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# Mitigation of Boron Toxicity in Two Sorghum Cultivars Using a Novel Bio-Composite Derived from Waste Sheep Wool and Rice Husk

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**Abstract**
Excess boron (B) is a major stress factor limiting crop growth, particularly in semi-arid regions, creating an urgent need for environmentally friendly and cost-effective solutions to mitigate B toxicity. This study evaluated a newly synthesized bio-composite (WRHP), produced from waste sheep wool and rice husk, for its potential to reduce B toxicity in two sorghum cultivars and assessed its structural properties and effects on plant nutrient dynamics. Treatments included B-contaminated soil alone and soil amended with 0.5, 1.0 and 2.0 g $kg^{-1}$ WRHP. Fourier transform infrared spectroscopy (FTIR) analysis revealed a dominant peak at 1066 $cm^{-1}$ along with several weaker bands, while X-Ray Diffraction (XRD) showed characteristic reflections at 21°, 23°, and 31° 2θ, confirming keratin-derived components and silicon (Si), phosphorus (P), and potassium (K) bearing minerals. WRHP decreased tissue B concentrations, reducing levels in cv. Akdari from 189 to below 99.3 mg $kg^{-1}$ and in cv. Ogretmenoğlu from 115 to 75.7 mg $kg^{-1}$. WRHP enhanced Si accumulation especially in cv. Ogretmenoglu and increased P concentration at the highest application rate. Oxidative stress indicators (Malondialdehyde (MDA) and $H_2O_2$) decreased markedly following WRHP application. At the same time, WRHP enhanced non-enzymatic antioxidants and was accompanied by significant reductions in ascorbate peroxidase (APX) activity, along with dose-dependent decreases in superoxide dismutase (SOD) and catalase (CAT) activities. Post-harvest soil analyses showed lower plant-available B and increased P and Si levels with increasing WRHP doses. The WRHP effectively alleviates B toxicity by reducing B uptake, improving Si nutrition, and mitigating oxidative stress in sorghum.

**Keywords** Oxidative stress · Antioxidant enzymes · Silicon · Keratin · Sorghum cultivars

## Introduction

Boron (B) plays essential roles in higher plants, participating in a wide range of physiological and biochemical processes. These include the transport and metabolism of carbohydrates, cell wall synthesis and lignification, maintenance of plasma membrane integrity and function, stimulation of nucleic acid metabolism, regulation of indoleacetic acid metabolism, involvement in the ascorbate-glutathione cycle and phenolic metabolism, pollen tube development, nitrogen metabolism; photosynthesis, and various enzymatic activities (Rehman et al. 2006; Reid 2014; Gunes et al. 2017) In sorghum, the adequate B concentration in plant tissues generally ranges between approximately 4–13 mg $kg^{-1}$ dry weight. Concentrations below this range induce deficiency symptoms, whereas levels exceeding 13 mg $kg^{-1}$ dry weight may result in toxicity (Bergmann 1992). This narrow optimal window clearly illustrates the dual role of B as both an essential micronutrient and a potentially toxic element. However, the physiological threshold separating B deficiency from toxicity is extremely narrow, particularly in arid and semi-arid regions where low rainfall and high evapotranspiration enhance B accumulation in the soil profile (Yermiyahu et al. 2008). Excess B disrupts root meristem activity, inhibits leaf expansion, induces necrotic lesions, and interferes with metabolic pathways, ultimately

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resulting in substantial reductions in plant growth and productivity (Nable et al. 1997; Reid 2014; Zhao et al. 2024). A major consequence of B toxicity is the excessive generation of reactive oxygen species (ROS), including hydrogen peroxide ($H_2O_2$) and superoxide ($O_2^-$) radicals, which induce oxidative stress and trigger lipid peroxidation. Malondialdehyde, a key biomarker of membrane peroxidation, typically increases under B stress, indicating severe cellular damage (Karabal et al. 2003; Gunes et al. 2006, 2007a; Guneri et al. 2025). To cope with oxidative stress, plants activate an array of antioxidant enzymes such as SOD, CAT, and APX, which collectively detoxify ROS and limit cellular injury (Gunes et al. 2006, 2007a; Mishra et al. 2023; Rao et al. 2025). Nevertheless, under high B concentrations, this antioxidant defense often becomes insufficient, highlighting the need for exogenous soil amendments capable of mitigating B uptake or enhancing plant tolerance.

Silicon (Si) has emerged as one of the most effective beneficial elements in ameliorating abiotic stress induced damage, including B toxicity. Numerous studies demonstrate that Si reduces B accumulation in shoots, enhances B binding within cell walls, modifies aquaporin-mediated B transport, and strengthens structural barriers such as the epidermis and endodermis (Gunes et al. 2007a; Khan et al. 2019; Shanmugaiah et al. 2023). Furthermore, Si markedly enhances antioxidant enzyme activities (SOD, CAT, and APX), stabilizes membranes, and reduces oxidative stress indicators such as $H_2O_2$ and MDA under toxic B conditions (Gunes et al. 2007a; Chen et al. 2019; Shanmugaiah et al. 2023). Importantly, the biological effectiveness of Si depends on its solubility and release dynamics, with amorphous silica or chemically activated silica being more beneficial than crystalline forms (Schaller et al. 2020; Spitzmüller et al. 2024).

Sorghum is recognized as a Si-accumulating cereal capable of absorbing large amounts of Si. Reported Si concentrations in sorghum shoots commonly range between 10 and 30 g $kg^{-1}$ dry weight, and in some cases may exceed this range depending on external Si supply and growth conditions (Morkovic et al. 2022; D'Agostini et al. 2025). These levels are substantially higher than those found in non-accumulator species and reflect the species' high Si uptake and translocation capacity.

In parallel with the expanding interest in sustainable agriculture, hybrid organic–inorganic bio-composite materials derived from agricultural residues have gained attention as low-cost, environmentally friendly soil amendments. Sheep wool (SW), rich in keratin proteins containing amide, hydroxyl, carboxyl, and thiol functional groups, can be chemically modified under alkaline and acidic treatments to produce reactive organic matrices with strong affinity for metal ions, silicates, and phosphates (Akca et al. 2023a; Sahin et al. 2025; Gunes et al. 2025a). Rice husk (RH), a silica-rich by-product, contains predominantly amorphous or poorly crystalline silica, which becomes bioavailable upon alkaline activation or silicate treatment (Kalapathy 2000; Taskin et al. 2023; Akca et al. 2023b; Gunes et al. 2025b, c). The WRHP bio-composite synthesized from SW and RH using sodium silicate, KOH activation, and subsequent phosphoric acid neutralization may increase Si availability, a higher density of functional groups, and an improved capacity to bind or immobilize excess B. Such hybrid materials not only serve as a source of bioavailable Si but may also act as B-adsorbing matrices due to their rich surface chemistry, providing a dual mechanism for mitigating B toxicity. The integration of agricultural residues into functional composites also aligns with the principles of the circular economy, transforming low-value wastes into high-value soil conditioners.

Sorghum (*Sorghum bicolor* L.) is a widely cultivated cereal particularly suited to regions where high soil B concentrations are common. It is moderately sensitive to B toxicity and displays well-characterized physiological and biochemical responses under stress, including alterations in Si and B uptake, modulation of antioxidant enzyme activity, and changes in oxidative stress markers. Given its strong Si-accumulating nature, sorghum also provides an excellent biological system to evaluate how externally supplied, bioavailable Si sources influence Si accumulation dynamics under B stress conditions. These characteristics make sorghum an ideal model crop for evaluating the effectiveness of novel Si-rich materials such as WRHP.

In this context, the present study investigates the potential of WRHP a bio-composite material to mitigate B toxicity in sorghum. Specifically, we examine the impacts of WRHP on (1) plant B and Si accumulation, (2) the activities of non-enzymatic antioxidants and key antioxidant enzymes (SOD, CAT, APX), and (3) oxidative stress indicators such as $H_2O_2$ and MDA. The findings aim to elucidate the biochemical and physiological mechanisms underlying WRHP-mediated stress alleviation and to contribute to the development of innovative, sustainable soil amendments derived from locally available agricultural by-products.

## Materials and Methods

### Preparation and Characterization of WRHP Material

For WRHP material, 200 g air-dried SW and 100 g air-dried RH were weighed, 1 L 0.5 M Na-Silicate (50%) was added to this mixture and boiled in the hotplate for 1 h. Afterwards, 1 mol KOH was added and boiled again. After cooling the mixture, 1 mol $H_3PO_4$ was added and thoroughly mixed. At the end of the reaction, the solution was dried in an oven at

65 °C and ground. Here, two agricultural wastes (SW and RH) were evaluated to produce a new material with fertilizer potential (WRHP). The functional groups of WRHP were characterized through Fourier transform infrared spectroscopy (FTIR) analysis. IR spectra ranging from 2000 to 500 $cm^{-1}$ were collected using a Shimadzu Infinity FTIR Spectrometer equipped with three reflective ATR units. The structural properties of WRHP were determined by an X-ray diffractometer (Rigaku, D/max-2200 Ultima) with Cu-Ka radiation (k:0.15406 nm) in the range of between 0 and 90° 2theta. The software OriginPro 2025b was used for creating the IR and XRD spectra. Total macro and micro elements concentration of WRHP was quantified using polarized energy dispersive X-ray fluorescence (PEDXRF), following the methodology as described by Gunes et al. (2009) (Table 1).

## Boron Adsorption Test in the WRHP Bio-Composite

A 7-day adsorption experiment was conducted to evaluate the B-adsorption capacity of the WRHP material. For this purpose, a 100 mg $L^{-1}$ B solution ($Na_2B_8O_{13}{\cdot}4H_2O$) was prepared and 10 g of WRHP was added. The experiment lasted for 7 days, including day zero, and the solution in contact with the material was filtered daily and analyzed for B concentration (Wolf 1971). In addition, at the end of the adsorption test, WRHP was removed from the solution, thoroughly washed, and dried; this material was designated as WRHP7. Both materials were then analyzed by FTIR to identify functional groups associated with B.

**Table 1** Some chemical and physical properties of the experimental soil and biocomposite WRHP material

| Properties (Method) | Amount | |
|---|---|---|
| | Soil | WRHP |
| Texture (Bouyoucos) | Clay loam | - |
| $CaCO_3$ (Scheibler), g $kg^{-1}$ | 70.8 | - |
| pH (1:2.5 water) | 7.53 | - |
| EC (1:2.5 water), dS $m^{-1}$ | 0.38 | - |
| Organic matter (Walkley-Black), g $kg^{-1}$ | 10.7 | - |
| Si ($CaCl_2$-extractable), mg $kg^{-1}$ | 60.5 | - |
| Total-Si (XRF), g $kg^{-1}$ | - | 10.7 |
| B (Hot water extractable), mg $kg^{-1}$ | 2.52 | - |
| Total B (Spectrophotometric), mg $kg^{-1}$ | - | 0.12 |
| P ($NaHCO_3$-extractable), mg $kg^{-1}$ | 6.40 | - |
| Total-P (XRF), mg $kg^{-1}$ | - | 4.00 |
| Total-N (Kjeldahl), g $kg^{-1}$ | 3.30 | - |
| K ($NH_4OAc$-extractable), mg $kg^{-1}$ | 626 | |
| Total-K (XRF), g $kg^{-1}$ | - | 69.2 |
| Ca ($NH_4OAc$-extractable) mg $kg^{-1}$ | 5040 | |
| Total-Ca (XRF), g $kg^{-1}$ | - | 6.48 |
| Mg ($NH_4OAc$-extractable), mg $kg^{-1}$ | 490 | |
| Total-Mg (XRF), g $kg^{-1}$ | - | 2.15 |
| Zn (DTPA-extractable), mg $kg^{-1}$ | 0.96 | - |
| Total-Zn (XRF), mg $kg^{-1}$ | - | 34.8 |
| Fe (DTPA-extractable), mg $kg^{-1}$ | 3.62 | - |
| Total-Fe (XRF), g $kg^{-1}$ | - | 0.16 |
| Cu (DTPA-extractable), mg $kg^{-1}$ | 1.89 | - |
| Total-Cu (XRF), mg $kg^{-1}$ | - | 7.50 |
| Mn (DTPA-extractable), mg $kg^{-1}$ | 7.44 | - |
| Total-Mn (XRF), mg $kg^{-1}$ | - | 0.01 |

## Experimental Concept and Plant Growing Conditions for the Sorghum Plants

In this study, which aimed to mitigate B toxicity in two sorghum cultivars (*Sorghum bicolor* L. cvs. Akdari and Ogretmenoglu) using WRHP, a greenhouse experiment was conducted under natural lighting conditions at the Faculty of Agriculture, Ankara University (39°57′44.51″ N; 32°51′46.95″ E) between August 6 and October 10, 2024. Soil samples used in the experiment were collected from the research field of the same faculty at a depth of 0–20 cm. Selected physical and chemical properties of the soil were determined using the methods outlined in Table 1. The soil was air-dried, sieved through a 4-mm mesh, and 2 kg of soil was placed into each pot. Basal fertilization was applied as a liquid solution at rates of 150 mg N $kg^{-1}$ (from $NH_4NO_3$) and 100 mg P $kg^{-1}$ and 125 mg K $kg^{-1}$ (from $KH_2PO_4$).

Boron was added to the all pots as a liquid solution at a rate of 10 mg B $kg^{-1}$ using $Na_2B_8O_{13}{\cdot}4H_2O$ as the source, and the soils were incubated for two weeks. After fertilization and B application, ten sorghum seeds were sown in each pot. One week after emergence, the seedlings were thinned to five plants per pot to maintain uniform plant density. The experimental treatments consisted of: (1) Control, (2) WRHP at 0.5 g $kg^{-1}$, (3) WRHP at 1.0 g $kg^{-1}$, and (4) WRHP at 2.0 g $kg^{-1}$. The experiment was arranged in a randomized plot design with four replications. During the experimental period, pots were weighed daily and irrigated with the required amount of water to maintain 70% of field capacity.

For the biochemical analysis, fresh and fully matured leaves were randomly collected before harvest for immediate analysis, while the remaining leaves were stored at -20 °C. The plants were harvested by cutting them at the soil surface, thoroughly washed with tap water followed by distilled water, and dried at 65 °C until all moisture was removed. The dry weight of the samples was determined using a precision balance (accuracy: 0.01 g). The dried samples were ground using a static grinder, sieved through a 0.20 mm mesh, and prepared for plant analysis. Additionally, 100 g of soil samples were collected after harvesting for the soil analysis.

### Biochemical Analysis

Malondialdehyde (MDA) determination was estimated according to an optimized method of Dhindsa and Matowe (1981); Gunes et al. (2025a)

The concentration of $H_2O_2$ was determined using Loreto and Velikova (2001); Gunes et al. (2025a)

The non-enzymatic total antioxidant activity was estimated by the method of Prieto et al. (1999). The assay is based on the reduction of Mo(VI) to Mo(V), and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. 0.5 g of dry shoot samples was homogenized in 10 mL ethanol and centrifuged at 10,000 g for 5 min. Ethanolic extract (0.1 mL) was combined with 3 mL of reagent solution [0.6 M sulfuric acid ($H_2SO_4$), 28 mM sodium phosphate, and 4 mM ammonium molybdate]. The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid on a DW basis.

Frozen leaf powder samples (1 g) were homogenized in a cold mortar with 4 mL of 1 M phosphate buffer (pH 7.0) containing 0.1 mM Na-EDTA (10 mL). The homogenate was centrifuged at 15.000 rpm for 15 min at 4 °C, and the supernatant was used to measure antioxidant enzyme activities (Tejera García et al. 2004).

Superoxide dismutase activity estimation was based on the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) and was assayed by the method of Cakmak and Marschner (1992). One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction at 25 °C. The activity of SOD was expressed at unit $min^{-1}$ $g^{-1}$ fresh weight (FW). The absorbance was read at 650 nm using a spectrophotometer (Shimadzu UV-VIS 1201, Japan).

Catalase activity was determined by monitoring the rate of absorbance decrease at 240 nm for 3 min following the consumption of $H_2O_2$ (Aebi 1984). Enzyme activity was calculated using an extinction coefficient of 40 $mM^{-1}$ $cm^{-1}$.

Ascorbate peroxidase activity was assayed as a decrease in absorbance at 290 nm for 1 min according to Amako et al. (1994). APX activity was calculated using an extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$ for ascorbate.

### Total Elemental Analysis

Ground plant samples were digested in a furnace at 550 °C for 8 h, then the ashes were dissolved in 10 N $HNO_3$ and diluted to 50 mL with ultra-pure water. The extracts were then filtered and stored in plastic vials until analysis. Due to the higher analytical reliability of spectrophotometric methods for P, B, and Si determinations, these were analyzed using spectrophotometric techniques. Total B and P concentrations of sorghum plants were measured spectrophotometrically using a Shimadzu UV-VIS 1201 (Japan) at 420 and 430 nm for B (Wolf 1971) and P (Barton 1948), respectively. For Si determination, plant samples were heated in a muffle furnace at 500 °C for 3 h, and the ashes were dissolved in 2 mL of HF. Silicon was then determined using the colorimetric molybdate blue method as described by van der Vorm (1987). The use of HF in Si analysis may damage the quartz torch of ICP-OES systems; therefore, spectrophotometric determination was preferred to avoid instrumental limitations and to ensure accurate measurements. Wheat flour with the code 1567a (NIST) was used as the standard reference material for element analysis.

### Post-Harvesting Soil Sample Analysis

Plant available B concentration of soil was determined according to Wolf (1971) and available P concentration of soil was determined according to Olsen (1954). Besides available Si concentration of soil was determined according to Snyder (2001).

### Statistical and Multivariate Analysis

The experiment was conducted in a completely randomized design with four replicates, each replicate representing an individual pot. The significance of treatments was determined with two-way ANOVA, and the significant differences among the treatment means were calculated by Tukey's honestly significant difference (HSD) test at $p < 0.05$ using OriginPro 2025b software (Steel et al. 1997). Principal component analysis (PCA) was performed on the correlation matrix of WRHP treatments in sorghum plants to examine multivariate relationships among the measured variables. The analysis was conducted using standardized variables, and principal components were derived as linear combinations of the original variables weighted by their respective loadings. PCA plots and correlation triangle were generated using OriginPro 2025b software (OriginLab, Northampton, MA).

## Results

### Characterization of Bio-Composite WRHP

The WRHP bio-composite was produced by the alkaline hydrolysis of SW and RH using sodium silicate and potassium hydroxide, followed by neutralization with phosphoric acid. The resulting material contains hydrolyzed keratin

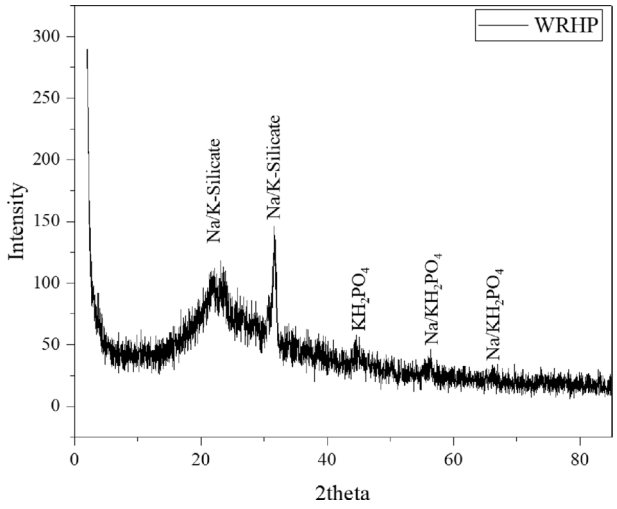



**Fig. 1** X-ray diffraction (XRD) pattern of WRHP

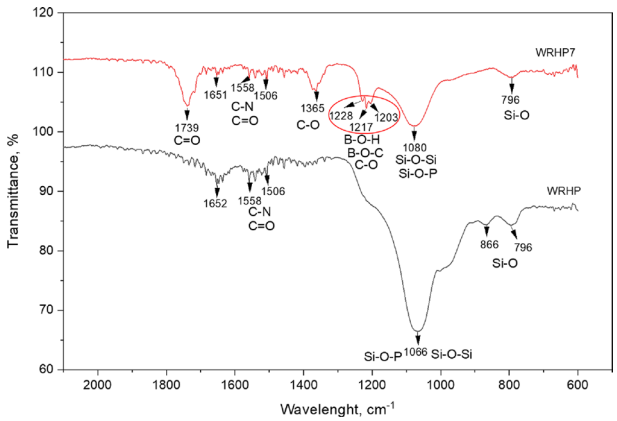



**Fig. 2** Fourier transform infrared spectroscopy (FTIR) spectrum of WRHP and WRHP7

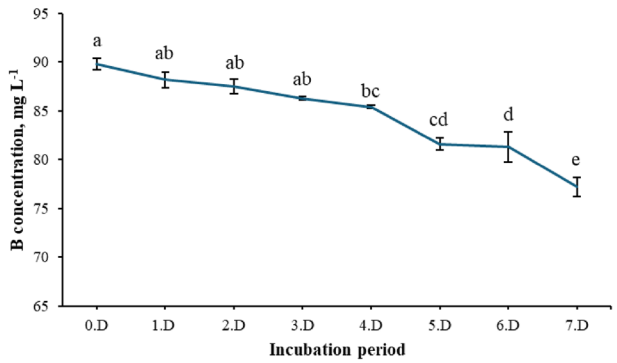



**Fig. 3** The 7-day boron (B) adsorption test in the WRHP (D: day)

along with Si, P, and K bearing inorganic phases. Its structural features were examined using XRD and FTIR spectroscopy. The XRD pattern of WRHP showed two strong peaks with shoulder-like features at 21° and 23° 2θ, and a sharper and more intense peak at 31° 2θ. In addition, weak reflections at 46°, 56°, and 68° 2θ (Fig. 1). FTIR analysis of the material revealed a dominant peak at 1066 $cm^{-1}$, accompanied by relatively weaker peaks at 796, 866, 1506, 1558, and 1652 $cm^{-1}$ (Fig. 2).

## Boron Adsorption Test of Bio-Composite WRHP

During the 7-day B adsorption experiment, the B concentration in the solution progressively declined over time, and this decrease was found to be statistically significant. On day zero, 89.9 mg $L^{-1}$ B was obtained in the solution, while this value decreased to 77.2 mg $L^{-1}$ B by the end of day 7. Additionally, the B content of the raw B solution was determined as 91.6 mg $L^{-1}$ B (Fig. 3). After the adsorption test, WRHP was separated from the solution, thoroughly washed, and dried; this material was designated as WRHP7. Both materials were analyzed by FTIR to identify the presence of functional groups related to B. Accordingly, in WRHP7, new peaks were observed at 1203, 1217, and 1228 $cm^{-1}$, which were not present in WHRP (Fig. 2).

## Dry Weight of Sorghum Plants

The cultivar × WRHP interaction was not statistically significant for plant dry weight. However, regardless of sorghum cultivar and WRHP dose, plant dry weight increased significantly with WRHP application (Table 2, Figure S1 and S2).

## Boron, Silicon, and Phosphorus Concentrations in Sorghum Plants

WRHP application was effective in reducing B concentration in both varieties. In the cv. Akdari grown in B-contaminated soil, the B concentration was 189 mg $kg^{-1}$, which decreased below 99.3 mg $kg^{-1}$ with WRHP application, regardless of the dose. In the cv. Ogretmenoglu, the B concentration in the control was 115.7 mg $kg^{-1}$, which decreased to 75.7 mg $kg^{-1}$ with 2 g $kg^{-1}$ WRHP application (Fig. 4).

WRHP applications also positively affected Si concentration in plants. Although the increase was not statistically significant in cv. Akdari, Si concentration in cv. Ogretmenoglu increased from 9.60 g $kg^{-1}$ in the control to 11.9 and 14.1 g $kg^{-1}$ following the application of 1 and 2 g $kg^{-1}$ WRHP, respectively (Table 2).

The interaction between cultivar and WRHP applications was not statistically significant for plant P concentration. Regardless of sorghum cultivar, plant P concentration showed a significant increase compared to the control, particularly at the highest WRHP dose (2 g $kg^{-1}$) (Table 2).

**Table 2** Effect of WRHP on plant dry weight, total silicon (Si), and total phosphorus (P) concentrations of sorghum cultivars growing in boron-toxic soil

| Treatments | | Dry weight,g pot$^{-1}$ | Si,g kg$^{-1}$ | P,g kg$^{-1}$ |
|---|---|---|---|---|
| Cultivar x WRHP Interaction | | | | |
| Akdari | Control ($WRHP_0$) | 10.4±0.39 a | 12.0±1.22 a | 2.64±0.06 ab |
| | $WRHP_{0.5}$ | 11.5±1.14 a | 12.3±2.23 a | 2.48±0.14 b |
| | $WRHP_1$ | 11.4±1.17 a | 12.4±1.59 a | 2.88±0.12 ab |
| | $WRHP_2$ | 12.0±0.72 a | 12.5±1.45 a | 2.97±0.11 a |
| Ogretmenoglu | Control ($WRHP_0$) | 8.34±0.16 b | 9.60±0.87 c | 2.06±0.14 b |
| | $WRHP_{0.5}$ | 10.6±1.17 a | 10.9±0.65 bc | 2.26±0.09 b |
| | $WRHP_1$ | 10.4±1.15 a | 11.9±0.78 b | 2.45±0.07 ab |
| | $WRHP_2$ | 11.1±0.39 a | 14.1±0.98 a | 2.85±0.17 a |
| Cultivar | Akdari | 11.3±0.25 a | 12.3±0.36 a | 2.74±0.07 a |
| | Ogretmenoglu | 10.1±0.33 b | 11.6±0.46 a | 2.41±0.09 b |
| WRHP Dose | Control ($WRHP_0$) | 9.38±0.40 b | 10.8±0.57 b | 2.35±0.13 b |
| | $WRHP_{0.5}$ | 11.1±0.41 a | 11.7±0.60 ab | 2.37±0.09 b |
| | $WRHP_1$ | 10.9±0.42 a | 12.1±0.42 ab | 2.67±0.10 ab |
| | $WRHP_2$ | 11.5±0.26 a | 13.3±0.51 a | 2.91±0.09 a |
| F | Cultivar | 15.6$^{**}$ | 1.96$^{ns}$ | 16.9$^{**}$ |
| | WRHP | 8.88$^{**}$ | 5.07$^{*}$ | 10.6$^{**}$ |
| | Cultivar x WRHP | 0.82$^{ns}$ | 3.34$^{*}$ | 1.66$^{ns}$ |

The values are means of four replicates±standard errors. Different letters in each column represent significant difference at $p<0.05$ level based on Tukey's honestly significant difference (HSD) test. ns: not significant; *: $p<0.05$; **: $p<0.01$.

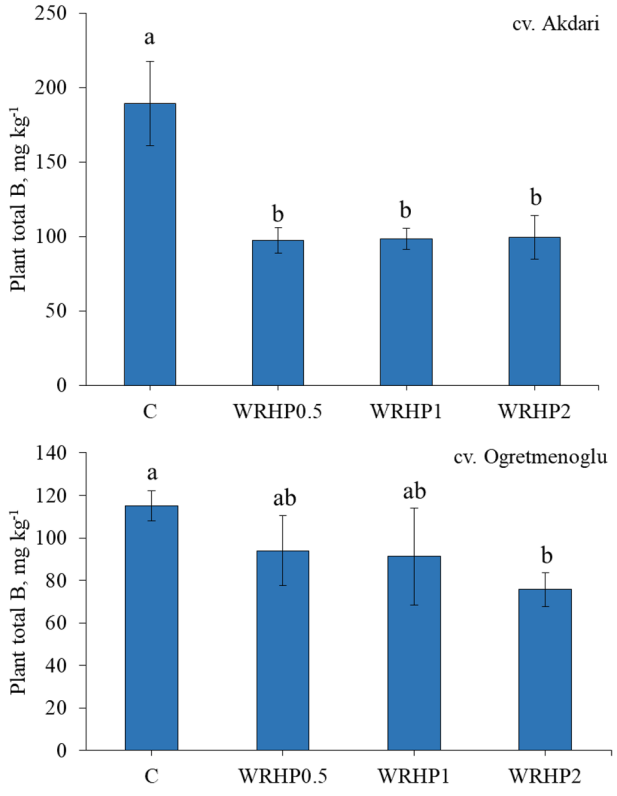



**Fig. 4** Effect of WRHP on plant boron concentration of sorghum cultivars growing under boron-toxic soil. The values are means of four replicates ± standard error. Different letters in each column represent a significant difference at the $p<0.05$ level based on the Tukey HSD test

## Hydrogen Peroxide ($H_2O_2$) and Malondialdehyde (MDA) Concentrations

In cv. Akdari, the $H_2O_2$ concentration was 37.9 mmol kg$^{-1}$ FW$^{-1}$ in the control and decreased to 22.2 and 24.6 mmol kg$^{-1}$ FW$^{-1}$ with the 0.5 and 2 g kg$^{-1}$ WRHP applications, respectively. In cv. Ogretmenoglu, regardless of WRHP dose, the $H_2O_2$ concentration decreased from 46.6 mmol kg$^{-1}$ FW$^{-1}$ to around 20.0 mmol kg$^{-1}$ FW$^{-1}$ (Table 3). The cultivar × WRHP dose interaction was not significant for MDA content. Regardless of cultivar differences, plant MDA content significantly decreased, particularly at the 1 and 2 g kg$^{-1}$ WRHP doses (Table 3).

## Non-Enzymatic Antioxidants Level

In cv. Akdari, regardless of the WRHP application rates, non-enzymatic antioxidants increased in plants treated with WRHP compared to the control. Similarly, in cv. Ogretmenoglu, WRHP applications also resulted in an increase; however, these increases were statistically significant at the 1 and 2 g kg$^{-1}$ WRHP doses (Table 3).

## Superoxide Dismutase, Catalase and Ascorbate Peroxidase Enzyme Activities

Superoxide dismutase activity in both cultivars decreased with WRHP treatments; however, this reduction was statistically significant only in the cv. Ogretmenoglu variety at the highest dose. The cultivar × WRHP dose interaction was not significant for CAT activity. Catalase enzyme activity significantly decreased at 1 and 2 g kg$^{-1}$ WRHP application doses in the cv. Ogretmenoglu. Ascorbate peroxidase enzyme activities were significantly reduced in both varieties, regardless of the applied WRHP doses (Table 4).

**Table 3** Effect of WRHP on $H_2O_2$ and MDA concentration and nonenzymatic total antioxidants of sorghum cultivars growing in boron-toxic soil

| Treatments | | $H_2O_2$ mmol $kg^{-1}$ $FW^{-1}$ | MDA mmol $kg^{-1}$ $FW^{-1}$ | Nonenzymatic antioxidants µmol $g^{-1}$ $DW^{-1}$ |
|---|---|---|---|---|
| Cultivar x WRHP Interaction | | | | |
| Akdari | Control ($WRHP_0$) | 37.9±2.20 a | 7.61±0.44 a | 34.4±1.29 b |
| | $WRHP_{0.5}$ | 22.2±1.03 c | 5.84±0.42 ab | 47.7±1.36 a |
| | $WRHP_1$ | 31.4±2.71 ab | 5.45±0.40 b | 50.2±1.94 a |
| | $WRHP_2$ | 24.6±2.01 bc | 5.29±0.50 b | 47.8±0.33 a |
| Ogretmenoglu | Control ($WRHP_0$) | 46.6±1.22 a | 6.23±0.59 a | 38.3±0.95 c |
| | $WRHP_{0.5}$ | 20.8±0.99 b | 5.77±0.28 a | 44.4±1.91 bc |
| | $WRHP_1$ | 21.7±1.84 b | 5.19±0.47 a | 50.2±2.24 ab |
| | $WRHP_2$ | 22.2±1.53 b | 4.90±0.19 a | 54.3±1.58 a |
| Cultivar | Akdari | 29.0±1.84 a | 6.05±0.31 a | 45.0±1.67 a |
| | Ogretmenoglu | 27.8±2.88 a | 5.52±0.91 a | 46.8±1.74 a |
| WRHP Dose | Control ($WRHP_0$) | 42.3±2.02 a | 6.92±0.43 a | 36.4±1.05 c |
| | $WRHP_{0.5}$ | 21.5±0.71 c | 5.81±0.23 ab | 46.0±1.25 b |
| | $WRHP_1$ | 26.6±2.37 b | 5.32±0.29 b | 50.2±1.09 a |
| | $WRHP_2$ | 23.4±1.26 bc | 5.09±0.26 b | 51.0±1.43 a |
| F | Cultivar | $0.89^{ns}$ | $2.99^{ns}$ | $3.07^{ns}$ |
| | WRHP | $56.0^{**}$ | $7.18^{**}$ | $44.6^{**}$ |
| | Cultivar x WRHP | $8.91^{**}$ | $0.95^{ns}$ | $4.54^{**}$ |

The values are means of four replicates±standard errors. Different letters in each column represent significant difference at $p<0.05$ level based on Tukey's honestly significant difference (HSD) test. ns: not significant; **: $p<0.01$.

**Table 4** Effect of WRHP on CAT, APX, and SOD enzyme activities of sorghum cultivars growing in boron-toxic soil

| Treatments | | CAT mmol $g^{-1}$ FW $min^{-1}$ | APX mmol $g^{-1}$ FW $min^{-1}$ | SOD unit $g^{-1}$ |
|---|---|---|---|---|
| Cultivar x WRHP Interaction | | | | |
| Akdari | Control ($WRHP_0$) | 0.15±0.01 a | 1.64±0.17 a | 326±8.79 a |
| | $WRHP_{0.5}$ | 0.13±0.01 a | 0.43±0.04 b | 307±12.7 a |
| | $WRHP_1$ | 0.11±0.02 a | 0.38±0.04 b | 298±19.1 a |
| | $WRHP_2$ | 0.10±0.03 a | 0.36±0.03 b | 295±7.29 a |
| Ogretmenoglu | Control ($WRHP_0$) | 0.30±0.02 a | 1.37±0.23 a | 1686±74.9 a |
| | $WRHP_{0.5}$ | 0.23±0.01 ab | 0.75±0.04 b | 1315±184 ab |
| | $WRHP_1$ | 0.18±0.01 b | 0.66±0.05 b | 1392±110 ab |
| | $WRHP_2$ | 0.17±0.02 b | 0.62±0.09 b | 939±61.7 b |
| Cultivar | Akdari | 0.12±0.01 b | 0.70±0.15 a | 306±6.52 b |
| | Ogretmenoglu | 0.22±0.02 a | 0.85±0.09 a | 1333±86.7 a |
| WRHP Dose | Control ($WRHP_0$) | 0.22±0.03 a | 1.50±0.14 a | 1006±259 a |
| | $WRHP_{0.5}$ | 0.18±0.02 ab | 0.59±0.07 b | 811±209 ab |
| | $WRHP_1$ | 0.15±0.02 b | 0.52±0.06 b | 845±213 ab |
| | $WRHP_2$ | 0.14±0.02 b | 0.49±0.07 b | 617±125 b |
| F | Cultivar | $55.6^{**}$ | $3.32^{ns}$ | $299^{**}$ |
| | WRHP | $9.80^{**}$ | $37.4^{**}$ | $7.24^{**}$ |
| | Cultivar x WRHP | $2.27^{ns}$ | $3.01^{ns}$ | $6.21^{**}$ |

The values are means of four replicates±standard errors. Different letters in each column represent significant difference at $p<0.05$ level based on Tukey's honestly significant difference (HSD) test. ns: not significant; **: $p<0.01$

## Plant-Available Boron, Silicon and Phosphorus Concentrations in Soils After the Experiment

After the experiments were completed and the plants were harvested, soil samples were taken from the pots, and the plant-available concentrations of B, Si, and P in the soils were determined. The cultivar × WRHP interaction was not statistically significant for the plant-available B concentration of the soils. However, the effects of cultivar and WRHP doses were significant. After harvest, the plant-available B concentration was 2.27 mg $kg^{-1}$ in soils where cv. Akdari was grown, whereas it was determined as 1.41 mg $kg^{-1}$ in soils where cv. Ogretmenoglu was cultivated. Depending on the WRHP application rates, soil B concentration significantly decreased at the 1 and 2 g $kg^{-1}$ doses compared to the control (Table 5).

Following harvest, the Si concentration in the soils increased significantly compared to the control, particularly at higher WRHP application doses. Similarly, WRHP applications enhanced P levels in soils where both genotypes were grown, with increases being more pronounced at higher application rates (Table 5).

**Table 5** Effect of WRHP on post-harvest soil available boron (B), silicon (Si) and phosphorus (P) concentrations

| Treatments | | Post-harvest soil available B mg $kg^{-1}$ | Post-harvest soil available Si mg $kg^{-1}$ | Post-harvest soil available P mg $kg^{-1}$ |
|---|---|---|---|---|
| Cultivar x WRHP Interaction | | | | |
| Akdari | Control ($WRHP_0$) | 2.92±0.29 a | 55.6±2.67 b | 25.1±3.49 c |
| | $WRHP_{0.5}$ | 2.65±0.27 a | 62.5±3.34 ab | 32.9±2.67 bc |
| | $WRHP_1$ | 1.84±0.18 b | 63.9±2.53 a | 40.1±2.77 b |
| | $WRHP_2$ | 1.67±0.21 b | 68.7±4.62 a | 56.1±5.85 a |
| Ogretmenoglu | Control ($WRHP_0$) | 2.11±0.27 a | 58.7±4.31 b | 16.2±0.91 d |
| | $WRHP_{0.5}$ | 1.95±0.28 a | 62.9±1.23 ab | 26.8±4.45 c |
| | $WRHP_1$ | 0.85±0.18 b | 66.1±6.24 ab | 34.9±4.76 b |
| | $WRHP_2$ | 0.75±0.18 b | 68.8±2.33 a | 53.5±3.95 a |
| Cultivar | Akdari | 2.27±0.15 a | 62.6±1.43 a | 38.6±3.08 b |
| | Ogtermenoglu | 1.41±0.17 b | 64.1±1.33 a | 32.8±3.62 a |
| WRHP Dose | Control ($WRHP_0$) | 2.52±0.18 a | 57.1±13.8 c | 20.7±1.88 d |
| | $WRHP_{0.5}$ | 2.30±0.16 a | 62.7±5.46 b | 29.8±1.67 c |
| | $WRHP_1$ | 1.34±0.20 b | 65.0±20.8 ab | 37.5±1.60 b |
| | $WRHP_2$ | 1.21±0.18 b | 68.8±11.5 a | 54.8±1.71 a |
| F | Cultivar | 102$^{**}$ | 1.21$^{ns}$ | 17.3$^{**}$ |
| | WRHP | 61.0$^{**}$ | 13.7$^{**}$ | 111$^{**}$ |
| | Cultivar x WRHP | 0.55$^{ns}$ | 0.29$^{ns}$ | 0.89$^{ns}$ |

The values are means of four replicates±standard errors. Different letters in each column represent significant difference at $p<0.05$ level based on Tukey's honestly significant difference (HSD) test. ns: not significant; **: $p<0.01$.

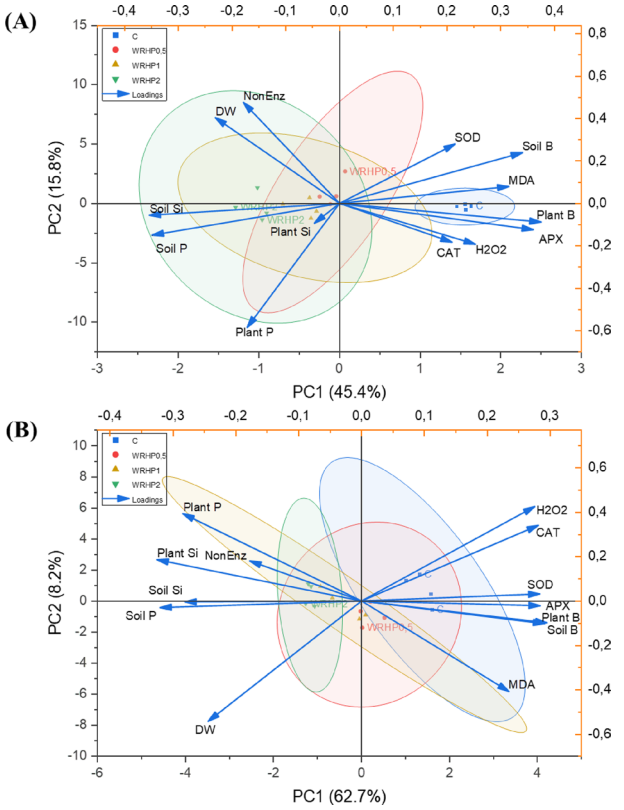



**Fig. 5** The biplots of principal component analyses (PCA) of sorghum cultivars (A: cv. Akdari; B: cv. Ogretmenoglu; DW: Dry weight; Plant B: Plant boron; Plant Si: Plant silicon; Plant P: Plant phosphorus; Soil B: Soil boron; Soil Si: Soil silicon; Soil P: Soil phosphorus; H2O2:Hydrogen peroxide; MDA: Malondialdehyde; NonEnz: non-enzymatic total antioxidant; CAT: Catalase; SOD: Superoxide dismutase; APX: Ascorbate peroxidase)

## Principal Component and Pearson Correlation Analysis of Experiment

In the cv. Akdari, principal component analysis (PCA) showed a total variability of 61.2% (PC1 = 45.4% and PC2 = 15.8%) (Fig. 5A). According to Kaiser's rules, eigenvalues greater than 1.0 are considered descriptive of the variance in a data set (Kaiser 1964) and eigenvalues of dry weight (5.90), plant B (2.06), plant Si (1.47) and plant P (1.06) concentration were greater than 1.0. The greatest eigenvalue was obtained from dry weight (Table S1 and S3). According to the PCA analysis, all WRHP treatments were effective on dry weight, plant and soil Si and P concentrations, and non-enzymatic antioxidant concentrations. It was shown that WRHP reduced the soil B concentration, the levels of stress markers such as MDA and $H_2O_2$ and enzymes such as CAT, SOD, and APX compared to B treatment solely (Fig. 5A). According to the correlation analysis performed, a negative correlation was detected between dry weight and plant B, P, MDA, $H_2O_2$, CAT, APX, and soil B in the cv. Akdari. Similarly, a negative relationship was determined between plant B and dry weight of plant B, P, Si, soil P and Si and non-enzymatic antioxidants (Fig. 5A).

In the cv. Ogretmenoglu, the PCA showed a total variability of 70.9% (PC1 = 62.7% and PC2 = 8.20%) (Fig. 5B). The eigenvalues of dry weight (8.15) and plant B (1.06) concentration were greater than 1.0. As in the cv. Ogretmenoglu, the greatest eigenvalue was obtained from dry weight (Table S2 and S4). According to the PCA analysis, especially increasing doses of WRHP treatments ($WRHP_1$ and $WRHP_2$) were effective on dry weight, plant, and soil

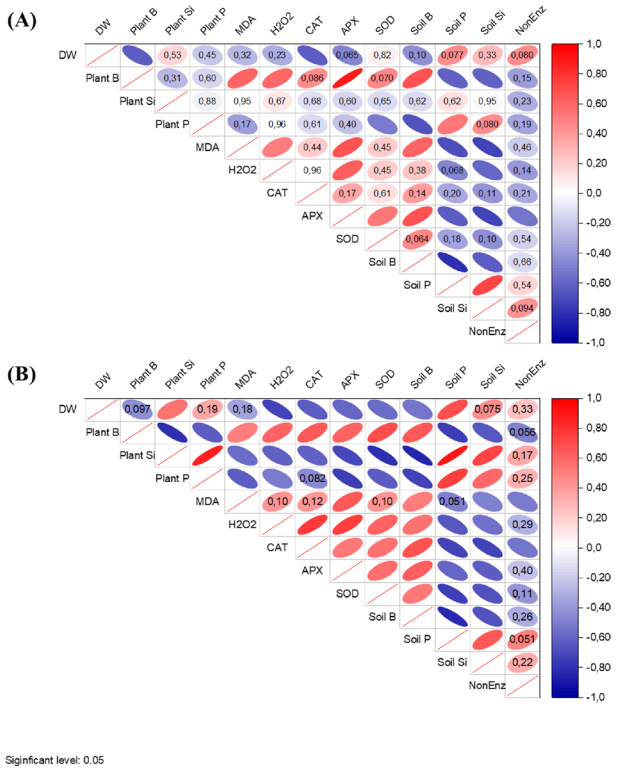



**Fig. 6** Pearson correlation of sorghum cultivars (A: cv. Akdari; B: cv. Ogretmenoglu; DW: Dry weight; Plant B: Plant boron; Plant Si: Plant silicon; Plant P: Plant phosphorus; Soil B: Soil boron; Soil Si: Soil silicon; Soil P: Soil phosphorus; H2O2:Hydrogen peroxide; MDA: Malondialdehyde; NonEnz: non-enzymatic total antioxidant; CAT: Catalase; SOD: Superoxide dismutase; APX: Ascorbate peroxidase)

Si and P concentrations and non-enzymatic antioxidants. It was shown that WRHP reduced the plant and soil B concentration and levels of stress markers such as MDA and $H_2O_2$ and enzymes such as CAT, SOD, and APX compared to B treatment solely (Fig. 5B). The PCA analysis revealed that the response of both cultivars to WRHP treatments under B toxicity was quite similar, with the only difference being that cv. Ogretmenoglu exhibited a higher response to increasing WRHP treatment doses (Fig. 5A and B). As a result of the correlation analysis, a negative correlation was determined between dry weight and plant B, MDA, $H_2O_2$, CAT, APX, SOD, and soil B (Fig. 5B). In addition, in the cv. Ogretmenoglu, a negative relationship was observed between plant B and dry weight, and between plant and soil P and Si, and non-enzymatic antioxidants. It was also determined that these relationships were similar in the cv. Akdari (Fig. 5A and B, and Fig. 6A and B).

## Discussion

The present study demonstrates that the WRHP bio-composite, synthesized from waste sheep wool and rice husk via alkaline hydrolysis and phosphoric acid neutralization, effectively mitigates B toxicity in sorghum cultivars while enhancing plant Si uptake. The FTIR spectrum of the WRHP bio-composite exhibited a dominant and sharp band at 1066 $cm^{-1}$, which is characteristic of Si-O-Si and Si-O-P asymmetric stretching vibrations and reflects the combined silica–phosphate structure formed during alkaline hydrolysis and subsequent phosphoric acid neutralization. Additional bands at 796 and 866 $cm^{-1}$ correspond to Si-O bending modes and possible carbonate-associated vibrations, while peaks at 1506, 1558, and 1652 $cm^{-1}$ indicate the presence of organic functional groups derived from the keratin fraction of SW, particularly amide-related C-N and C=O vibrations. The FTIR profile confirms the coexistence of silica, phosphate, and keratin-derived functional groups within the WRHP composite structure.

In the XRD pattern of the WRHP, the sharp and intense peak at 31° is typically indicative of a well-ordered atomic lattice belonging to a crystalline phase. In contrast, the shoulder-like features observed around 21–23° are most likely the result of the superposition of two components: (1) a broad amorphous hump, characteristic of amorphous silica and generally appearing within the 20–25° range, and (2) one or more narrow crystalline peaks (e.g., quartz or alkali–silicate phases) that form the shoulder. This pattern indicates the coexistence of both amorphous and crystalline contributions. Amorphous Si usually produces a single broad hump, whereas sharp, high-intensity peaks signify crystalline structures. Thus, the shoulder formation is typically caused by the overlap of a broad amorphous hump with adjacent crystalline peaks. Various crystalline or hydrated forms of Na and K-silicates can also generate reflections within the 20–30° region, and the presence of $Na_2SiO_3$ and KOH in WRHP strengthens this possibility. Following neutralization, phosphate salts formed from $K^+$ and $PO_4^{3-}$ may crystallize and often exhibit weak reflections in the 40–70° region; therefore, the weak peaks observed at 46°, 56°, and 68° may correspond to phosphate salts or other secondary crystalline phases. The XRD and FTIR results confirm the formation of a bio-composite containing both amorphous and crystalline silicate phases along with organic keratin-derived structures.

Although the plants were supplied with basal fertilization of N, P, and K during the experiment, WRHP treatments significantly improved the growth of sorghum in B-contaminated soil, as reflected by increased dry weights, particularly in the cv. Ogretmenoglu. This enhancement is likely associated with the reduction in B accumulation in

plant tissues. Both cultivars exhibited substantial decreases in shoot B concentration with WRHP application, with the cv. Akdari, showing reductions below 99.3 mg $kg^{-1}$ regardless of dose, and cv. Ogretmenoglu showing a dose-dependent decline to 75.7 mg $kg^{-1}$ at the highest application dose. These findings suggest that WRHP effectively adsorbs B in the soil and/or modifies its bioavailability, consistent with previous reports on keratin- and silica-based materials as B-immobilizing amendments (Inal et al. 2009; Soylemezoglu et al. 2009; Liu et al. 2017; Sahin et al. 2025; Gunes et al. 2025a). The effect of WRHP on B adsorption was clearly demonstrated in the adsorption experiment. During a one-week incubation period, WRHP decreased the B concentration in the solution from 91.6 mg $L^{-1}$ to 77.2 mg $L^{-1}$, indicating its considerable B retention capacity. Hovewer, It should be noted that this test was designed as a preliminary assessment of B adsorption behavior and does not represent a comprehensive adsorption isotherm study. Furthermore, FTIR analysis performed following the adsorption assay provided evidence for the formation of WRHP-B complexes. To the best of our knowledge, this study presents the first spectroscopic indication of complex formation between WRHP and B. In addition to this, the peaks at 1228, 1217, and 1203 $cm^{-1}$ in WRHP7 generally indicate C-O, B-O-C, or B-O-H stretching vibrations (Romanos et al. 2013; Tan et al. 2020; Zielinkiewicz et al. 2023). The FTIR changes and reduced B concentration in adsorption test strongly support that WRHP adsorbed B from the solution. The new peaks appearing in WRHP7 indicate that B formed chemical or coordinative bonds within the polymer matrix.

In addition to reducing B accumulation, WRHP enhanced Si concentrations in plant tissues, especially in the cv. Ogretmenoglu, where Si increased from 9.60 g $kg^{-1}$ in the control to 14.1 g $kg^{-1}$ at 2 g $kg^{-1}$ WRHP. This result indicates that the bio-composite not only mitigates toxic B effects but also acts as a Si source, which may strengthen plant cell walls and membranes, improve stress tolerance, and facilitate selective nutrient uptake (Gunes et al. 2007a; Souri et al. 2021; Ali and Bijay-Singh 2025). WRHP effects on P were less pronounced, although a significant increase was observed the plants at the highest dose, likely reflecting minor contributions of phosphoric acid-treated WRHP to plant-available P.

Malondialdehyde is a reactive aldehyde and a well-known biomarker of oxidative stress and lipid peroxidation in plants. MDA is primarily produced during peroxidation of polyunsaturated fatty acids in cell membranes. Reactive oxygen species like $O_2^-$ and $H_2O_2$ attack membrane lipids, breaking them down into aldehydes, including MDA. Its accumulation indicates membrane damage caused by oxidative stress (Mansoor et al. 2022; Gulcin 2025). In plants, high MDA levels are commonly observed under abiotic stresses like B toxicity (Karabal et al. 2003; Gunes et al. 2006, 2007a, b; Choudhary et al. 2020; Zhao et al. 2024). The observed decreases in oxidative stress markers, including MDA and $H_2O_2$, further support the protective role of WRHP. Both genotypes showed reduced MDA and $H_2O_2$ levels, with significant reductions in the cv. Akdari variety at 1 and 2 g $kg^{-1}$ doses. In parallel with the decline in oxidative stress markers, WRHP application also enhanced the non-enzymatic antioxidant pool in both genotypes. In cv. Akdari, non-enzymatic antioxidants increased compared to the control regardless of WRHP dose. Similarly, in cv. Ogretmenoglu, WRHP treatments promoted increases in non-enzymatic antioxidants, with statistically significant improvements at the 1 and 2 g $kg^{-1}$ doses. This coordinated increase in non-enzymatic antioxidants, together with the reductions in $H_2O_2$ and MDA, indicates that WRHP not only limits ROS generation through B immobilization and Si supply but also strengthens the overall antioxidant buffering capacity of the plants. This reduction indicates that WRHP alleviates B-induced oxidative damage, likely through both the immobilization of B and the supply of Si, which enhances the antioxidant defense system. Correspondingly, activities of key antioxidant enzymes (SOD, CAT and APX) decreased following WRHP applications. Importantly, this decline was accompanied by simultaneous reductions in $H_2O_2$ and MDA levels and improvements in plant growth, indicating that ROS generation was lowered rather than detoxification capacity being suppressed. If Si had exerted a direct inhibitory effect on these enzymes, oxidative stress markers would be expected to accumulate due to impaired ROS scavenging. Instead, the coordinated decrease in ROS, lipid peroxidation, and enzyme activities suggests a reduced oxidative burden. Therefore, the lower antioxidant enzyme activities observed in WRHP-treated plants reflect stress alleviation and normalization of redox homeostasis, rather than direct enzymatic inhibition by Si (Gunes et al. 2007a, b, 2025a; Rao et al. 2025).

Analysis of soil after harvest confirmed that WRHP reduced plant-available B and increased soil Si content, particularly at higher doses. The dose-dependent decline in B concentrations in soils planted with both sorghum genotypes aligns with the observed reductions in plant tissue B, suggesting that WRHP retains B in the soil matrix or promotes its immobilization. The concomitant increases in soil Si further highlight the dual function of WRHP as both a detoxifying agent and a Si fertilizer, consistent with previous studies on silica- and keratin-based soil amendments. The results indicate that WRHP is an effective, multifunctional bio-composite for mitigating B toxicity in sorghum. Its capacity to reduce B uptake, enhance Si accumulation, improve growth, and alleviate oxidative stress demonstrates its potential as a sustainable soil amendment, particularly in

regions prone to B contamination. The dual action of organic functional groups from hydrolyzed keratin and inorganic silicate phases emphasizes the importance of hybrid bio-composites derived from agricultural residues in improving crop resilience and promoting circular economy principles.

## Conclusion

The WRHP bio-composite, derived from waste sheep wool and rice husk, effectively mitigates B toxicity in sorghum by reducing B accumulation in plant tissues and enhancing plant-available Si. Application of WRHP improved plant growth, decreased oxidative stress markers (MDA and $H_2O_2$), and modulated antioxidant enzyme activities, reflecting lower stress levels. Soil analyses confirmed that WRHP immobilizes B while increasing Si availability, demonstrating its dual functionality as a detoxifying amendment and Si source. These results highlight WRHP as a sustainable, multifunctional bio-composite with potential for improving crop resilience in B-contaminated soils and promoting the valorization of agricultural residues. Although no formal economic analysis was conducted, WRHP is produced from waste sheep wool and rice husk, suggesting its potential as a low-cost and sustainable soil amendment suitable for circular economy approaches. Further studies under multi-cycle or field conditions are needed to assess its long-term stability, residual effects, and practical scalability.

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**Author Contributions** Hanife Akca: Investigation; Formal analysis, Validation, Visualization, Writing-original draft, and Review & editing; Mehmet Burak Taskin: Investigation; Formal analysis; Review & editing Burcu Aydogan: Formal analysis; Ezgi Kucukel: Formal analysis; Esra Guneri: Formal analysis; Aydin Gunes: Conceptualization, Methodology, Supervision, Writing-original draft, and Review & editing.

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**Data Availability** The data that support the findings of this study are available from the corresponding authors upon reasonable request.

## Declarations

**Conflict of interest** All authors declared that there is no conflict of interest in current study.

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