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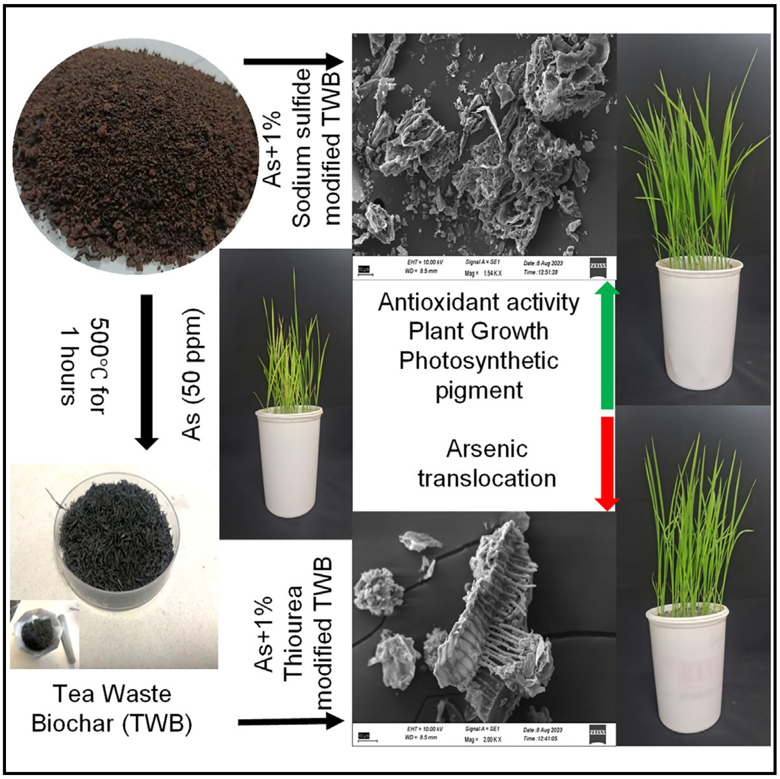
Sulfur-modified tea-waste biochar improves rice growth in arsenic contaminated soil and reduces arsenic accumulation

Graphical abstract



Highlights

- Sulfur modified tea waste biochar in soil boosts photosynthesis and overall plant health
- The modified biochar reduces oxidative stress in rice plants caused by arsenic toxicity
- The modified biochar amendments reduced arsenic content in rice plant shoots by 19–30%
- In roots, these amendments also decreased arsenic content by 10–30%

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In brief

Soil science; Soil chemistry; Environmental science; Environmental chemistry

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Sulfur-modified tea-waste biochar improves rice growth in arsenic contaminated soil and reduces arsenic accumulation

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SUMMARY

Arsenic (As) is a non-essential carcinogenic metalloid and an issue of concern for rice crops. This study investigated the effects of sulfur-loaded tea waste biochar (TWB) due to modification with sodium sulfide (SSTWB) or thiourea (TUTWB) on As stress and accumulation in rice plants. The results showed that sulfur-modified TWB improved plant morphology compared to plants grown in As-contaminated soil alone. Biochar amendments elevated the activity of antioxidant enzymes in rice plants harvested at 15 and 30 days after transplant (DAT). Additionally, SSTWB and TUTWB significantly reduced As content in shoots by 26% and 19% at 15 DAT, respectively, as compared to TWB. This trend continued at 30 DAT with SSTWB achieving the maximum decrease of 30%. Similar reductions were observed in plant roots. The study suggests that sulfur-modified biochar amendments offer a promising strategy to mitigate the negative effects of As on, and reduce its accumulation in, rice.

INTRODUCTION

Arsenic (As) is a non-essential, toxic, and carcinogenic metalloid.[1] Arsenic contamination in soils and its buildup in crops have drawn significant public attention worldwide.[2] Arsenic contamination in paddy fields has been reported globally, posing a concern to the people using rice as the staple food.[3] This is due to the fact that rice accumulates As much more efficiently than other cereal crops owing to its cultivation conditions and genetic and physiological features.[3,4] In areas where As contamination and rice cultivation coexist, the main source of As exposure in humans becomes the consumption of rice.[5,6]

In plants, one of the most prominent ways in which As can be potentially dangerous is by causing oxidative stress and disrupting the redox state. The oxidative stress conditions can harm proteins, lipids, membranes, and ultimately cause cell death.[7] Arsenic can also interact directly with proteins, especially in the reduced As(III) form, and disrupt their functions.[8] The negative impacts of As on rice have been reported to result in reduced yields also.[9] Therefore, it is necessary to reduce the bioavailability of As in paddy fields to reduce its impacts on plants and to also avoid subsequent toxicity in humans. Although *ex situ* cleanup technologies can be used to remove As from soil, it is impractical in view of the huge extent of the problem and also due to the necessity of agriculture for the farmers involved in this profession. Thus, there is a need for environmentally friendly and economically viable *in situ* methods to reduce the detrimental effects of As on rice productivity and quality.[5] There are several *in-situ* methods that have been tested to address the As problem in agricultural soil and its accumulation in rice. These techniques include microbial supplementation, irrigation management, chemical treatment, and biochar amendment. Microbial supplementation has been found to reduce As in rice in studies.[10] The addition of inorganic or organic chemicals like gypsum,[11] thiourea[9] and elements such as silica[12] have proven successful. Simple agronomic management practices like intermittent irrigation[13] and soil inversion[14] provide easy solutions. However, apart from the As issue, the agricultural fields face the problem of unhealthy soil. To this end, biochar addition can be a long-lasting sustainable method to improve soil health and tackle the As issue simultaneously. Biochar is a charred carbonaceous material obtained by pyrolyzing plant biomass or agricultural waste like rice straw, grass, wood, or manure at low oxygen levels. Because of its remarkable capacity to increase soil fertility while reducing greenhouse gas emissions from agricultural lands, including nitrous oxide (N_2O) and methane (CH_4), biochar application in agricultural fields has attracted a lot of attention in recent years. The use of biochar prepared from waste organic biomass also helps in reducing the waste. Biochar has been found to improve the chemical and physical characteristics of soil, enhancing nutrient retention via cationic adsorption or pH increase of acidic soils.[15,16] Furthermore, biochar remains stable in soil for extended periods, providing a prolonged remediation effect. This long-term stability reduces the need for frequent reapplication compared to other methods, ensuring consistent soil improvement over time. Moreover, heavy metals were found to become immobile, and their bioavailability in soil was decreased by biochar.[17] Khan et al.[16] observed significant decreases in As accumulation in rice straw, leaves, and grains (by 30–34%, 24–28%, and 70–74%, respectively) in a pot experiment. These decreases were caused by a drop in soil bioavailable As (by 30–38%) upon biochar application.

In order to attain further improvement, apart from raw biochar, biochar modified with various chemicals has been used in recent studies.[18] These modifications cover a variety of techniques, such as adding reactive materials and changing the structure of biochar both chemically and physically. With the changes in variables like surface area, surface charge, and surface functional groups, modified biochar has been found to exhibit an improved capacity to seize accessible nutrients from the soil and alter their bioavailability.[19] This procedure helps limit the overuse of fertilizer and stops nutrients from being lost through leaching.[20] Additionally, biochar made from plant materials like leaves or stems directly adds nutrients like potassium (K), calcium (Ca), magnesium (Mg), etc. to the soil, improving its fertility.[21,22]

In the plant system, sulfur is involved in several important processes. After being absorbed, sulfur is assimilated into cysteine and methionine.[23] Cysteine is found in the active sites of numerous enzymes and is essential for the formation of sulfhydryl groups and disulfide bonds in proteins.[24] Moreover, sulfur containing compounds are essential for plants to protect themselves against stress, including As.[25] Sulfur supply has been found to affect As accumulation and translocation in paddy soil and rice plants.[26] Furthermore, sulfur might have an impact on the chemical and physical characteristics of soil.[27,28] However, only a few reports have analyzed the overall behavior of sulfur and As in a rice-soil system. Considering the importance of sulfur in As stress tolerance and accumulation, the present study was planned to modify biochar with sulfur and evaluate its effects on rice. As the properties of raw and modified biochar differ greatly, the research work was conducted to precisely understand the effects of sulfur-modified tea waste biochar on rice.

In this study, the effect of sulfur-impregnated biochar on As mobility and transfer from soil to the rice system was investigated. The hypothesis was that biochar would enhance the nutrient bioavailability to plants including that of sulfur and help plants in better growth and lesser As accumulation when grown in As-contaminated soil.

RESULT

Rice plant response to varying biochar doses

Arsenic contamination hampered rice plant growth at the early stages. Biochar amendments may mitigate some of these negative effects, but their influence on plant morphology depends on the application rate. At 7 DAG, As+1B exhibited the highest root length, followed by the control, As+0.5B, As+2B and As (Figures S1B and S2A). However, at 14 DAG, the control group had the longest roots, followed by 0.5B and As (which had similar root lengths) (Figures S1A, S1C, and S2B). Plant sample grown in soil amended with 2% biochar showed the shortest root length. Similarly At 21 DAG, the control group again had the longest roots, followed by As+2B, As+1B, and then As+0.5 B with As induced plant showing the shortest root length. The control group consistently had the greatest shoot and total length across all time points (7 DAG, 14 DAG, and 21 DAG) (Figure S1D and S2C).

The preliminary study also investigated the impact of As contamination and biochar amendments (0.5%, 1%, and 2%) on pigment content (chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and carotenoids) in rice plants harvested at 7 and 14 DAG. Plants grown in As-amended soil exhibited significantly lower pigment content (Chla, Chl b, and carotenoids) compared to the control group. Biochar amendments effectively increased chlorophyll content in rice plants exposed to arsenic stress. Control plants displayed a 38% increase in Chl a compared to As-amended plants harvested at 7 DAG. Biochar treatments further enhanced chl a content compared to As-amended plants by 33.87, 54.84 and 29.03% in As+0.5B, As+1B and As+2B setup respectively. Similar to chl a, control plants have substantially higher (64.29%) chl b compared to the As treatment. Biochar amendments significantly increased chl b content compared to As-amended plants by 125%, 65% and 55% respectively in As+0.5B, As+1B and As+2B. The control group also had higher (47.06%) carotenoid content compared to As-amended plants. Biochar amendments again led to increased carotenoid content compared to the As treatment. Maximum increase was found in sample harvested at 7 DAG from As+1B by 59.26% followed by As+0.5B (40.74%) and As+2B (29.63%) (Figure S3A). The analysis of Chl a, Chl b, and carotenoid concentrations across different treatments revealed notable variations in pigment levels compared to the control group plant harvested at 14 DAG. At 14 DAG, comparison of the different biochar doses (0.5B, 1B, and 2B) with the As treatment, demonstrated variable effects in Chl a, Chl b, and carotenoid levels. The treatment with 0.5B biochar showed minimal changes, with Chl a level increasing slightly by approximately 9.09%, Chl b levels by 156.52%, and carotenoid levels by 15.25% (Figure S3B). Similarly, other biochar treatments also induced an increase in pigment levels. These findings underscore the varied impacts of biochar application at different rates on the levels of Chl a, Chl b, and carotenoids in rice plant shoots exposed to As toxicity, highlighting the potential of biochar as a mitigating agent.

Additionally, MDA concentration was measured in both roots and shoots at 7 and 14 DAG. The results revealed a significant increase in MDA in both roots and shoots of rice plants grown in As-amended soil compared to the control group at both 7 and 14 DAG. These increases were substantial, reaching 11% and 34% for shoot and root respectively, at 7 DAG, and further rising to 36.31% and 56.13% for shoot and root, respectively, at 14 DAG, compared to the control. The study observed a dose-dependent decrease in MDA content with increasing

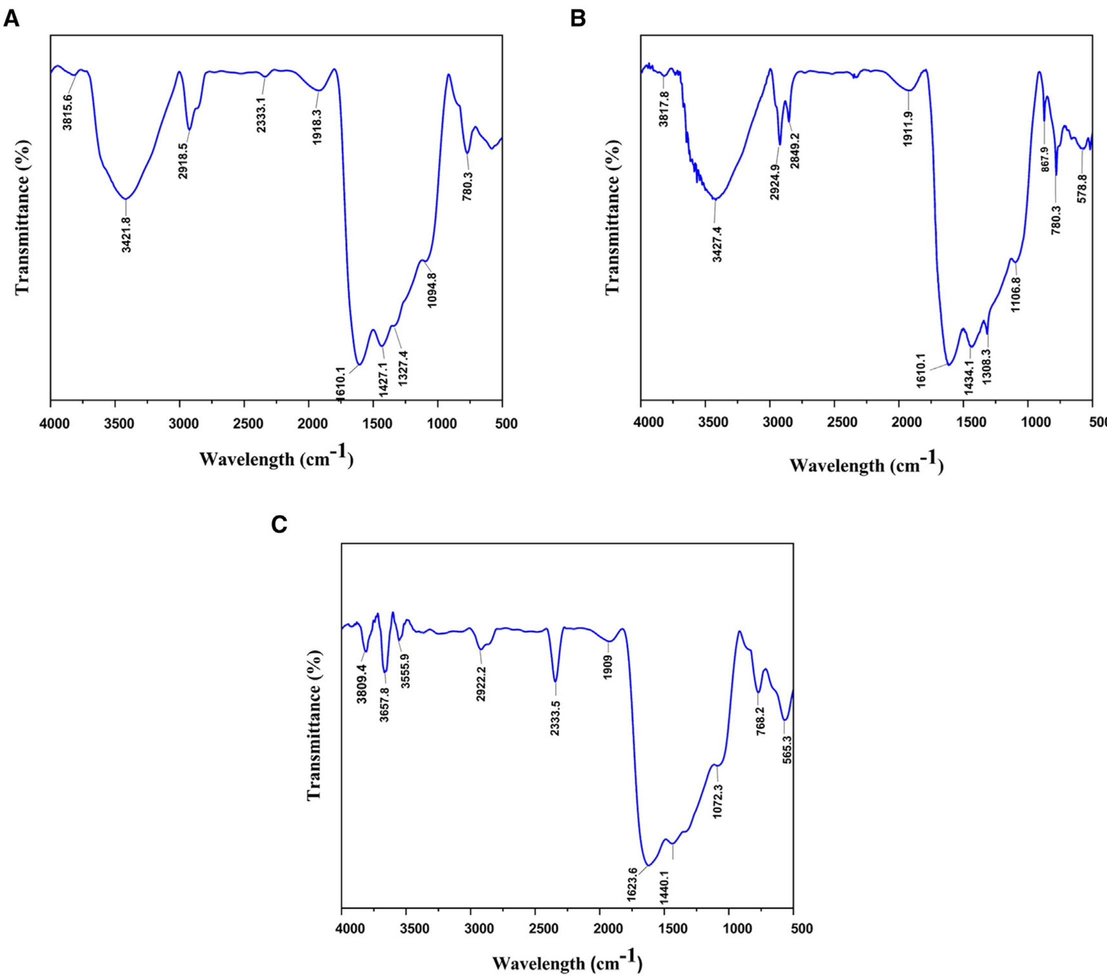

Figure 1. FTIR analysis of different types of biochar, where vertical and horizontal axis represents absorbance and wavenumber, respectively

(A) TWB; (B) SSTWB; (C) TUTWB. TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar.

biochar amendments (0.5%, 1%, and 2%). This suggests that biochar application helps mitigate the negative effects of As stress on rice plants. At both 7 DAG and 14 DAG, the highest reductions in MDA content (both shoot and root) were observed in plants treated with the 1% biochar dose. These reductions were followed by those observed with the 2% biochar and then the lowest dose (As+0.5B) of biochar amendment (Figures S3C and S3D). Overall, this study highlighted that As contamination significantly elevates MDA content in rice plants, indicating increased oxidative stress. However, biochar amendment, particularly at 1% doses, demonstrated promise in alleviating this stress response.

Characterization of biochar and modified biochar

FTIR was used to analyze the raw and modified biochar for the presence of any chemical groups, as shown in Figure 1. The FTIR of the TWB, SSTWB, and TUTWB contained several adsorption peaks. The peak at 780.80 cm^{-1} for TWB, 780.30 for SSTWB, and at 768.20 cm^{-1} for TUTWB were assigned to C–H bending aromatic CH out-of-plane deformation.[29] The peaks of biochar around 3400, 2900, 1600, and 1100 cm^{-1} might be attributed to hydroxyl groups (OH), C-H, C=C., and C-O respectively.[30] Compared with TWB, the new peaks found at 868 and 579 cm^{-1} on the surface of SSTWB were attributed to sulfonyl groups (O=S=O) and Si–O, respectively.[31,32] Similarly, the new peak on the surface of TUTWB at 579 cm^{-1} reflected Si-O and around 3500-3600 cm^{-1} showed N-H stretching, which confirms the loading of TU in TWB. The results suggested that types and strengths of functional groups present in modified biochar offered more adsorption sites in comparison to unmodified biochar.

The SEM of biochar (Figure 2) revealed a variety of porous structures, although the variations in size and shape of the pores suggested that the adsorption capacities of different biochar might differ.[33] The surfaces of SSTWB (Figure 2B) and TUTWB (Figure 2C) showed greater porous structures in comparison to TWB (Figure 2A). It was clear that the two materials had different morphologies. Additionally, the surfaces of SSTWB and TUTWB were rough and had more irregular particles, while the surface of

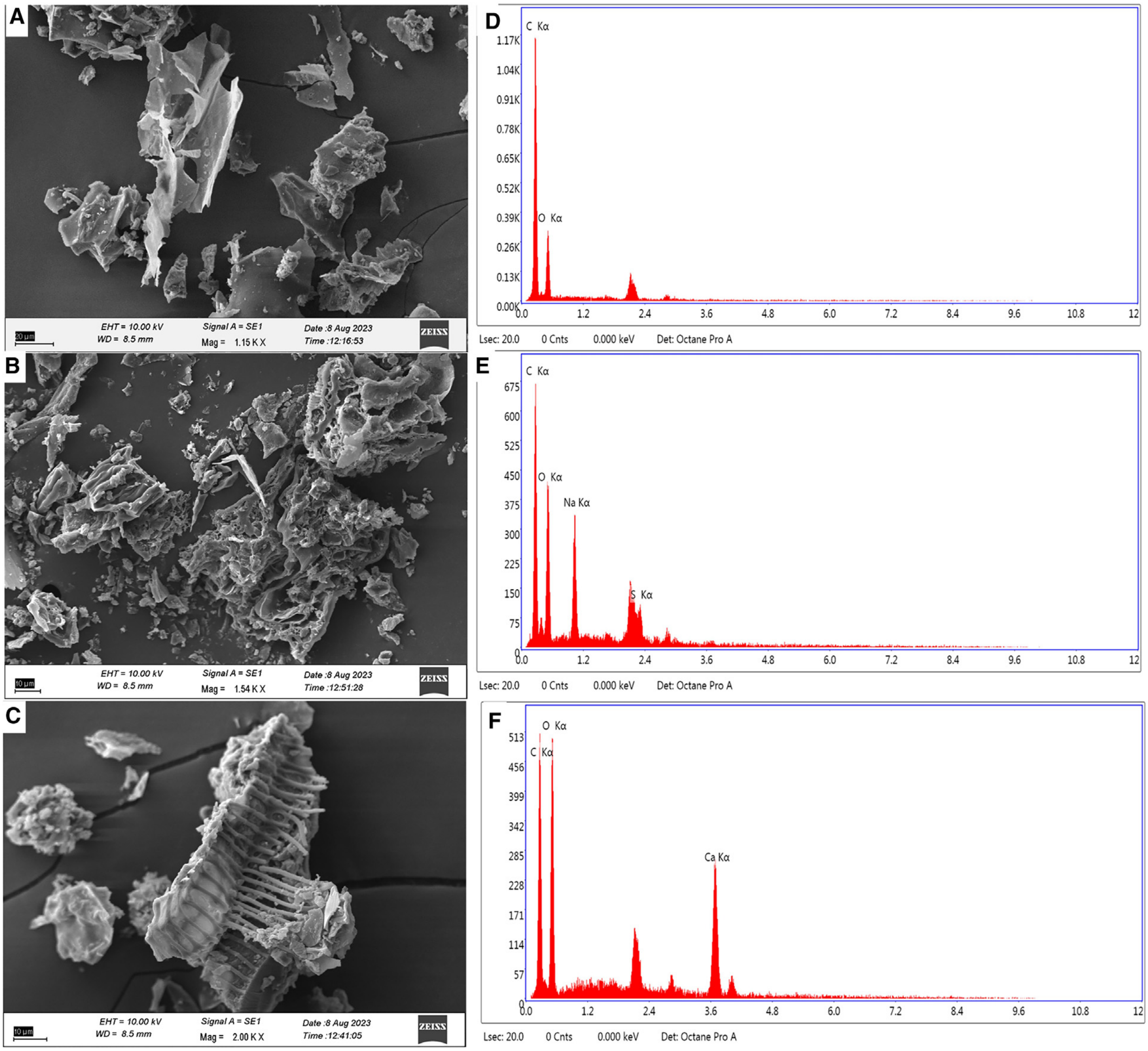

Figure 2. SEM-EDX images of unmodified and modified tea waste biochar

Scanning electron microscopy image and EDX spectra of TWB (A, D), SSTWB (B, E) and TUTWB (C, F). TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar.

TWB was smooth and had few pores. The EDX spectra of TWB, SSTWB, and TUTWB were further analyzed. The sulfur concentration in SSTWB and TUTWB was found to be higher than that of TWB, as illustrated in Figures 2D and 2E, and 2F. This indicates that sulfur was successfully loaded onto the surface of both modified biochar's. Our findings are consistent with Zhang et al.,[34] who previously showed successful sulfur loading onto biochar using sodium sulfide. It was important to note that SSTWB surface had significantly more sodium than TWB, which may have improved biochar's ability to exchange ions.[34]

Using a Quantachrome Instrument (USA, Model-Autosorb (IQ2)) surface area and porosimetry analyzer (Norcross, GA), the surface area of the biochar was determined. Liquid N_2 adsorption was employed to quantify the molecular surface area, and the BET (Brunauer−Emmett−Teller) equation was applied to determine the surface area of the biochar sample.[35] The specific surface area (SSA) of SSTWB (2.99 m^2 g^{-1}) and TUTWB (2.34 m^2 g^{-1}) was about 47% and 15% higher than the specific surface area of TWB (2.04 m^2 g^{-1}), respectively. This indicates that the SSA of modified biochar acted as an important parameter of As adsorption in soil.

Soil physico-chemical properties and elemental status

The physicochemical analysis of soil samples collected at three distinct time points revealed significant variations in SOC, available phosphorus, pH, EC, and ORP across different experimental treatments (Table S1 in supplementary file). At Zero DAT, the control group exhibited a pH range of 6.44 ± 0.12, while As alone,

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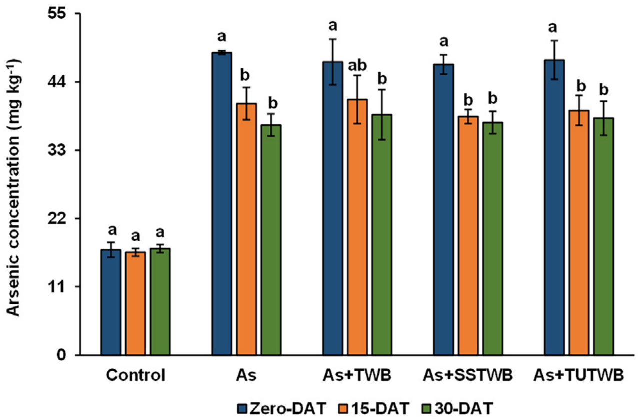

Figure 3. Arsenic concentration (mg kg^{-1}) in soil at different time points

All the values are means of triplicates ± SD. One-way ANOVA was found significant at 95% significant level. Dissimilar letter above means within a column are significantly different at $p \leq 0.05$ as performed by Tukey's post-hoc test. As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

As+TWB, As+SSTWB, and As+TUTWB treatments showed pH ranges of 6.40 ± 0.07, 6.53 ± 0.23, 7.11 ± 0.36, and 7.00 ± 0.27, respectively. Similarly, ORP values varied significantly among treatments, with the control group exhibiting −10.70 ± 1.21 mV and As+TUTWB showing −7.00 ± 0.56 mV. EC values ranged from 74.85 ± 8.56 to 119.80 ± 5.85 μS cm^{-1} across treatments at Zero DAT. At 15 DAT, pH levels fluctuated between 5.87 ± 0.05 and 6.35 ± 0.14, showing a slight decrease in pH in all treatments as compared to Zero DAT, possibly due to biochar treatments. EC values ranged from 214.00 ± 8.89 to 303.00 ± 36.06 μS cm^{-1}, suggesting a moderate increase in ion concentration over time. ORP values ranged from 20.00 ± 3.11 to 25.45 ± 3.75 mV, with some treatments exhibiting higher oxidation potential. At 30 DAT, pH values ranged from 5.88 ± 0.04 to 6.23 ± 0.06, slightly lower than those observed at 15 DAT. EC values ranged from 228.75 ± 22.98 to 289.00 ± 36.06 μS cm^{-1}, indicating a slight to moderate increase compared to the previous time point. ORP values ranged from 14.50 ± 0.85 to 21.30 ± 2.05 mV, showing consistency or slight variations from the previous time point. Notably, the As+SSTWB treatment exhibited the highest pH on all time points, while the control group showed the highest ORP. Additionally, EC values showed fluctuations across treatments and different time points, reflecting the dynamic nature of soil properties under experimental conditions.

This study also examined how different biochar's (TWB, SSTWB, and TUTWB) affect SOC and available phosphorus levels in the presence of As contamination. At Zero DAT, the highest SOC content was found in the As+SSTWB (2.86 ± 0.43%) treatment, while the control group (1.63 ± 0.27%) had the lowest. Available phosphorus levels also varied, with the As+TUTWB 78.83 ± 7.20 kg ha^{-1} the highest and the As-only treatment having the lowest (78.83 ± 7.20 kg ha^{-1}) in the As+TUTWB. These trends continued throughout the experiment with some variations. At 15 DAT, the SOC content ranged from a minimum of 1.88 ± 0.26% in the control treatments to a maximum of 3.19 ± 0.38% in the As-only treatment. Available phosphorus levels varied from 52.87 ± 6.50 kg ha^{-1} in the control group to 88.17 ± 4.79 kg ha^{-1} in As+SSTWB. At 30-DAT, the SOC content ranged from a minimum of 1.88 ± 0.28% in the control treatment to a maximum of 2.75 ± 0.27% in both SSTWB and TUTWB treatment. Available phosphorus levels varied from 42.40 ± 9.02 kg ha^{-1} in the As-only treatment to 76.15 ± 10.79 kg ha^{-1} in As+SSTWB. Overall, the application of different biochar treatments (TWB, SSTWB, and TUTWB) with arsenic resulted in varied soil organic carbon and available phosphorus levels compared to the arsenic alone and control groups across different time points.

Table 1. Concentration of various major oxides present in experimental soil

Major oxide	concentration (%)
MgO (%)	1.89 ± 0.06
K_2O (%)	2.47 ± 0.09
CaO (%)	1.42 ± 0.17
Fe_2O_3 (%)	2.66 ± 0.70
Al_2O_3 (%)	13.80 ± 0.43
SiO_2 (%)	66.33 ± 1.38
P_2O_5 (%)	0.19 ± 0.011
MnO (%)	0.06 ± 0.033

Values are mean of triplicate ±SD.

Soil As levels were monitored at three intervals (0, 15, and 30 DAT) across different experimental conditions. As shown in Figure 5, in Zero DAT, no significant differences in soil As contents were observed. However, at both 15 DAT and 30 DAT, amendments involving biochar showed notable reductions in soil As content compared to As-only treatment. Among the biochar amendments, SSTWB exhibited a slight decrease in soil As content from 38.40 mg kg^{-1} at 15 DAT to 37.45 mg kg^{-1} at 30 DAT. TUTWB showed a moderate decrease with reductions from 39.40 to 38.15 mg kg^{-1}. In contrast, TWB displayed a greater reduction in soil As, from 41.15 mg kg^{-1} at 15 DAT to 38.70 mg kg^{-1} at 30 DAT. Application of modified biochar resulted in a minimal decrease in total soil arsenic concentration between 15 and 30 DAT (Figure 3). However, a significant reduction in As content was observed in the roots and shoots of the rice plants. Additionally, concentrations of various major oxides (MgO, K_2O, CaO, Fe_2O_3, Al_2O_3, SiO_2, P_2O_5 and MnO) present in the soil are provided in Table 1. The result suggested that biochar amendments primarily reduced the bioavailability of As in the soil, potentially by binding As to its surface within the rhizosphere. Based on these results, the addition of biochar to soil may be a viable way to reduce As contamination. These findings emphasize the effectiveness of biochar amendments, particularly modified biochar, in reducing soil As content over time. The improved As adsorption capacity of SSTWB and TUTWB likely contributes to their enhanced performance compared to TWB.

Plant morphological changes under different experimental conditions

The growth parameters of the plant samples were measured to assess the performance of plants under the influence of biochar

Figure 4. Morphological appearance of rice plants at 15 DAT and 30 DAT under As stress

The morphological appearance of rice plants harvested at 15 DAT (A) and 30 DAT (B). As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

amendments. These growth parameters were assessed in plant samples collected at 15 and 30 DAT. Compared to the control, plants grown in As-amended soil exhibited significant reductions in root and shoot length at both time points (Figure 4). At 15 DAT, total, shoot, and root length decreased by 17%, 16%, and 24%, respectively (Figure 5A). Furthermore, these reductions were further intensified in plants at 30 DAT (33%, 28%, and 48%, respectively) as compared to control plants (Figure 5B). In biochar amendments, growth improvement was seen. At 15 DAT, shoot and root length increased by 11% and 34%, respectively, in plants grown in As+SSTWB compared to the As alone treated plant. TWB also promoted growth, with increases of 6% and 13% observed for shoot and root length, respectively. Interestingly, As+TUTWB resulted in an 8% increase in shoot length but a 13% decrease in root length compared to the As alone treatment (Figure 5A). A similar pattern of biochar amendments reducing As adverse impacts was observed at 30 DAT. The length of the shoots and roots increased by 14% and 24%, respectively, in TWB and by

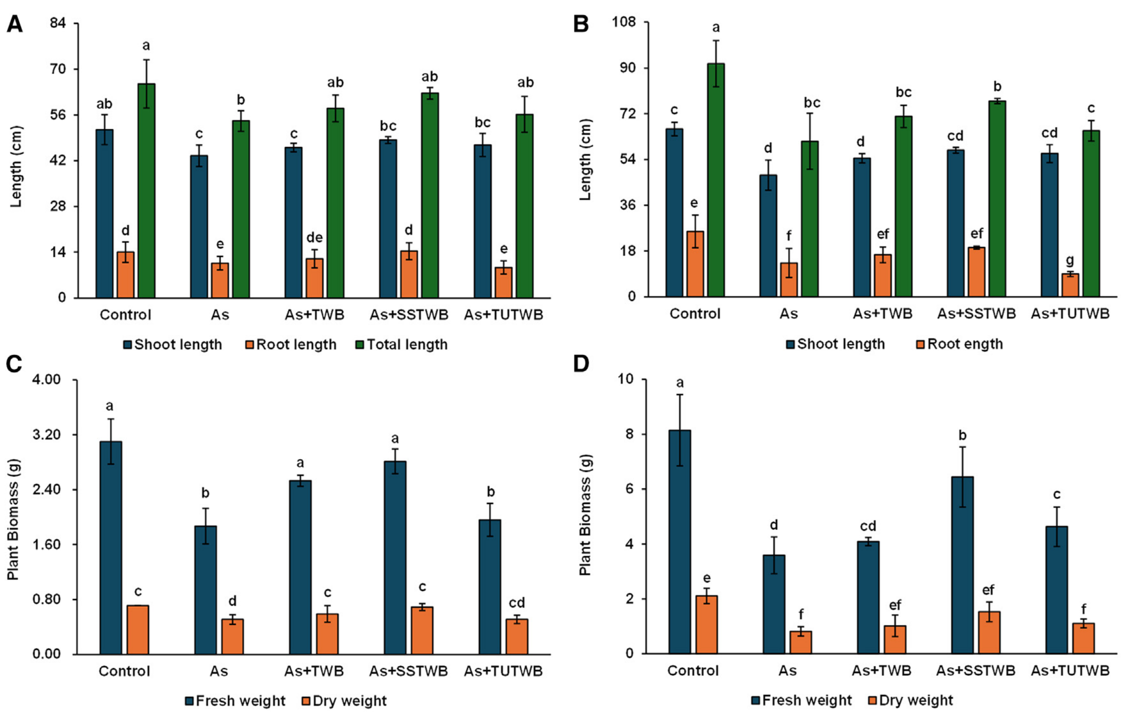

Figure 5. Biochar mediated change in rice plant growth parameters (root, shoot, and total length) and biomass at 15 DAT and 30 DAT under As stress

Effect of control, As and As plus different types of biochar on root length, shoot length and total length of rice plants at 15 DAT (A) and 30 DAT (B) and plant biomass at 15 DAT (C) and 30 DAT (D). All the values are means of triplicates ± SD. One-way ANOVA was found significant at 95% significant level. Dissimilar letter above means within a column are significantly different at $p \leq 0.05$ as performed by Tukey's post-hoc test. As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

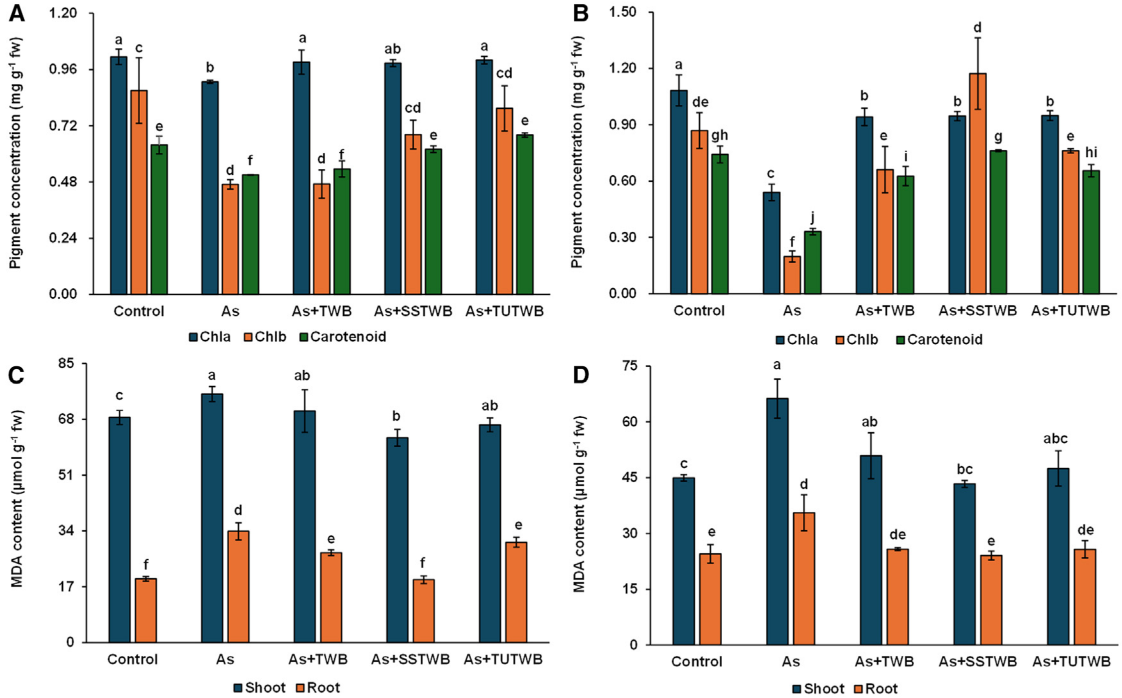

Figure 6. Biochar-mediated change in photosynthetic pigments and MDA content in rice plant at 15 DAT and 30 DAT under As stress

Effect of control, As and As plus different types of biochar on photosynthesis pigment of rice plants at 15 DAT (A) and 30 DAT (B) and MDA content at 15 DAT (C) and 30 DAT (D). All the values are means of triplicates ± SD. One-way ANOVA was found significant at 95% significant level. Dissimilar letter above means within a column are significantly different at $p \leq 0.05$ as performed by Tukey's post-hoc test. As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

21% and 45%, respectively, in As+SSTWB as compared to As-only treatment. It is interesting to note that As+TUTWB showed again a distinct effect, increasing shoot length by 18% but decreasing root length by 34% when compared to the As-only treatment (Figure 5B).

The fresh weight and dry weight of control plant samples were 40% and 28% higher, respectively, in 15 DAT samples as compared to As-amended pot samples. The fresh weight of plant samples taken from As+TWB, As+SSTWB, and As+TUTWB amended pots was 35%, 50%, and 5% higher, respectively, while the dry weight was 16% in As+TWB and 35% in As+SSTWB as compared to the As-only treatment. Similarly, the fresh weight and dry weight of control plant samples were observed to be 56% and 61% higher, respectively, in the 30 DAT sample compared to the plant grown in As-amended pot. Meanwhile, the fresh weight of plant samples collected from TWB, SSTWB, and TUTWB amended pots showed increases of 14%, 79%, and 29%, respectively, while the dry weight showed higher values by 25%, 87%, and 35.37%, respectively, compared to the arsenic-amended pot (Figures 5C and 5D).

Photosynthetic pigment and lipid peroxidation

Compared to the control group, plants grown in As-amended soil exhibited a significant decrease in pigment content. Chlorophyll *a*, *b*, and carotenoid levels declined by 11%, 46%, and 20%, respectively, at 15 DAT and by 50%, 77%, and 55%, respectively, at 30 DAT, indicating a negative impact of As on plant photosynthesis (Figures 6A and 6B). Interestingly, rice plants grown in pots amended with biochar alongside As (As+TWB, As+SSTWB, and As+TUTWB) showed a reversal of this trend compared to plants grown in As-only amended soil. An increase of about 0.1– to 0.75-fold in chlorophyll *a*, 0.45– to 4.95-fold in chlorophyll *b*, and 0.05– to 1.39-fold in carotenoids in As+TWB, As+SSTWB, and As+TUTWB treatments as compared to As alone treatment (Figures 6A and 6B). These results suggested that sulfur modified biochar can effectively mitigate the negative effects of arsenic on chlorophyll content in rice plants.

The toxicity of As was also assessed in terms of lipid peroxidation (an indicator of membrane damage), as indicated by the malondialdehyde (MDA) content, in rice plants harvested at 15 and 30 DAT. Plants grown in As-amended soil exhibited significantly higher MDA content in both shoots (10%–47%) and roots (45%–74%) compared to the control group at 15 and 30 DAT (Figures 6C and 6D). Biochar amendments (As+TWB, As+SSTWB, As+TUTWB) displayed a protective effect reducing MDA content compared to plants grown only with As. Shoot MDA decreased by 7%–17% across the biochar treatments at 15 DAT. However, root MDA showed a more significant reduction, with decreases ranging from 10% to 43% (Figure 6D). At 30 DAT also, biochar amendments induced a decline in MDA levels. Shoot MDA decreased by 23%–35% in plants with biochar amendments compared to the As-only treatment. Root MDA also showed reductions ranging from 27% to 32% in biochar treatments (Figure 6D).

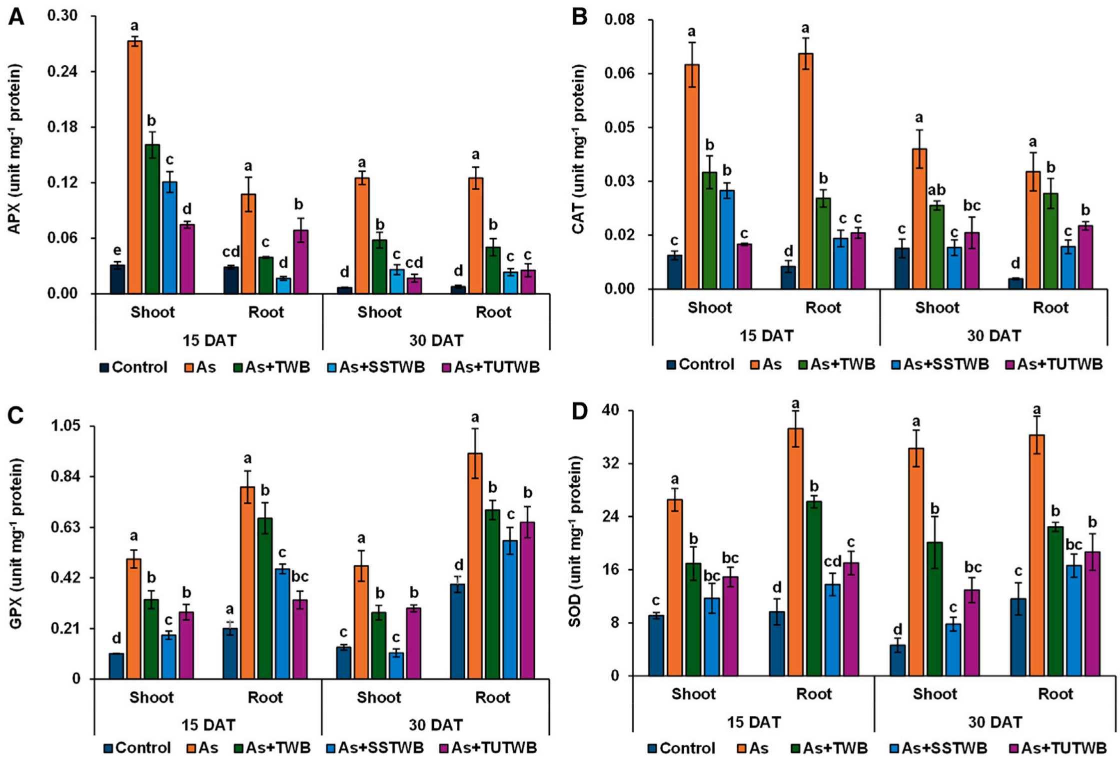

Figure 7. Biochar-mediated changes in antioxidant enzyme activities in rice plant (APX, CAT, GPX, and SOD) under As stress

Effect of control, As and As plus different types of biochar amendments on APX (A), CAT (B), GPX (C), and SOD (D) activity in rice plants. All the values are means of triplicates ± SD. One-way ANOVA was found significant at 95% significant level. Dissimilar letter above means within a column are significantly different at $p \leq 0.05$ as performed by Tukey's post-hoc test. As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

Effects of arsenic and biochar amendments on antioxidant enzyme activity in rice plants

Plants grown in As-amended soil exhibited the highest SOD activity in both shoots and roots compared to all biochar treatments. Biochar amendments resulted in decreased SOD activity compared to the As-only treatment. SOD activity in shoot and root of plants harvested at 15 DAT showed a decreasing order: As+SSTWB (56% and 63%) > As+TUTWB (44% and 53%) > As+TWB (36% and 30%). Similarly, plants harvested at 30 DAT depicted a decline in SOD activity in As+biochar plants (Figure 7D). The activity of APX, GPX, and CAT also depicted a response similar to that of SOD. The maximum activity of these enzymes in shoot and root was found in As-alone treated plants. In different biochar amendments, a significant reduction in APX (Figure 7A), GPX (Figure 7B), and CAT (Figure 7C) activity was observed as compared to that of As alone treatment. Among the biochar treatments, the application of modified biochars (SSTWB and TUTWB) resulted in greater decline in enzyme activity as compared to unmodified TWB.

Arsenic content in root and shoot of rice plant

Arsenic content in shoots of plant samples harvested from As amended soil at 15 DAT was found to be 118.29 $\mu g\ g^{-1}$ dw. In biochar amendment, As content showed a decline, which was found to be the maximum in SSTWB (87.46 $\mu g\ g^{-1}$; 26%), followed by 19% in TUTWB (96.64 $\mu g\ g^{-1}$) and 12% in TWB (103.71 $\mu g\ g^{-1}$). At 30 DAT also, a similar pattern was followed. The maximum decrease of As content in shoot was found in SSTWB (167.75 $\mu g\ g^{-1}$) and the minimum decrease was observed in TWB (201.75 $\mu g\ g^{-1}$) in comparison to As alone treatment (241.08 $\mu g\ g^{-1}$). Arsenic content in the root of plant samples harvested from As amended soil was 1656.00 $\mu g\ g^{-1}$ at 15 DAT and 2893.00 $\mu g\ g^{-1}$ at 30 DAT. In SSTWB, TUTWB, and TWB, a decrease in root As content was observed, which was 26% (1227.00 $\mu g\ g^{-1}$), 18% (1353.10 $\mu g\ g^{-1}$) and 10% (1494.03 $\mu g\ g^{-1}$), respectively, at 15 DAT and 30% (2013.03 $\mu g\ g^{-1}$), 22% (2246.00 $\mu g\ g^{-1}$) and 15% (2463.08 $\mu g\ g^{-1}$), respectively, at 30 DAT in comparison to As alone treated plant (Figure 8).

DISCUSSION

This study aimed to evaluate the effectiveness of sulfur modified biochar in modifying these soil properties. Many previous studies have already proven that biochar impregnated with inorganic elements like iron, iron oxide, iron manganese oxide, bismuth, and sulfur affect the abundance of microbes in soil. Thus, biochar modified with these elements has the ability to immobilize potentially toxic elements like As and Cd.[36] Biochar amendments may improve soil acidity, enhance nutrient content, and potentially

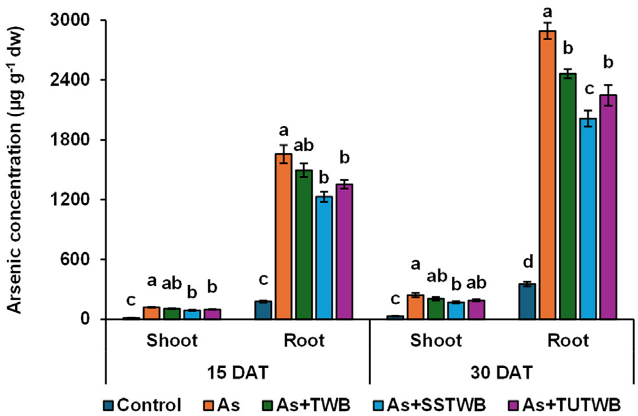

Figure 8. Effect of control, As and As plus different types of biochar on As accumulation by shoot and root in rice plants at 15 DAT and 30 DAT

All the values are means of triplicates ± SD. One-way ANOVA was found significant at 95% significant level. Dissimilar letter above means within a column are significantly different at $p < 0.05$ as performed by Tukey's post-hoc test. As - arsenic; TWB - tea waste biochar; SSTWB - sodium sulfide modified tea waste biochar; TUTWB - thiourea modified tea waste biochar; DAT - days after transplant.

reduce As bioavailability. Soil physicochemical properties, such as pH and ORP, significantly influence As mobility and plant uptake. Studies have shown that higher soil pH (alkaline conditions) can lead to increased total As accumulation in plants.[37] This is likely due to increased desorption of arsenite [As(III)] and arsenate [As(V)] at higher pH since the negative surface charge on soil particles is enhanced.[38] In our study, biochar application led to an increase in soil pH due to its inherent alkaline nature. Biochar contains basic cations (such as Ca^{2+}, Mg^{2+}, and K^{+}) that readily dissociate and release carbonates and oxides in the soil.[27] These released cations can exchange with protons (H^{+}). However, negatively charged functional groups (carboxyl, hydroxyl, and phenolic) of biochar contribute to pH elevation by attracting and neutralizing H^{+} ions.[39] In this experiment, the elevated ORP values compared to the control might have also contributed to As behavior, as ORP is known to influence As speciation and mobility.[40] According to Li et al.[41] biochar application can alter soil ORP and affect As speciation and mobility. In another study, El-Naggar et al.[42] suggested that flooded soils experience dynamic redox conditions, which significantly affect the speciation and mobilization of elements like As, Co, and Mo. Additionally, SOC and associated microbial activity can significantly affect As mobilization and plant bioavailability.[43] Soil organic carbon directly reflects the organic carbon content of applied biochar, indicating a positive correlation. The observed increase in SOC may be due to biochar stimulating the decomposition of existing soil organic matter. Sulfur-modified biochar showed reduced volatile and soluble organic carbon content, suggesting decreased mineralization and decomposition processes compared to unmodified biochar.[42] Biochar amendments not only raised soil alkalinity but also enhanced available phosphorus (P) content. This improvement is attributed to biochar's high specific surface area and abundant functional groups. Additionally, differences in EC among soil samples suggest variations in their ability to enhance water-soluble ion content.[44] The observed changes in soil characteristics following TWB, SSTWB, and TUTWB application could be attributed to the direct effect of biochar itself or the complex interactions. Varying biochar surface properties affected As bioavailability, leading to improved adsorption behavior.[45] between its physical and chemical properties with the existing soil environment.

Rice plants exposed to As toxicity exhibited reduced growth attributes such as root length, shoot length, and plant biomass (both fresh and dry weight). The cellular damage induced by As toxicity leads to the over-generation of reactive oxygen species (ROS), disrupting plant metabolism, including photosystems, photorespiration, and respiration.[46] Consequently, plant growth is hindered. Furthermore, As exposure directly interferes with the photosynthetic activity of plants, affecting chlorophyll and carotenoid contents, which are principal factors contributing to lower biomass accumulation under stressed conditions.[47] Our findings support this concept, as rice plants exposed to As exhibited lower chlorophyll and carotenoid content, indicating a decline in photosynthetic pigments. Furthermore, As exposure can disrupt stomatal function, leading to abnormalities, dysfunction, and closure of stomata along with guard cell distortion.[48] Similar trends of growth retardation due to As have been observed in previous research on rice,[49] maize (*Zea mays* L.; Khan et al.[50]), and mungbean (*Vigna radiata*; Alam et al.[51]). In this study, both sodium SSWB and TUTWB restored the growth attributes of rice even under As stress. The high metal adsorption affinity of SSTWB and TUTWB amendments and their potential to improve plant characteristics by enhancing soil nutrient status, enzymatic activities, and reducing heavy metal bioavailability[52] contribute to improved plant growth under stressed conditions. Our findings align with earlier research by Irshad et al.,[36] where biochar-amended soil effectively mitigated cadmium and As toxicity and improved rice plant growth. Additionally, the positive effect of iron-modified biochar on plant growth improvement has been documented in a previous study,[53] indicating that essential plant nutrients accumulate more when biochar is applied to soil, even under As exposure, promoting plant growth while mitigating toxicity. The positive effect of biochar on increasing rice growth characteristics under stress conditions in our study is consistent with the findings of Shukla et al.,[54] where the potential of biochar to increase metal adsorption or reduce its translocation from soil was cited as the reason for rice plants' improved stress tolerance and growth promotion.

Previous studies have shown that biochar amendments can alleviate the negative effects of metal(loid) toxicity and enhance plant photosynthetic pigments.[55,56] Consistent with these reports, our data revealed an upward trend in carotenoid, chlorophyll *a*, and chlorophyll *b* content in rice plants exposed to As stress when treated with SSTWB and TUTWB. This suggests that biochar application can mitigate the detrimental effects of As on rice plant photosynthesis. A possible reason for the observed growth improvement is reduced ROS load and oxidative stress that lead to decreased plant toxicity and hence improved growth.[7] Reactive oxygen species (ROS) typically act as cellular signaling molecules under normal conditions. However, under stress conditions such as As exposure, plants can accumulate excessive

ROS, leading to cellular dysfunction and damage to lipids, proteins, and carbohydrates.[57] This is evident in the As-treated rice plants, where the significant increase in ROS disrupts cell membrane integrity through enhanced lipid peroxidation (reflected by elevated MDA content). Similar observations of As-induced oxidative stress, characterized by increased MDA levels have been reported by Dixit et al.[25] ROS are inevitable byproducts of normal cellular metabolism. However, under stress conditions like As exposure, their accumulation can reach harmful levels, leading to cellular dysfunction. Plants possess a sophisticated antioxidant defense system to counteract ROS and maintain cellular homeostasis. This system consists of various antioxidant enzymes present in different cellular compartments.[58,59] These enzymes work synergistically to convert highly reactive ROS into less harmful or non-toxic forms, effectively mitigating oxidative stress. This study demonstrates that application of both SSTWB and TUTWB biochar effectively alleviated the detrimental effects of excessive ROS accumulation in rice plants exposed to As. These findings align with the work of Hafez et al.,[60] who reported that biochar can significantly enhance plant cell membrane stability under stress conditions. Improved membrane integrity likely contributes to better regulation of plant water pressure and relative water content (RWC). This, in turn, can mitigate oxidative stress by reducing the production of ROS and minimizing lipid peroxidation within the cells.

This study also examined the impact of As contamination and biochar amendments (TWB, SSTWB, and TUTWB) on the activity of antioxidant enzymes (APX, GPX, SOD, and CAT) in rice plant root and shoot harvested at 15 and 30 DAT. Superoxide radicals are produced by plant cells in response to abiotic stressors like arsenic poisoning. SOD acts on superoxide radicals and converts them to hydrogen peroxide and oxygen, which is then managed via several peroxidases, including GPX.[61] The significant increase in the activity of SOD and GPX in response to As alone treatment is in confirmation of earlier work.[61,62] Furthermore, CAT is an essential antioxidant enzyme found in plant cells that scavenges excess hydrogen peroxide (H_2O_2) and transforms it into harmless oxygen (O_2) and water molecules (H_2O). Furthermore, in different cell compartments, APX and GPX are essential in lessening the negative effects of hydrogen peroxide.[53] This maintains a dynamic balance between the generation and removal of H_2O_2 in peroxisomes. The findings suggest that As contamination elevated antioxidant enzyme activity in rice plants, potentially as a response to oxidative stress. SSTWB and TUTWB amendments led to a decrease in activity of antioxidant enzymes along with MDA, indicating lower ROS production compared to the TWB and As-only induced plant. The coordinated enzymatic response observed in the study highlights the plant's attempt to mitigate oxidative stress by scavenging and neutralizing ROS generated under As exposure. Although there is limited study on biochar's ability to reduce oxidative stress caused by As, several studies point to a possible connection. Increased catalase (CAT) activity was seen in mung bean plants grown in As-contaminated soil amended with biochar.[51] The observed positive effects of SSTWB and TUTWB were considered to be due to decreased As accumulation within plant tissues. To confirm this, the concentration of As in root and shoot tissues was examined.

The root is the primary tissue directly exposed to As in the soil, and it regulates As accumulation through altered uptake and self-restriction.[63,64] This explains the higher As accumulation in the roots at 15 and 30 DAT as compared to that in the shoot. This aligns with prior research, which also found a higher concentration of As in roots compared to shoots and grains.[56,58,65] Introducing SSTWB and TUTWB to the soil significantly reduced the uptake and accumulation of As in plant shoots and roots. This is consistent with earlier studies where biochar was found to immobilize As in the soil, thereby reducing its uptake by plants.[66,67] The decrease in plant As uptake could be due to an increase in As immobilization with soil organic matter or a reduction in As levels in the soil solution. Beesley et al.[68] similarly reported that As formed complexes with dissolved organic carbon, rendering it unavailable for plant uptake. Therefore, biochar can limit plant As uptake through either direct or indirect interactions. Direct interactions encompass electrostatic attraction, complexation, ion exchange, and precipitation, while indirect interactions involve soil pH, CEC, mineral dissolution, and soil organic carbon.[67]

Conclusions

In conclusion, this study suggested that tea waste biochar (TWB) modified with either sodium sulfide (SSTWB) or thiourea (TUTWB) holds promise for mitigating As toxicity in rice plants. These modified biochar amendments improved plant growth by enhancing chlorophyll content and reducing lipid peroxidation, a marker of oxidative stress. Furthermore, SSTWB and TUTWB application improved antioxidant enzyme activity within the rice plants, potentially by reducing the generation of ROS. The observed positive effects were attributable to the reduced As accumulation in roots and shoots of plants and it was the most desirable result. Overall, these findings suggest that TWB and its modified versions (SSTWB and TUTWB) could be a viable strategy for alleviating the toxicity of As in rice plants and improving overall soil health. Biochar's stability in soil translates to long-term advantages, with a single application providing sustained benefits over the years. Additionally, the production of biochar from tea waste represents a sustainable practice to recycle waste and contributes to low environmental impact and circular economy practices.

Limitations of the study

One limitation of this study is the characteristic variability in the composition of sulfur-modified TWB. Meanwhile, as efforts were made to standardize the synthesis process, variations in biochar characteristics may have influenced the results. Future studies should consider ensuring the consistency of sulfur-modified TWB in As remediation and on rice plant growth. The study was a pot experiment conducted under controlled conditions, which may vary in field conditions. Factors such as soil microbes, soil heterogeneity, and environmental conditions could affect the performance of sulfur-modified TWB. Field trials are needed to validate these findings under diverse environmental conditions. Furthermore, the present study focused on the short-term impacts of sulfur-modified TWB on soil properties and plant growth. The long-term stability and efficacy of both modified and unmodified TWB, particularly its potential for carbon sequestration and its relations with soil microbes over time, remain unknown. Long-term studies are suggested to consider these aspects.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Sudhakar Srivastava (sudhakar.iesd@bhu.ac.in).

Materials availability

This study did not generate new unique reagents and components.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- All custom-made scripts and codes for analysis are available for request by contacting the lead author, Sudhakar Srivastava (sudhakar.iesd@bhu.ac.in).
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

S.K.P.: executed all the experiments, formal analysis, data curation, data interpretation, drafting the article. Shraddha Singh: data analysis. V.D.R.: writing and drafting the article. Shengdao Shan: writing, drafting the article. Sudhakar Srivastava: conceptualized and supervised the study and finalized the article for submission.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- METHOD DETAILS
  - Biochar preparation, modification, and characterization
  - Biochar dose optimisation experiment
  - Experimental design and sampling methods
  - Estimation of rice plant stress-responsive markers and stress regulatory enzymes
  - Analysis of physicochemical parameters of soil
  - Arsenic estimation in soil and plant samples
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sodium sulfide	Sigma-Aldrich	1313822
Sodium (meta)arsenite	Loba Chemie	05775
Thiourea	Merck (GR grade)	62566
2-Thiobarbituric acid	Himedia	67527
Trichloroacetic acid	Loba Chemie	06356
Coomassie Brilliant blue G 250	Himedia	MB092
ortho-Phosphoric acid 85%	Merck (ACS grade)	7664-38-2
Polyvinylpyrrolidone	Merck	9003-39-8

METHOD DETAILS

Biochar preparation, modification, and characterization

In this study, tea-waste was used as a feedstock to produce biochar. Tea-waste was collected from tea shops from local markets around Banaras Hindu University. After collection, it was washed with running tap water several times and then washed with double distilled water (DDW). The tea-waste was dried in the oven at 100°C for 24 h (h). Dried biomass was packed well in an airtight box and pyrolyzed in a muffle furnace at 500°C under nitrogen atmosphere. The feedstock was heated to the desired temperature at a rate of 7 °C min^{-1} and held for 1 h. Then, it was allowed to cool inside the furnace overnight to avoid air oxidation. The produced tea-waste biochar (TWB) was washed with deionized water (DDW) until washings were clear and oven dried at 80°C for 12 h. Washed TWB was ground and sieved to retain the 0.5–1 mm mesh fraction. In the modification process, 100 mL of 0.78 M $Na_2S.9H_2O$ (Sodium Sulfide; SS) and 100 mL of 6.57 mM NH_2CSNH_2 (Thiourea; TU) were taken in 2 separate beakers. 10 g of biochar was weighed and added in each. After stirring for 6 h at 500 rpm, the mixture was filtered and washed with DDW for several times to remove excess SS and TU. After that, filtered biochar was dried at 80°C for 12 h and kept in desiccators for further use. Modified biochar was named as sodium sulfide modified tea-waste biochar (SSTWB) and thiourea modified tea-waste biochar (TUTWB). Using Fourier transform infrared spectroscopy (FTIR) (BRUKER Ettlingen, Germany), the different functional groups in the biochar were observed. The surface area of the biochar was determined by using BET; Brunauer–Emmett–Teller analysis (Quantachrome Instruments (USA) Model-Autosorb (IQ2)). To monitor the surface morphology of biochar, scanning electron microscopy (SEM) (Model Carl Zeiss EVO 18, Germany) analysis was performed.

Biochar dose optimisation experiment

A preliminary experiment was conducted to determine optimal biochar application rates for rice grown in As-amended soil. Five treatments were established, each with three replicates (total n = 15), to investigate the effects of biochar on rice plants grown in As-amended soil. Treatments were named as control (no biochar and no As amendment), As (50 mg kg^{-1}), As+Biochar in which As amendment with biochar applied at three different rates (w/w based on dry soil weight). The three different doses were As+0.5B (0.5% biochar), As+1B (1% biochar) and As+2B (2% biochar). For each treatment, 700 g of soil was placed in a plastic pot. Biochar (at the designated level for As+Biochar treatments) was thoroughly mixed with the soil before planting. Rice seeds were surface sterilized and incubated. After incubation an equal number of seeds were sown in each pot. Plant sample was taken as three different time point at 7, 14 and 21 days after germinations (DAG). Three replicates were collected for each treatment at each time point. Plant height in terms of root length, shoot length, total length and chlorophyll were measured. MDA content was also analyzed as a marker of cellular stress and lipid peroxidation. These optimized biochar application rates provided a baseline for later experiments involving biochar and sulfur-modified biochar under similar conditions and sampling intervals.

Experimental design and sampling methods

Soil used in pot experiments was collected from the top 0–20 cm of farmland located in Banaras Hindu University, Varanasi, India. The collected soil was sieved through a 2-mm sieve to obtain a uniform particle size distribution. The experiment was carried out using polyvinyl chloride (PVC) pots of 20 cm in diameter and 25 cm in height. A total of 3 kg of sieved (2 mm) soil (dry-weight basis) was filled into each pot. In this study, a total of five different treatments, each consisting of three replicates, were used. The treatments were named as control (without biochar and As), soil spiked with 50 mg kg^{-1} sodium meta arsenite (As), As+TWB, As+SSTWB and As+TUTWB. The amount of biochar was kept at 1% (w/W). This rate was determined from the preliminary dose optimization experiment. Each of the five setups was sampled for soil at three different time points; zero-days after transplant (DAT), 15 DAT, and 30 DAT. The sampling of rice plants was done at 15 and 30 DAT. Rice seeds (Moti, local variety) were first sterilized in 30% ethanol, followed by washing several times using DDW to remove pathogens, and then incubated under controlled conditions for 24 h. After the incubation, rice seeds were sown directly into prepared flooded field plots for nursery, followed by gentle watering to facilitate germination. Six plants of identical height were transplanted into each pot after the rice seedlings had grown for 20 days. The morphological characteristics of the plant sample assessed were fresh weight (g), dry weight (g), shoot length (cm), and root length (cm). The height of the plant was measured in centimeters, starting from the base and ending at the tip of the tallest leaf (or panicle, depending on which was longer). The total number of panicles in each plant sample was determined by counting the tillers of each plant.

Estimation of rice plant stress-responsive markers and stress regulatory enzymes

Using Arnon's[69] method, the concentrations of photosynthetic pigments were measured. A spectrophotometer was used to detect the optical density at wavelengths of 480 nm, 510 nm, 645 nm, and 663 nm in order to determine the concentration of carotenoids, chlorophyll *a* and *b*. The carotenoid concentration was determined using the Duxbury and Yentsch[70] formula. MDA content in plant tissue was determined using protocol given by Heath and Packer.[71] Approximately 100 mg of fresh rice plant leaves were ground in 20% trichloroacetic acid (TCA) containing 0.5% 2-thiobarbituric acid (TBA). The homogenate was centrifuged for 15 min at 4 °C at 10,000 rpm. The supernatant was collected and incubated in a water bath for 30 min at 95°C. Absorbance was measured at 532 nm and corrected for nonspecific background absorbance at 600 nm. A blank containing only the TBA and TCA mixture was used for reference. The MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.[1] For enzyme analyses, a 100 mg sample of fresh plant material was homogenized at 4°C in 100 mM chilled potassium phosphate buffer (pH 7.0) with 1 mM EDTA, 2% polyvinyl pyrrolidone (PVP) (w/v), 2% glycerol (v/v), and 5 mM sodium ascorbate. A cooling centrifuge was used to centrifuge the homogenate for 15 min at 10,000 rpm at 4°C. Total protein content in enzyme extract was determined following the method described by Bradford.[72] Following the procedures outlined in Srivastava et al.,[73] the activity of superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), and ascorbate peroxidase (APX) were measured in the supernatant by following the methods of Beauchamp and Fridovich,[74] Hemeda and Klein,[75] Aebi[76] and Nakano and Asada[77] respectively.

Analysis of physicochemical parameters of soil

A 10 g soil sample was collected using a scraper from each of the 15 pots at three distinct intervals. These samples were kept in a zipper bag and brought to the laboratory for further analysis. The samples were first sun-dried and then ground to a fine powder with the help of a mortar pestle, followed by sieving with a 2 mm sieve. Mettler Toledo's portable, waterproof PCSTestrTM 35 series was used to measure the physicochemical properties such as pH, and electrical conductivity (EC μS cm^{-1}). An Aquasol handheld ORP meter (AM-ORP-01) was used to measure the oxidation-reduction potential (ORP mV). The Walkley and Black[78] and Oleson[79] protocols were followed for the estimation of percentage soil organic carbon (SOC) and available phosphorus (Kg ha^{-1}), respectively.

Arsenic estimation in soil and plant samples

Wavelength Dispersive X-ray Fluorescence (WD-XRF) (S8 TIGER, Bruker, Mannheim, Germany) was used for elemental analysis in soil samples. Press pellet method (for trace elements) and fused bead method (for essential elements) were used to prepare the sample as detailed previously.[9] For WD-XRF analysis, this procedure was carried out with the support of the xrFuse2 Electric Automatic Fusion Furnace.[80]

The concentration of total As in root and shoot samples was determined following acid digestion, as described by Srivastava and Singh.[81] After digestion, 10 mL of Milli-Q water was added to each sample's residue. The samples were then filtered via filter paper with a 0.22 μm pore size (Merck-MF-MilliporeTM, MCE membrane filter). A graphite furnace atomic absorption spectrophotometer (Analytic Jena ContrAA 300, Analytic Jena) fitted with a hydride production device was used to analyze arsenic. Based on ten replicates, the recovery rate of spiked samples was determined to be between 90 and 95%. Standard reference material containing 1 μg mL^{-1} of arsenic was frequently evaluated to ensure the quality of analytical data, and the results were determined to be consistent with certified values.

QUANTIFICATION AND STATISTICAL ANALYSIS

All the data is presented as the mean of three replicates along with the standard deviation; in plant analysis, each replicate represents six plants. To compare mean values between treatments, one-way ANOVA was performed, followed by Tukey's HSD post-hoc test for pairwise comparisons. All tests were conducted at a confidence level of 95%. The analyses were performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software (In Figures 3, 5, 6, 7, and 8).