± 0.01d | 0.7 ± 0.01d |
| 10HEC | 0NTA-BC | 1.12 ± 0.03f | 0.81 ± 0.03f | 1.94 ± 0.02f | 0.76 ± 0.01f |
| 5HEC | 0NTA-BC | 1.11 ± 0.01e | 0.80 ± 0.01e | 1.89 ± 0.01e | 0.72 ± 0.02e |
| 0HEC | 0.75NTA-BC | 1.15 ± 0.02a | 0.85 ± 0.01a | 2.01 ± 0.01a | 0.83 ± 0.03a |
| 10HEC | 0.75NTA-BC | 1.18 ± 0.03c | 0.90± 0.02c | 2.08 ± 0.02c | 0.96 ± 0.02c |
| 5HEC | 0.75NTA-BC | 1.17 ± 0.02b | 0.88 ± 0.01b | 2.04 ± 0.02b | 0.92 ± 0.02b |
| | | 600Pb | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 0.96 ± 0.01c | 0.66 ± 0.01d | 1.63 ± 0.02d | 0.34 ± 0.02d |
| 10HEC | 0NTA-BC | 0.99 ± 0.02e | 0.72 ± 0.02f | 1.71 ± 0.05f | 0.44 ± 0.01f |
| 5HEC | 0NTA-BC | 0.98 ± 0.03d | 0.71 ± 0.01e | 1.67 ± 0.01e | 0.41 ± 0.04e |
| 0HEC | 0.75NTA-BC | 1.01 ± 0.01a | 0.74 ± 0.03a | 1.74 ± 0.02a | 0.54 ± 0.01a |
| 10HEC | 0.75NTA-BC | 1.06 ± 0.04b | 0.76 ± 0.01c | 1.83 ± 0.04c | 0.66 ± 0.02c |
| 5HEC | 0.75NTA-BC | 1.04 ± 0.02b | 0.75 ± 0.04b | 1.79 ± 0.02b | 0.59 ± 0.03b |

**Table 6. Effect of different treatments on gas excheange attributes (E – Transpiration rate, gs – Stomatal conductance, Pn – Net photosynthetic rate) of the maize plant.**

| HEC | NTA-BC | E (m mol $H_2O$ $m^{-2}$ $s^{-1}$) | gs (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | Pn (µmol $CO_2$ $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 4.05 ± 0.08a | 241.85 ± 2.47a | 24.44 ± 0.55a |
| 10HEC | 0NTA-BC | 3.56 ± 0.07d | 210.61 ± 6.64d | 20.62 ± 0.66d |
| 5HEC | 0NTA-BC | 4.38 ± 0.06c | 268.78 ± 2.42c | 26.77 ± 1.11c |
| 0HEC | 0.75NTA-BC | 3.89 ± 0.04f | 228.46 ± 1.67f | 22.84 ± 0.65f |
| 10HEC | 0.75NTA-BC | 4.23 ± 0.05b | 250.19 ± 3.39b | 25.64 ± 0.48b |
| 5HEC | 0.75NTA-BC | 3.73 ± 0.06e | 223.28 ± 3.15e | 21.95 ± 0.34e |
| | | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 2.89 ± 0.03a | 181.12 ± 3.15a | 16.78 ± 0.37a |
| 10HEC | 0NTA-BC | 2.22 ± 0.07c | 123.71 ± 2.65cd | 17.09 ± 7.16a |
| 5HEC | 0NTA-BC | 3.36 ± 0.02c | 201.80 ± 1.05c | 19.55 ± 0.34a |
| 0HEC | 0.75NTA-BC | 2.80± 0.02e | 168.68 ± 2.85e | 15.69 ± 0.58a |
| 10HEC | 0.75NTA-BC | 3.12 ± 0.17b | 191.63 ± 3.45b | 18.36 ± 0.73a |
| 5HEC | 0.75NTA-BC | 2.48 ± 0.16d | 149.22 ± 15.5de | 14.73 ± 0.18a |

**Table 7. Effect of different treatments on N, P, K, and uptake of Pb in the leaves of the maize plant.**

| HEC | NTA-BC | Leaves N (%) | Leaves P (%) | Leaves K (%) | Leaves Pb (µg/g) |
|---|---|---|---|---|---|
| | | 0Pb | 0Pb | 0Pb | 0Pb |
| 0HEC | 0NTA-BC | 2.92 ± 0.04a | 0.32 ± 0.02a | 2.77 ± 0.04a | 1.58 ± 0.21c |
| 10HEC | 0NTA-BC | 2.28 ± 0.07d | 0.26 ± 0.02c | 2.27 ± 0.06d | 3.21 ± 0.26f |
| 5HEC | 0NTA-BC | 3.42 ± 0.11c | 0.38 ± 0.01b | 2.93 ± 0.01c | 0.88 ± 0.18a |
| 0HEC | 0.75NTA-BC | 2.66 ± 0.15f | 0.29 ± 0.03d | 2.54 ± 0.09e | 2.03 ± 0.16d |
| 10HEC | 0.75NTA-BC | 3.23 ± 0.03b | 0.36 ± 0.01ab | 2.89 ± 0.01b | 1.24 ± 0.08b |
| 5HEC | 0.75NTA-BC | 2.44 ± 0.07e | 0.28 ± 0.02d | 2.43 ± 0.06e | 2.59 ± 0.27e |
| | | 600Pb | 600Pb | 600Pb | 600Pb |
| 0HEC | 0NTA-BC | 1.68 ± 0.09a | 0.22 ± 0.03a | 1.92 ± 0.07a | 5.25 ± 0.03bc |
| 10HEC | 0NTA-BC | 1.04 ± 0.04d | 0.12 ± 0.01d | 1.59 ± 0.06d | 6.40 ± 0.07e |
| 5HEC | 0NTA-BC | 2.08 ± 0.08c | 0.24 ± 0.02c | 2.12 ± 0.03c | 4.11 ± 0.31a |
| 0HEC | 0.75NTA-BC | 1.50 ± 0.05f | 0.20 ± 0.01f | 1.79 ± 0.05f | 5.52 ± 0.23c |
| 10HEC | 0.75NTA-BC | 1.87 ± 0.05b | 0.23 ± 0.03b | 2.01 ± 0.04b | 5.01 ± 0.18b |
| 5HEC | 0.75NTA-BC | 1.23 ± 0.1e | 0.16 ± 0.01e | 1.70 ± 0.03e | 6.00 ± 0.23d |

**E, gs, and Pn Levels:** In the treatment with 0HEC, 0NTA-BC, and no Pb (lead), the E value was 3.56 m mol $H_2O$ $m^{-2}$ $s^{-1}$. When 10HEC+0NTA-BC was introduced, there was a 9.35% increase in E content, and in the 5HEC+0NTA-BC, there was a 4.85% increase over the control under no Pb stress. When 0HEC+0.75NTA-BC was added, it represented a 13.91% increase in E levels over the control. For the 10HEC+0.75NTA-BC, there was a substantial 23.19% increase in E level, and the 5HEC+0.75NTA-BC treatment showed a 19.04% increase over the control under no Pb stress. In the 0HEC+0NTA-BC, the control had a mean E value of 2.22 m mol $H_2O$ $m^{-2}$ $s^{-1}$. When 10HEC was introduced to this treatment, there was a 26.13% increase in E levels; in the case of 5HEC+0NTA-BC, there was an 11.71% increase over the control under 60Pb stress. The introduction of 0.75NTA-BC+0HEC treatment resulted in a 30.07% increase in E levels over the control under 60Pb stress. In the 10HEC+0.75NTA-BC, there was a significant 51.35% increase in E levels, and in the 5HEC+0.75NTA-BC, there was a substantial 40.54% increase under 60Pb stress (Table 6).

The mean gs level in the control group with 0HEC and 0NTA-BC was 210.60 mmol $H_2O$ $m^{-2}$ $s^{-1}$. When 10HEC was introduced alongside 0NTA-BC, there was an 8.48% increase in gs level over the control, and the 5HEC treatment with 0NTA-BC showed a 6.02% increase under no Pb stress. When 0.75NTA-BC was added to 0HEC, a significant 14.84% increase in gs level was observed, and a combination of 10HEC+0.75NTA-BC resulted in a remarkable 27.63% increase over the control without Pb stress. Similarly, in contrast to the control, the addition of 5HEC 0.75NTA-BC treatment resulted in an 18.80% increase in gs level under no Pb stress. When 600Pb stress was introduced into the 0HEC+0NTA-BC treatment, there was a significant 41.03% increase in gs level, and applying 10HEC with 0NTA-BC under 60Pb stress led to a substantial 36.36% increase more than the control. Similarly, the 5HEC+0NTA-BC treatment demonstrated a noticeable 20.62% increase in gs level in 60Pb stress. When 0.75NTA-BC was combined with 0HEC under 60Pb stress, there was a significant 46.39% increase in gs levels, and 10HEC+0.75NTA-BC treatment exhibited a substantial 63.13% increase parallel to the control under 60Pb stress. Over the control treatment, with the addition of 5HEC+0.75NTA-BC treatment, there was a 54.91% increase in gs levels under 60Pb stress (Table 6).

In the absence of heavy metals (Pb) and without the addition of NTA-BC (0HEC and 0NTA-BC), the mean photosynthetic rate was 20.62 µmol $CO_2$ $m^{-2}$ $s^{-1}$. When 10HEC+0NTA-BC treatment was applied, there was a 10.78% increase in photosynthetic rate (Pn) evaluated to the control, and the 5HEC+0NTA-BC treatment showed a 6.44% increase under no Pb stress. In the presence of 0.75NTA-BC without Pb stress (0HEC 0.75NTA-BC), the Pn was increased by 18.52% over the control. The 10HEC+0.75NTA-BC treatment resulted in a significant 29.82% increase, while the 5HEC 0.75NTA-BC treatment exhibited a 24.33% increase in Pn over the control under no Pb stress. When heavy metals (60Pb) were introduced with NTA-BC (0HEC 0NTA-BC), the photosynthetic rate was 14.73 µmol CO2 m-2 s-1. The 10HEC+0NTA-BC treatment showed a 6.52% increase in Pn paralleled to the control, while the 5HEC+0NTA-BC treatment resulted in a 13.94% increase under 60Pb stress. With 0HEC+0.75NTA-BC, there was a substantial 16.06% increase in Pn over the control under 60Pb stress. The highest increase was observed in the 10HEC+0.75NTA-BC treatment, with a 24.65% boost in Pn, and the 5HEC+0.75NTA-BC treatment exhibited a remarkable 32.74% increase in Pn in contrast to the control under 60Pb stress (Table 6).

**POD, SOD, CAT, and APX activity:** In the baseline treatment, when no HEC or NTA-BC treatment was added, the POD activity was recorded at 33.86 EU/mg protein. When 10HEC was introduced with no NTA-BC and no Pb, there was a decrease of 3.87% in POD activity, and the treatment with 5HEC with no NTA-BC produced a 7.62% decrease over the control. With 0.75NTA-BC and 0HEC, the POD activity was dropped by 13.78%, and introducing 10 HEC alongside 0.75NTA-BC led to a 20.50% reduction in POD activity compared to the control. The most significant decrease was observed when 5HEC and 0.75NTA-BC were combined, resulting in a 31.20% decrease in POD activity under no stress. Introducing lead (60Pb) into the baseline treatment of 0HEC and 0NTA-BC led to a mean POD activity of 53.84 EU/mg protein. When 10HEC was added with 0NTA-BC, it led to a 54.88% decrease over the control, and treatment with 5 HEC+0NTA-BC resulted in a 48.85% decrease in POD

activity under 60Pb stress. When 0.75NTA-BC was introduced alongside 0HEC, it led to a 16.96% decrease in POD activity. Increasing HEC to 10% in this treatment resulted in a 26.24% decrease, and the treatment with 5HEC and 0.75 NTA-BC, along with 60Pb stress, caused a 38.26% decrease in POD activity (Fig. 1).

In the absence of HEC and NTA-BC, mean SOD activity was measured by 18.61 EU/mg protein. When 10HEC was added with 0NTA-BC, there was a 15.24% decrease in sod activity above the control, and 5HEC in combination with 0NTA-BC led to a 5.47% decrease. When 0.75NTA-BC was introduced without HEC, a 20.36% reduction in SOD activity was observed, and with 10HEC and 0.75NTA-BC, SOD activity was further decreased by 56.71% over the control under no stress. In comparison to the control, 5HEC and 0.75NTA-BC combination also showed a significant drop, with a 28.82% decrease. In 60Pb stress without HEC and 0NTA-BC, the SOD activity was decreased significantly to 29.02 EU/mg protein. Combining 10HEC and 0NTA-BC resulted in a 7.28% decrease, and 5HEC combined with 0NTA-BC displayed a 3.61% reduction in SOD activity over the control under 60Pb stress. In the case of 0HEC+0.75NTA-BC, SOD activity was 15.52% lower when compared to the control treatment. Adding 10HEC to 0.75NTA-BC resulted in a substantial 58.81% decrease parallel to the control, and 5HEC with 0.75NTA-BC of lead exhibited a 28.05% decrease in POD activity under the 60Pb stress (Fig. 1).

Without HEC and NTA-BC, the mean CAT activity was measured at 42.97 EU/mg protein, while with the addition of 10HEC and 0NTA-BC, there was an 11.92% decrease without any stress. Introducing 5HEC and 0NTA-BC resulted in a 6.36% decrease related to the control under no stress. Over the control, when 0.75NTA-BC was added without HEC, CAT activity was decreased by 19.54%. When 10HEC+0.75NTA-BC were applied, CAT activity was significantly reduced by 29.55% over the control under no Pb stress, and with 5HEC and 0.75NTA-BC, it decreased by 25.12%. Under 60Pb, stress without HEC and NTA-BC led to a notable decrease of 36.67%. When 10HEC was combined with 0NTA-BC, there was an 8.54% decrease, and the addition of 5HEC with 0NTA-BC resulted in a 4.79% decrease over the control under 60Pb stress. The combination of 0.75NTA-BC and 0HEC caused a decline of 16.43% in CAT activity, and the addition of 10HEC with 0.75NTA-BC led to a substantial decrease of 39.54%, while combining 5HEC with 0.75NTA-BC resulted in a 23.72% decrease more than the control under 60Pb stressed condition (Fig. 2).

In the case of APX activity, treatment 10HEC 0NTA-BC showed a 20.31% reduction evaluated to the control, and the treatment 5HEC+0NTA-BC showed a 7.45% reduction. When 0HEC 0.75NTA-BC 0 treatment was applied, there was substantial decrease in APX activity by 37.66% compared to the control and 10HEC+0.75NTA-BC treatment, resulting in an 83.65% drop. Adding 5HEC+0.75NTA-BC treatment showed a 72.07% decrease in APX activity related to the control under no Pb stress. In the Pb stress condition, without HEC and NTA-BC treatment, it showed an increase of 2.89 EU/mg protein, while the 10HEC+0NTA-BC treatment exhibited a decrease of 10.73%. In contrast, the 5HEC+0NTA-BC treatment showed a minor decline of 6.64% evaluated to the control under 60Pb stress. Adding 0HEC 0.75NTA-BC treatment in 60Pb stress led to a 17.60% decrease in APX activity, while the 10HEC+0.75NTA-BC treatment displayed a substantial reduction of 67.29% over the control under 60Pb stress. The 5HEC 0.75NTA-BC treatment also demonstrated a 30.62% reduction in APX activity evaluated to the control in 60Pb stress (Fig. 2).

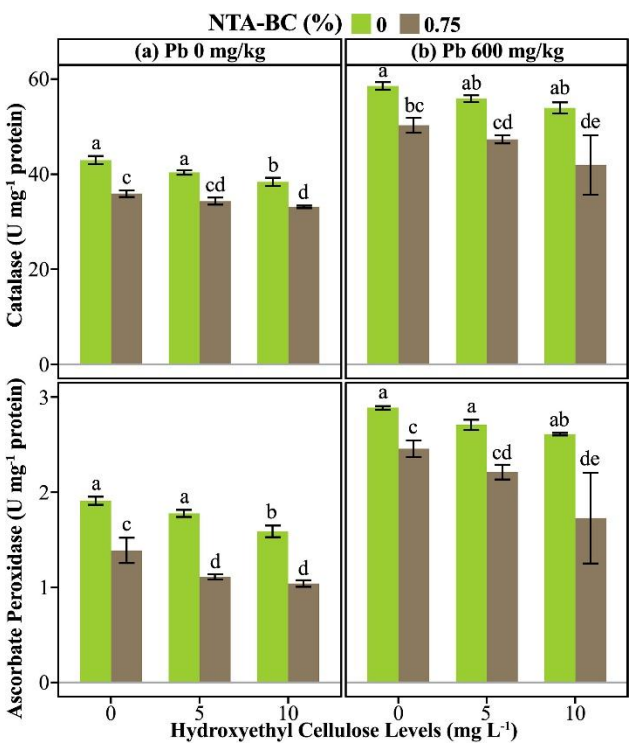


Fig. 1. Effect of different treatments on peroxidase and catalase in the maize plant.

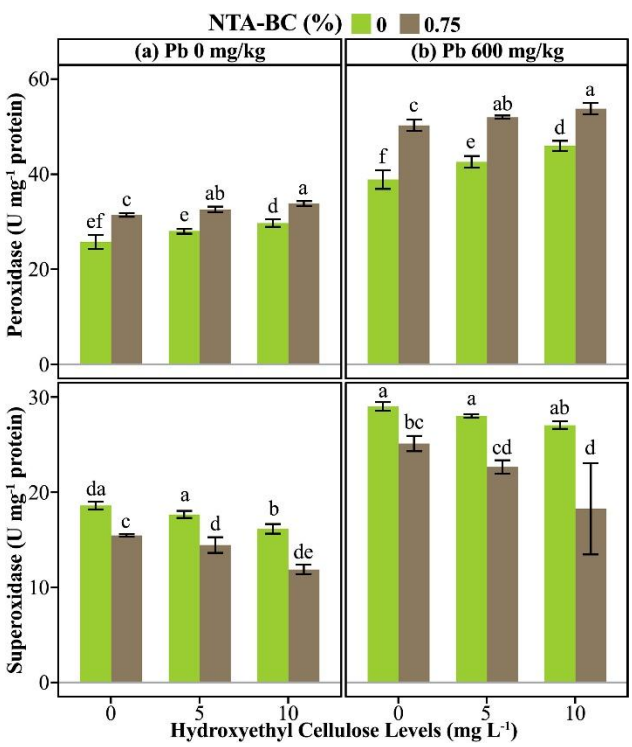


Fig. 2. Effect of different treatments on peroxidase and superoxidase in the maize plant.

**Leave N, P, and K and leave Pb uptake:** When no HEC and NTA-BC treatment was added, the leave N was 2.28%. However, when 10HEC was introduced to 0NTA-BC, the leave N was increased to 2.66%, and in the case of 5HEC+0NTA-BC, leave N showed a 2.44% rise over the control under no stress. Under Pb stress, without HEC and 0NTA-BC, the leave N was measured at 1.04%. When 10HEC+0NTA-BC was added, the leave N rose to 43.75%, related to the control treatment. With the addition of 5HEC+0NTA-BC treatment, an 18.75% increase was evaluated to the control under Pb stress. In treatments with 0.75NTA-BC, the leave N increased to 28.10%, related to the control without HEC. When 10HEC was introduced, it showed a significant 49.07% increase in leave N over the control under no Pb stress. Adding 5HEC+0.75NTA-BC treatment indicated a 41.82% increase in leave N over the control without 60Pb stress. Treatment 0HEC+0.75NTA-BC showed a substantial 61.78% increase compared to the control under 60Pb stress. When 10HEC was added with 0.75NTA-BC, the leave N was further increased to 99.76%, related to the control in 60Pb stress. In contrast to the control, treatment 5HEC=0.75NTA-BC showed a remarkable 80.05% increase related to the 60Pb-stressed control (Table 7).

In the case of leave phosphorus, treatment 10HEC+0NTA-BC was introduced under no Pb stress, resulting in an 11.54% rise, and 5HEC+0NTA-BC treatment, indicating a 6.73% increase evaluated to the control treatment. Adding 0HEC+0.75NTA-BC treatment represents a 24.04% increase related to the control under no stress. The 10HEC+0.75NTA-BC treatment signifies a remarkable 45.19% increase in leave P content over the control. The addition of 5HEC+0.75NTA-BC treatment showed a substantial 38.46% increase compared to the control under no Pb stress. Under Pb stress, the control group (0HEC+0NTA-BC) had a mean leaves P content of 0.13%. The introduction of 10HEC with 0NTA-BC under 60Pb stress indicated a substantial 56.00% rise in leaf phosphorus compared to the control under 60Pb stress. The 5HEC+0NTA-BC treatment in 60Pb stress showed a 28.00% increase evaluated to the control. The 0HEC+0.75NTA-BC treatment demonstrated a substantial 72.00% increase more than the control and treatment 10HEC+0.75NTA-BC, indicating an impressive 96.00% rise in leave P under 60Pb stress. Adding 5HEC, 0.75NTA-BC treatment, resulted in a remarkable 86.00% increase under 60Pb stress (Table 7).

Without any treatment (0HEC and 0NTA-BC), the mean leaf potassium content was 2.27%. Compared to the control, 10HEC was applied without NTA-BC, and there was an 11.99% increase; with 5HEC without NTA-BC, there was a 6.93% increase in leaf potassium content under no stress. When 0.75NTA-BC was used without any other treatment (0HEC+0.75NTA-BC), there was a substantial 21.78% increase in leaf potassium content with no Pb stress. Adding 10HEC to 0.75NTA-BC resulted in a 28.82% increase; when 5HEC was added to 0.75NTA-BC, it resulted in a 27.06% increase in leaf potassium over the control. With the introduction of 0HEC and 0NTA-BC, the mean leaf potassium content was 1.59% under 60Pb stress. When 10HEC+0NTA-BC was added, there was a 12.40% increase in leaf potassium content; 5HEC+0NTA-BC treatment showed a 6.59% increase under 60Pb stress over the control. In the 0HEC+0.75NTA-BC treatment, the leaf potassium content was increased by 1.92%, parallel to the control under 60Pb stress. Adding 10HEC+0.75NTA-BC treatment resulted in an impressive 32.97% increase, and with 5HEC+0.75NTA-BC treatment, there was a 26.37% increase in potassium content under 60Pb stress over the control (Table 7).

When no HEC or NTA-BC was applied, the mean leave Pb uptake was 3.21 µg/g under no stress. When 10HEC and 0NTA-BC were introduced, the leave Pb uptake was decreased to 58.45%, and with 5 HEC and 0NTA-BC treatment, the leave Pb uptake showed a 23.92% decrease over the control under no stress. The treatment with 0HEC and 0.75NTA-BC, showed a remarkable 103.65% increase contrasted to the control under no Pb stress. In comparison to the control, when 10HEC and 0.75NTA-BC were applied, the leave Pb concentration was dropped 265.06%, and in the 5HEC and 0.75NTA-BC treatment, the Pb concentration was decreased 158.55% in No Pb stress. Under 60pb stress, in the control group without (0HEC and 0NTA-BC) treatment, the leave Pb uptake was recorded at 6.40 µg/g. With 10HEC and 0NTA-BC, the Pb concentration decreased to 15.93%, and in the 5HEC and 0NTA-BC treatment, the Pb concentration was decreased to a 6.66% under 60Pb stress. For the treatment with 0HEC and 0.75NTA-BC, the Pb concentration was 5.25 µg/g, showing a 21.95% reduction. With 10HEC and 0.75NTA-BC, the Pb concentration was decreased to 55.97%, and the 5HEC and 0.75NTA-BC treatment showed a 27.73% reduction under 60Pb stress (Table 7).

## Discussion

**Mechanisms of Pb immobilization and reduced uptake:** A central mechanism by which NTA-BC mitigates Pb toxicity is through the enhanced chelation of lead ions in the rhizosphere, thereby improving their mobilization and subsequent uptake by plants while simultaneously ameliorating soil conditions (Sanjana *et al.*, 2024). Beyond chelation, NTA's influence extends to modifying the rhizosphere's biochemical properties, including a decrease in pH and an increase in dissolved organic carbon, which collectively enhance lead accumulation in plants (Chojnacka *et al.*, 2023). Furthermore, biochar, particularly iron-modified biochar, contributes to the immobilization and reduced bioavailability of heavy metals such as lead and cadmium in agricultural soils, thus protecting plants from their toxic effects (Algethami *et al.*, 2023).

**Effects on plant growth and photosynthetic pigments:** The enhancement in shoot and root growth under normal conditions following HEC application aligns with its known role as a plant growth stimulant. HEC promotes metabolic processes such as photosynthesis, nutrient uptake, and hormonal signaling, while NTA-BC further enhances growth by chelating essential nutrients and mitigating Pb toxicity (Hong & Fletcher, 2023, Xu & Wu, 2023). Under Pb stress, root elongation may also increase as an adaptive strategy to maximize nutrient and water acquisition, while the shoot/root ratio is modulated to maintain growth under adverse conditions (Francis *et al.*,

2023, Pinto *et al.*, 2023). Significant increases in fresh and dry biomass with HEC and NTA-BC treatments are attributed to a combination of growth stimulation and Pb immobilization. HEC promotes overall growth, while NTA-BC limits Pb uptake, thereby reducing its toxic effects on plant metabolism. This dual action contributed to enhanced stress tolerance, reflected in higher stress tolerance index (STI) values among treated plants (Gavrilescu, 2022). Chlorophyll and carotenoid contents were positively influenced by HEC and NTA-BC treatments. The increase in chlorophyll can be linked to improved nutrient availability, particularly nitrogen, facilitated by NTA-BC chelation and HEC-mediated nutrient uptake (Mashhadikhan *et al.*, 2022).

**Antioxidants:** Higher carotenoid levels suggest strengthened antioxidative defense, protecting the photosynthetic apparatus from oxidative damage caused by residual Pb stress (Ghasemi *et al.*, 2023, Shahzad *et al.*, 2023). This enhanced defense mechanism is crucial in environments contaminated with heavy metals, as it prevents the propagation of reactive oxygen species that can otherwise damage cellular components (Zulqurnain *et al.*, 2019). Enhancements in transpiration, stomatal conductance, and photosynthetic rate indicate that HEC and NTA-BC improve water and nutrient use efficiency. Pb immobilization by NTA-BC reduces toxic interference in stomatal function and nutrient transport, allowing normal gas exchange and sustaining photosynthetic activity even under Pb stress. The activities of antioxidant enzymes (POD, SOD, CAT, and APX) reflect modulation of the plant's oxidative stress response. Under non-stress conditions, lower enzyme activity indicates reduced ROS accumulation due to decreased Pb bioavailability. Under Pb stress, HEC and NTA-BC likely prime the plant's antioxidant system, enabling efficient ROS scavenging and maintaining cellular homeostasis (Khan *et al.*, 2022). Similarly, green synthesized silver nanoparticles have been shown to alleviate Pb toxicity in maize and wheat by regulating antioxidant activity and improving nutrient concentration (Danish *et al*., 2024).

## Conclusion

The research focuses on the beneficial effects of a combination of Hydroxyethyl cellulose (HEC) and nitrilotriacetic acid chelated biochar (NTA-BC) on maize growth under lead (Pb) stress. The most significant result was the substantial improvement in various growth parameters with the 10HEC+0.75NTA-BC treatment, including shoot length, shoot and root weights, root length, chlorophyll content, and leaf nutrient concentrations. This treatment can potentially mitigate the adverse effects of Pb stress in plants, with potential implications for enhancing crop yields and food safety in areas affected by heavy metal pollution. Future research directions include exploring the long-term effects of the treatment on maize and other crops, its impact on soil health and nutrient retention, and mechanisms underlying the synergistic effects of HEC and NTA-BC. Researchers should also investigate the feasibility of scaling up this approach for real-world agricultural use through field trials and economic assessments.

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