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Impact of Thiourea on Wheat's Morpho-Physiological and Ionic Attributes (*Triticum aestivum* L.) under Lead Stress: Reducing the Translocation of Lead from Soil to Roots, Shoots, and Grains

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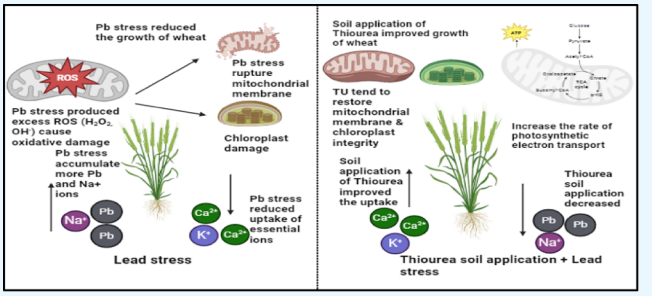
ABSTRACT: Wheat (*Triticum aestivum* L.) is a key cereal crop broadly consumed across the earth. Nonetheless, abiotic stressor influences such as toxic metals severely limit its production. Thiourea (TU) is a sulfur-rich organic molecule that reduces the negative effects of environmental stresses such as heavy metals, including lead (Pb). Thus, the ongoing experiment was designed to assess the impact of thiourea amendment through soil (0 and 100 mg/L) on wheat cultivars Akbar 19 (V_1) and Ghazi 11 (V_2) under lead stress (0 mM and 15 mM Pb). The morphological features of the two cultivars (V_1 and V_2), comprising the fresh weight of shoots (17 and 23%), fresh weight of roots (31 and 26%), leaf area (22 and 10.9%), and total chlorophyll content (16%), were all decreased due to the toxic effects of stress caused by heavy metals. However, treatment of thiourea through soil allowed counteracting the decrease in biomass caused by heavy metals. It improved the initial weight of the shoots upto (12.5 and 14.2%), roots by (37.5 and 24%), leaf surface area upto (17.6 and 7.9%), and total chlorophyll contents (18 and 9.9%) while decreasing the MDA levels by (16.9 and 22.3%) and the activities of H_2O_2 upto (16 and 11.5%), root Pb activity upto (8.9 and 35%) and shoot Pb activity by (12.9 and 23.8%), grain concentration upto (25 and 7.56%), soil Pb content were reduced by(17 and 16%), in both varieties (V_1 as well as V_2). Overall results indicate that treating wheat crops cultivated in pots with external thiourea decreased the damage from oxidation caused by lead and enhanced the antioxidant activity and ionic concentrations. Furthermore, all morpho-physiological parameters exhibited that Ghazi 11 (V_2) performed better relative to Akbar 19 (V_1). Nevertheless, note that research on wheat by application of thiourea-triggered changes in cultivation under heavy metal stress is still in its earliest stages, requiring more investigation to apply in wide fields.



INTRODUCTION

Heavy metal (HM) pollution of soil has grown in importance as a worldwide ecological issue in the past few decades.[1] The US EPA considers lead (Pb) a human carcinogen, making it the most dangerous heavy metal (HM) owing to its recyclable nature.[2] A great deal of productive farmland has been demolished and the collecting of heavy metals (HMs) like Pb, As, and cadmium (Cd) in soils has increased due to the recent quick increase in human-induced activities such as metal plating, the combustion of coal, water supply from wastewater, the production of biological fertilizers, the metallic industry, and automobile drains among others.[3] These heavy metals (HMs) cause environmental contamination and pose a major risk to human health and safety by interfering with the ecological and physiological features of soil.[4] Excessive accumulation of lead in plants causes less germination of seeds, restricts photosynthesis, and elevates oxidative stress and other negative physicochemical effects that lead to a reduction in plant development.[5] Lead (Pb) exposure in humans can result in anemia, bone fractures, neurological damage, kidney disease, irregularities of the cardiac and genital systems, and other health risks.[6] Many restoration strategies, including chemical precipitation, phytoremediation as electrokinetics, a process known for stabilizing it, and the exchange of ions, are being used worldwide in an ongoing effort to clean up Pb-contaminated soils.[7] Because stabilization is simple, inexpensive, and less harmful than other options, it has gained a lot of attention.[8]

Worldwide, wheat (*Triticum aestivum* L.) is a widely grown cereal used as staple food that provides vital nutrients such as

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important nutritional fibers, necessary protein molecules, and carbs for a healthy lifestyle.[9,10] Unique elements that have culinary uses, such as dietary food, minerals, vitamins B and C, lipids, and amino acids, are provided by the bran of wheat and grain germ.[11] Wheat is preferable to other grains because of its substantial nutritional content, which consists of 8−15% protein and 60−80% carbs.[12] Dryness, salt, cold, heat, and metal exposure all have a considerable influence on the yield of cultivars.[12−14] According to Qasim et al. (2022),[15] wheat crops are susceptible to heavy metal stress which modify the majority of biochemical reactions by producing a large amount of reactive oxidative species (ROS) as well as disrupting the electron transport chain, which results in limited development and crop loss.[16] The development of technological advances in agriculture and high-yielding, immune-to-illness wheat cultivars has led to a significant increment in grain cultivation in developing nations.[17] To alleviate world poverty and manage food scarcity, this revolution is crucial. However, the cultivation of wheat is often threatened by several abiotic variables, including heavy metals, drought, salt, and extreme heat stress.[18−20]

Enhancement of plant defenses against environmental stress can be achieved by adding nutrients from minerals or bioregulators, which regulate many biological and physiological processes at the metabolism and entire plant levels.[21] Thiourea (TU), a sulfur-rich cultivation promoter compound, efficiently inhibits oxidative damage caused by environmental factors as well as regulates plant development.[22,23] According to Ahmad et al.,[24] it is a nonphysiological thiol-based ROS scavenger that has a 42% sulfur (S) content as well as a 36% nitrogen (N) content. It can reduce the redox imbalance brought on by stress, as well as various plant ailments. Exogenously administered sulfur-TU increases plant resistance to stress.[25,26] This inference increased the crop yield and development, as well as increased the barrier stability, photosynthetic efficiency, antioxidant potential, and growth.[27,28]

According to several studies, applying S-TU to various crops, including wheat, improves their morpho-physiological, biological, and yield-contributing indicators, which aids them to cope with environmental stresses.[29] The study hypothesized that the treatment of thiourea may ameliorate the adverse effects of Pb stress on wheat varieties. The objective of the current study was to evaluate the alleviating effect of thiourea on wheat (i) to investigate the morpho-physiological and ionic attributes of wheat under Pb stress and TU treatments and (ii) to explore the role of TU applications in improving heavy metal stress tolerance by increasing antioxidant defense systems in wheat.

MATERIALS AND METHODS

The goal of the trial was to determine how thiourea affected wheat under lead stress. The experiment was executed in the University of Agriculture, Faisalabad's Old Botanical Garden. Wheat seeds were obtained from the Ayyub Agricultural Research Center, Faisalabad, namely, cultivars Akbar 19 and Ghazi 11. The study's parameters were: thiourea levels: TU1 = 0 mM (Control), TU2 = 2.5 mM, TU3 = 10 mM; lead stress: Pb1 = 0 mM (Control), Pb2 = 15 mM; V_1 = Akbar 19 and V_2 = Ghazi 11. After three or four soil waterways, plants were raised in plastic pots placed in the soil. Each variety had 18 pots; therefore, ten seeds were put in each of the 36 pots. After establishing seedlings, 6 plants were retained after thinning. Lead stress concentrations of 0 and 15 mM were applied when the seedlings were established after 70 days of sowing. Lead was available in the form of $PbCl_2$ calculated by the specific saturation method. The wheat crop received three irrigations in total during the crucial growth stages. Three concentrations of thiourea TU1 = 0 mM (Control), TU2 = 2.5 mM, and TU3 = 10 mM were applied exogenously (soil application) on the next day of Pb stress application. A completely randomized design (CRD) was used for the experimental trial, with 36 pots used to replicate 3 times, and three-factor factorial design was used to analyze the data statistically. To counteract the harmful effects of stress and accelerate growth, thiourea was utilized as a source of treatment by incorporating it into the soil at the maximal harvesting stage.

Harvesting and Data Collection. Plants were collected after 21 days of thiourea application, leaving 2 plants in each pot for grain analysis. Following harvesting, information was gathered on a range of growth, biological, physiological, and ionic characteristics. For the physio-biochemical investigation, two fresh plants per pot were placed in zipper bags and then frozen at −12 °C.

Morphological Matrices. After the plant specimens were harvested, the roots' and shoots' fresh weights were promptly measured using an electronic weighing balance. Following the determination of their fresh weights, the specimens were overdried for 2 weeks (up to a constant weight) at 65 °C in an oven (model: Memmer Western Germany). Using a computerized weighing balance (model: OHAUS Corporation), the dry mass of the roots as well as the shoots was determined. An inch measuring tape was used to measure the lengths of the shoots as well as the roots.

Photosynthetic Pigments. To test the amount of carotenoids, total chlorophyll, and chlorophyll (*a* and *b*), Velikova et al.[30] procedure was used. Fresh wheat leaves weighing 0.1 g were cut into small pieces, immersed in 5 mL of 80% acetone solution, and kept at 25 °C for the entire night. The resulting mixture's density of light (OD) was then computed using an ultraviolet (UV) spectrophotometer (IRMECO U2020, Germany) at wavelengths of 663, 645, and 480 nm. The following equations were applied to obtain a quantitative estimate of the quantity of chlorophyll from the OD readings:

$$\text{Chl. } a(\text{mg}/g\ \text{FW}) = [12.7(\text{OD}_{663}) - 2.69(\text{OD}_{645})] \times W \times V/1000$$

$$\text{Chl. } b(\text{mg}/g\ \text{FW}) = [22.9(\text{OD}_{645}) - 4.68(\text{OD}_{663})] \times W \times V/1000$$

$$\text{carotenoids } (\text{mg}/g\ \text{FW}) = \text{OD}_{480} + 0.114(\text{OD}_{663}) - 0.638(\text{OD}_{645})]/2500$$

where V is the volume of the extract (mL), and W is the weight of the fresh leaf tissue (g).

Oxidants of Wheat Varieties. The process outlined by Arnon's (1949)[31] was used to monitor the activity of H_2O_2. To evaluate H_2O_2, 0.25 g of fresh leaf samples were crushed in 3 mL of a 0.5% trichloroacetic acid solution. After 15 min of centrifugation, test tubes were filled with 0.5 mL of the specimen extract, 0.5 mL of potassium phosphate buffer, and 1 mL of KI. The spectrophotometer (model: IRMECO U2020, Germany) was used for taking readings at 390 nm after the vortex.

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Malondialdehyde (MDA) levels were determined by Cakmak and Horst.[32] A new sample of the leaf weighing 0.3 g was ground in a 0.5% trichloroacetic acid solution. After centrifuging the leaf samples at 12,000 rpm for 15 min, 1 mL of 0.5% thiobarbituric acid (TBA) in 20% tricarboxylic acid (TCA) solution was added to the test tubes. After being incubated in a hot water bath at 95 °C for 15 min, the samples in the test tubes were kept in ice for an additional 15 min. The value of the absorbance at 532 and 600 nm was measured using a spectrophotometer (IRMECO, Germany).

Antioxidants of Wheat Varieties. A chilled mortar and pestle was used to crush 250 mg of fresh wheat leaves. Each sample received 5 mL of potassium phosphate buffer before it was ground. The sample was homogeneous, and it was then placed into the Eppendorf tubes and centrifuged at 12,000 rpm for 15 min. The product was shifted to an additional Eppendorf tube and kept at −15 °C in the freezer. The procedures specific to each were used to measure the activity of POD, SOD, and CAT.

Chance and Maehly established a technique, as described in ref 33, to quantify the activity of the enzyme catalase (CAT). A cuvette was filled with 0.1 mL of plant material, 1 mL of H_2O_2 solution, and 1.9 mL of cold potassium phosphate buffer. The intensity of the absorbance was determined at 240 nm at a gap of 0, 30, 60, and 90 s using a UV−visible (UV−vis) spectrophotometer (IRMECO U2020, Germany).

The Spitz and Oberly[34] approach was used to quantify the SOD activity. Cuvettes made up of plastic were filled with 0.4 mL of filter water, 250 μL of cold potassium phosphate buffer, 0.1 mL of L-methionine solution, 0.1 mL of Triton X solution, 0.05 mL of nitroblue tetrazolium (NBT) solution, 0.05 mL of plant extract, and 0.05 mL of riboflavin solution. The cuvettes were left in front of a light bulb for 15 min. The blank sample was run, which did not include the plant sample. All samples and the blank sample had their absorbance calibrated at 560 nm using a spectro-analyzer (model: IRMECO U2020, Germany).

The peroxidase activity was calculated using the protocol outlined by Chance and Maehly.[33] The following solutions were added to the cuvette in the following order: 750 μL of K_3PO_4 buffer, 0.1 mL of H_2O_2, 0.1 mL of guaiacol, and 50 μL of plant extract. The quantity of intake was measured at a 470 nm wavelength at periods of 0, 30, 60, and 90 min using a spectrophotometer (IRMECO U2020, Germany).

Inorganic Ions and Soil Analysis. Lead and ionic contents were extracted according to the protocol followed by Allen et al.[35] For 2 weeks, samples of roots, leaves, and shoots were dried at 65 °C in an oven. Next, 5 mL of H_2SO_4 was used to immerse 0.1 g of dried biomass from roots and shoots in digestion flasks, and the mixture was left overnight. The digested flasks were heated to 350 °C on a hot plate the following day and kept there until the fumes began to dissipate. After that, H_2O_2 was progressively added to the flasks to create a clear, colorless solution. Following digestion, water was added to dilute the samples and make the volume up to 50 mL. With a flame photometer (Sherwood flame photometer-410, U.K.), the filtrate samples' shoot and root ionic content (Na^+, Ca^{2+}, and K^+) was measured.

Following the study's setup, 20 g of soil was taken from each pot and placed in brown wrappers. The soil was then crushed, allowed to air-dry, and then oven-dried for 48 h at 65 °C. Acid digestion with 4.5 mL of HNO_3 and 1.5 mL of HCL was applied to 0.25 g of dry soil following Mico et al.[36] The digested sample was diluted with distilled water to make a volume of 25 mL for the Pb analysis. An atomic absorption spectrophotometer was used to calibrate the amount of Pb in the solution.

Pb Analysis in Soil, Roots, Shoots, and Grains. To analyze the Pb content in roots and shoots, wheat leaves and roots were roasted at 550 °C. Five mL of 2 M hot HCl was combined with the resulting ash (Chapman and Pratt).[37] To ascertain the Pb content, an atomic absorption spectrophotometer was employed.

Ripe wheat grain samples were physically removed from every pot. Each grain sample was carefully cleaned in deionized water before being dried at 65 °C. Next, to test HMs (Pb in grains), dry samples were processed in a stainless-steel grinder. The materials were digested using a mixture of HNO_3−H_2O_2 and HCl. An atomic absorption spectrophotometer was used to determine the Pb concentration in grains (Niemelä et al.).[38]

Statistical Analysis. The study used a completely randomized design (CRD) with 3 replications. To create bar graphs, Microsoft Excel (version 2016) was utilized. A three-factorial analysis of variance was conducted using Statistix 8.1, and a Tukey's test was used to compare the means at the $P < 0.05$ level of significance. Utilizing the statistical application *R*-studio (V4.3.3), boxplot, the Pearson correlation factor, and principle component analysis were carried out.

RESULTS

Morphological Attributes. Statistical data demonstrated that lead stress and thiourea treatment exhibited significant results for the morphological characteristics of both wheat varieties (Table 1). The toxicity imposed by Pb (15 mM) significantly declined the fresh weight of shoots (17.7 and 23%), fresh weight of roots (31.1 and 26.1%), dry weight of shoots (35.9 and 12.8%), and dry weight of roots (2.5 and 43.6%) in V_1 and V_2, correspondingly, as compared to the control. Lead stress greatly reduced the leaf area of both wheat varieties, as 22% was decreased in V_1 and 10.9% in V_2, respectively (Table 1). Soil application of thiourea showed a great increment in morphological attributes of wheat, as the shoot fresh weight increased by 12.5 and 14.2%, the fresh weight of roots by 37.5 and 24%, the shoot dry weight by 18.2 and 11.6%, and the root dry weight 23 and 30% in V_1 and V_2, respectively, upon incorporation of thiourea. The leaf area was enhanced by 17.6 and 7.9% by 10 mM application of thiourea. The length of the shoot and the length of the root were reduced by 29 and 36% in V_1 and 27 and 46% in V_2, respectively, by the toxicity exposed by Pb stress as compared to the control, while application of thiourea (10 mM) caused maximum enhancement in the shoot length by 12 and 28% and in the root length by 13 and 23% among both varieties, respectively, relative to the control (Table 1).

Photosynthetic Pigments. Photosynthetic pigments were considerably affected by the toxicity of heavy metal (Pb) stress in both wheat varieties (Figure 1). The chlorophyll *a* content was reduced by 27 and 24%, chlorophyll *b* by 12.7 and 13%, total Chl. by 16%, carotenoids by 19 and 29%, and the Chl. *a*/*b* ratio by 16.8 and 13.2% in V_1 and V_2, respectively, as compared to the control, while treatment of thiourea after establishing seedlings significantly improved the photosynthetic pigments, that is, Chl. *a* by 19 and 13%, Chl. *b* by 17.8 and 8.9%, total Chl. by 18 and 9.9%, and carotenoids by 19 and 24.7%, and also the Chl. *a*/*b* ratio was improved by 0.8

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Table 1. Mean Square Value (ANOVA) of Thiourea and Lead (Pb) Stress on Morphological Parameters of Wheat Cultivars[a]

varieties	lead stress (Pb)	thiourea (TU)	shoot fresh weight (g)	root fresh weight (g)	shoot length (cm)	root length (cm)	shoot dry weight (g)	root dry weight (g)	leaf area (cm^2)
Akbar 19 (V_1)	control	control	4.81 ± 2.78ef	0.31 ± 0.17de	43.7 ± 25.3 cd	10.3 ± 0.17d	0.42 ± 0.01e	0.22 ± 0.13c-e	24.2 ± 13.9gh
		TU (2.5 mM)	5.15 ± 2.97bc	0.35 ± 0.20bc	46.07 ± 26.6bc	11.2 ± 0.20 cd	0.45 ± 0.008	0.26 ± 0.15bc	26.1 ± 15.1fg
		TU (10 mM)	5.55 ± 3.21 cd	0.37 ± 0.21a-c	48.5 ± 28.03ab	11.7 ± 0.20c	0.47 ± 0.08e	0.27 ± 0.15a-c	27.7 ± 16ef
	Pb (15 mM)	control	3.95 ± 2.28g	0.21 ± 0.12g	31.01 ± 17.9e	6.62 ± 0.18g	0.27 ± 0.08g	0.17 ± 0.1fg	18.8 ± 10.9j
		TU (2.5 mM)	4.08 ± 2.35g	0.26 ± 0.15f	33.2 ± 19.2e	7.21 ± 0.20fg	0.30 ± 0.01f	0.2 ± 0.11ef	20.6 ± 11.9ij
		TU (10 mM)	4.45 ± 2.57fg	0.29 ± 0.16ef	34.8 ± 20.1e	7.52 ± 0.27fg	0.32 ± 0.1f	0.21 ± 0.12d-f	22.2 ± 12.8hi
Ghazi 11 (V_2)	control	control	6.74 ± 3.89b	0.37 ± 0.21a-c	48.1 ± 27.8a-c	13.6 ± 0.30b	0.62 ± 0.006bc	0.25 ± 0.14b-d	32 ± 18.5a-c
		TU (2.5 mM)	7.11 ± 4.11a	0.38 ± 0.22ab	49.6 ± 28.7ab	14.3 ± 0.33ab	0.66 ± 0.008ab	0.28 ± 0.16ab	33.2 ± 19.2ab
		TU (10 mM)	7.74 ± 4.47a	0.4 ± 0.23a	51.6 ± 29.8a	15.33 ± 0.33fg	0.68 ± 0.01a	0.30 ± 0.17a	34.4 ± 19.9a
	Pb (15 mM)	control	5.14 ± 2.97de	0.27 ± 0.15ef	35.1 ± 20.3e	7.33 ± 0.33fg	0.54 ± 0.01d	0.13 ± 0.07g	28.5 ± 16.5def
		TU (2.5 mM)	5.54 ± 3.2 cd	0.30 ± 0.17de	40.6 ± 23.4d	8.22 ± 0.22ef	0.57 ± 0.01 cd	0.15 ± 0.08g	29.6 ± 17.1c-e
		TU (10 mM)	5.87 ± 3.39 cd	0.34 ± 0.19 cd	45.1 ± 26.1bcd	9.04 ± 0.14e	0.60 ± 0.008c	0.17 ± 0.1fg	30.8 ± 17.8b-d

[a]Values representing means ± standard error of three replicates of wheat varieties that share the same lettering for a parameter showed nonsignificant variation at a significance level of $p \leq 0.05$. V_1 = Akbar 19, V_2 = Ghazi 11; Control = no Pb stress; lead stress = Pb (15 mM); and Control = no thiourea, TU = thiourea (2.5 mM) and TU = thiourea (10 mM).

and 3.7% among both varieties, V_1 and V_2, correspondingly (Figure 1).

Oxidants and Antioxidants. The data derived from statistical analysis explained that oxidants and antioxidants of both wheat varieties were significantly impressed by Pb heavy metal stress (Figure 2). A considerable enhancement was observed in the activity of H_2O_2 (27.1 and 28%) and MDA content by 83 and 10% in V_1 and V_2, respectively, under lead stress as compared to the control, while substantial decreases of 16 and 11.5% and 16.9 and 22.3% were observed in H_2O_2 and MDA content by exogenous application of thiourea (Figure 2).

Recorded data exhibited that Pb stress greatly improved the enzymatic antioxidants of wheat varieties (Figure 2). The toxic effect of Pb stress increased the CAT, SOD, and POD by 32.7, 64, and 20.5% in V_1 and 68.6, 41.8, and 29.9% in V_2, respectively. Application of thiourea further improved the activity of antioxidants under both stress and control conditions. A maximum increase of CAT (25 and 24.7%), SOD (35.5 and 24.4%), and POD (27.6 and 20.1%) was noted in V_1 and V_2, correspondingly (Figure 2).

Inorganic Ions. Accumulation of heavy metals (Pb) through rooting and growth media noticeably improved the uptake of Pb content in wheat varieties while reducing the other mineral ions including Na^+, Ca^{2+}, and K^+ ions (Figures 3 and 4). Under lead stress (15 mM), the root Pb and shoot Pb content and the grain Pb and soil Pb content were increased by (20.2, 17.9%), (29.2, 38.8%), (22.9, 16.1%), and (8.5, 6.8%) in V_1 and V_2, respectively, in contrast with control conditions (Figures 3−5). The toxic effects of Pb reduced the other organic ions' uptake as compared to the control such as shoot Na^+ reduced by (29.8, 16%), root Na^+ up to (24.5, 18%), shoot Ca^{2+} reduced by (14.3 12.8%), and root Ca^{2+} up to (17, 18.7%), while shoot K^+ reduced by (28, 26%) and root K^+ up to (22.3, 20.7%) among both wheat varieties, V_1 and V_2, correspondingly (Figures 3 and 4). The treatment of thiourea greatly decreased the activity of inorganic ions (Na^+ and Pb) in both varieties (V_1, V_2), that is, the root and shoot Pb content and the grain and soil Pb were reduced by (8.9, 35%) and (12.9, 23.8%) and (25, 7.56%) and (17, 16%), shoot Na^+ up to (20.9, 13%), and root Na^+ up to (13.8, 10%) (Figures 3−5). On the other hand, the uptake of Ca^{2+} and K^+ through the root and shoot by the usage of thiourea was improved, that is, root Ca^{2+} up to (21.5 22.3%), shoot Ca^{2+} up to (7%), root and shoot K^+ up to (9.3, 8.9%), and (14, 16%) among both wheat varieties (Figure 4).

Correlation Analysis. The findings of the Pearson correlation analysis observed that the morpho-physiological and biochemical indices of both wheat varieties had a strong correlation (Figure 5). The majority of indicators showed a substantial positive correlation with a number of parameters, including the fresh weight of roots, dry and fresh weights of shoots, root and shoot lengths, CAT, POD, SOD, total chlorophyll, carotenoids, root K^+, shoot K^+, root and shoot Ca^{2+}, and shoot and root Na^+ content (Figure 5). On the contrary, the majority of physiological indicators showed negative correlations with the root dry weight, MDA, shoot and root Pb, and H_2O_2 (Figure 5). The majority of the indicators did not exhibit significant relationships with chlorophyll *a*, chlorophyll *b*, and the Chl. *a*/*b* ratio (Figure 6).

PCA and Heatmap Analysis. A two-way heatmap with a dendrogram was made to examine the impact of thiourea on different observations of wheat varieties under lead stress

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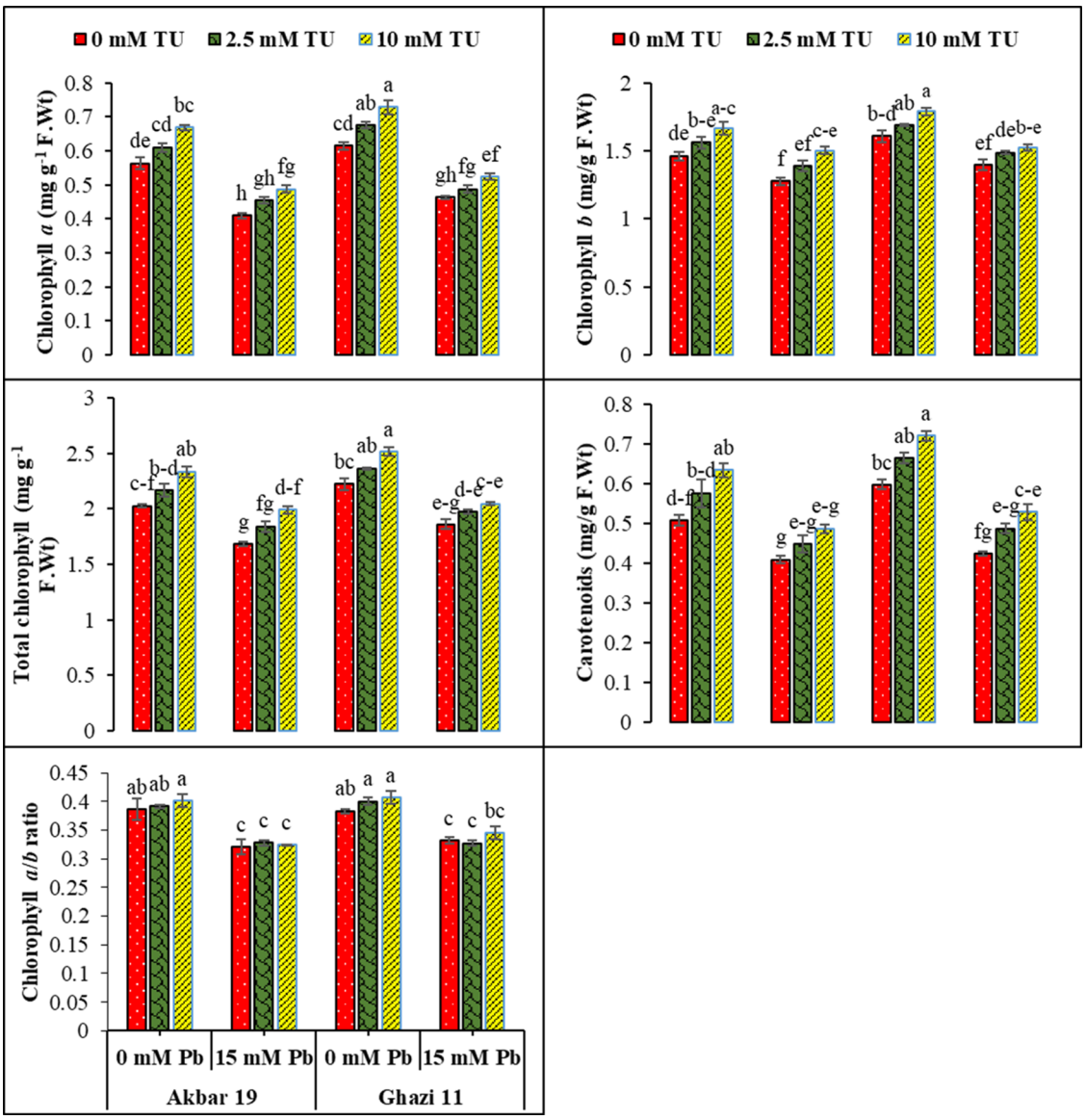

Figure 1. Effect of thiourea on chlorophyll *a* (mg g^{-1} F.Wt), (chlorophyll *b* (mg g^{-1} F.Wt), total chlorophyll (mg g^{-1} F.Wt), carotenoids (mg g^{-1} F.Wt), and chlorophyll *a*/*b* ratio under lead (Pb) stress. Error bars that share the same lettering for a parameter showed non-significant variation at a significance level of $p \leq 0.05$.

conditions (Figures 6 and 7). The observations were categorized into groups based on the similarities at the various stages of the treatment, and colored squares represented the interactions between the groups. When lead stress and thiourea treatment were taken into consideration, the maroon color showed a strong negative correlation, while the navy blue color showed a high positive link for a variety of observations (Figure 6). The heatmap has clustered into four groups. In the first group, MDA, shoot, and root Pb contents were clustered. These metrics are strongly positively correlated with lead stress (15 mM) where there is no treatment of thiourea and moderately correlated at the thiourea (2.5 mM) level. Both varieties were strongly negatively correlated at the thiourea (10 mM) level. This group revealed that the application of thiourea (10 mM) reduced the toxicity caused by Pb stress by mitigating the uptake of root and shoot Pb as well as the MDA level. The second group included chlorophyll *b* content, which was greatly affected by Pb stress (15 mM) and thiourea application (2.5 and 10 mM). The third group included SOD, CAT, and POD, which were significantly impacted by the administration of thiourea (10 mM) and Pb stress (15 mM) as well as strong negatively correlated under control conditions when there was no Pb and thiourea application. The fourth group has H_2O_2, shoot and root Na^+, shoot dry weight, leaf area, shoot fresh weight, and root Ca^{2+} ion activity. H_2O_2, shoot, and root Na^+ ions were strongly positively correlated under controlled conditions (no stress and treatment) and strongly negatively correlated at (15 mM) stress and (10 mM) thiourea application, while the shoot fresh weight and root Ca^{2+} were strongly positively correlated at (10 mM) thiourea application and negatively correlated under Pb stress (15 mM) when there was no treatment application. In the fifth group, shoot Ca^{2+}, shoot and root K^+ and length, root fresh and dry weight, total chlorophyll, carotenoids, chlorophyll *a*, and the *a*/*b* ratio were gathered. These metrics were strongly positively correlated at the control (no Pb stress) and thiourea (10 mM) concentration and weakly correlated at Pb stress (15 mM) and no thiourea treatment. These interpretations exhibited that

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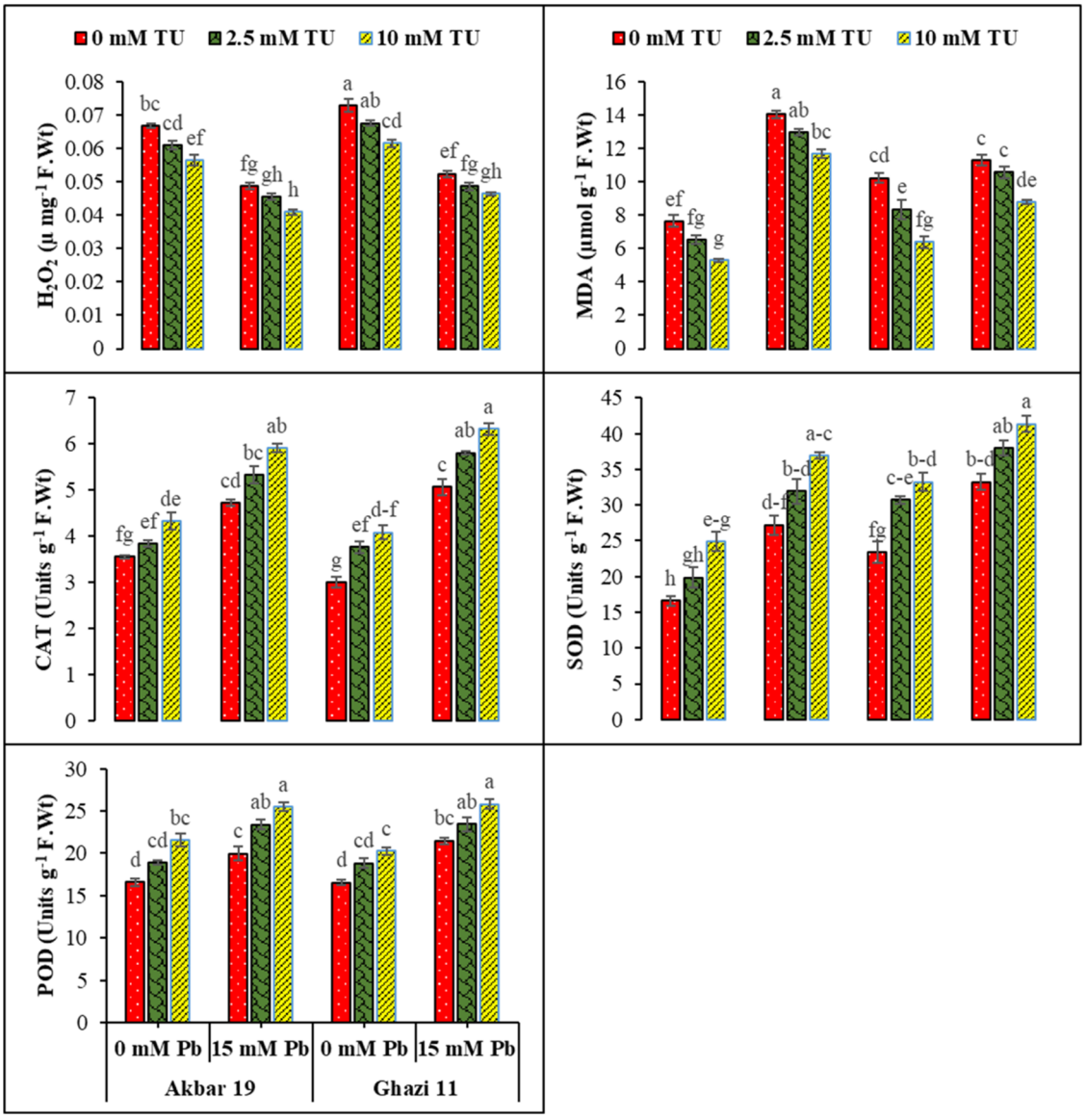

Figure 2. Effect of thiourea on H_2O_2 (μ mg^{-1} F.Wt), MDA (μmol g^{-1} F.Wt), CAT (Units mg^{-1} protein), SOD (Units mg^{-1} protein) and POD (Units mg^{-1} protein) under lead (Pb) stress. Error bars that share the same lettering for a parameter showed non-significant variation at a significance level of $p \leq 0.05$.

under Pb stress (15 mM), growth attributes and photosynthetic pigments of wheat varieties were reduced, while they were enhanced by the application of thiourea, which reduced the oxidative damage caused by H_2O_2 (Figures 7 and 8).

DISCUSSION

The development of plants is adversely affected by heavy metal stress, just like it is by other stresses.[39] According to our research, Pb stress reduced plant development, which is consistent with Pb-induced reductions in crop and wheat growth.[40] Poor plant development under Pb toxicity may be caused by the reduced uptake of minerals and water as a result of stress.[41] Plant morphological, biological, and biochemical parameters are negatively impacted by high Pb levels in soil, which results in stunted development.[42] Increased plant biomass may be caused by decreased Pb concentration in various wheat plant sections and elevated Fe concentration in the soil.[43] The excess of heavy metals damages the ultrastructure of chloroplasts, membrane permeability, and assimilation flow, which has a major effect on photosystems I and II and adversely affects net photosynthesis in plants, which in turn lowers morphological and physiological parameters of plants. This is why photosynthetic pigments decreased under Pb stress, as in our study.[44] The increased level of photosynthetic pigments seen in this study may have contributed to the improved photosynthetic performance of wheat plants as a result of the use of plant growth regulators.[45] Giving plants sulfur, thiol, and thiourea supplementation reduces the adverse impacts of heavy metals on crops and promotes plant growth by improving mineral uptake and photosynthesis.[46]

Decreased water and mineral uptake may lead to diminished chlorophyll production, which will slow down plant growth overall.[47] In the current investigation, the administration of TU has lessened the detrimental effects of Pb on wheat development. On the other hand, when TU was applied alone, the growth of wheat cultivars that were Pb-toxic had been enhanced more successfully. According to earlier

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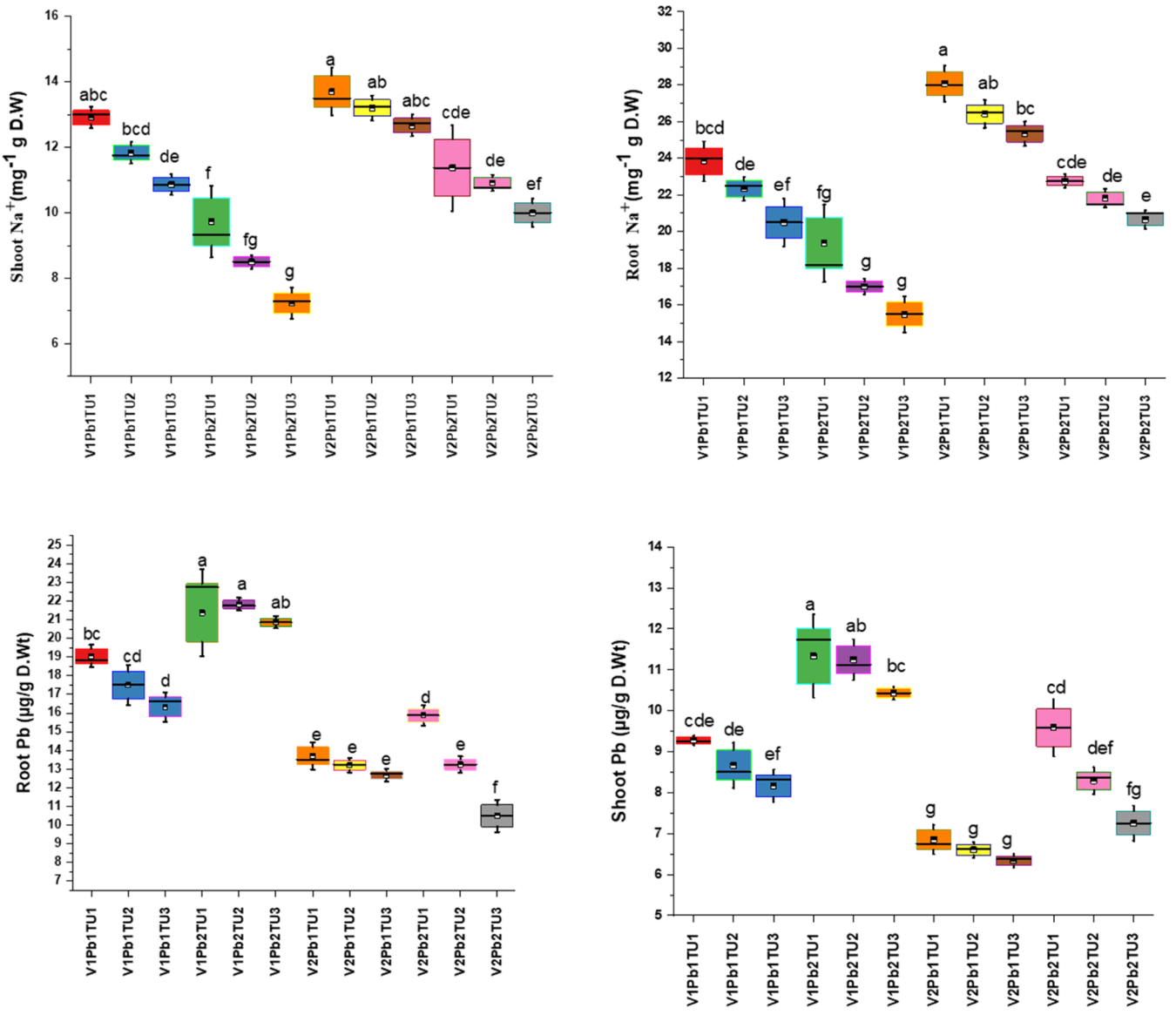

Figure 3. Effect of thiourea on shoot Na^+ ($mg^{-1}g$ D.W), root Na^+ ($mg^{-1}g$ D.W), (c) root Pb ($\mu g^{-1}g$ D.W) and shoot Pb ($\mu g^{-1}g$ D.W) under lead (Pb) stress. V1 = Akbar 19, V2 = Ghazi 11, Pb1TU1 = Control (no Pb+ no thiourea), Pb1TU2 = (no lead+ 2.5 mM thiourea), Pb1TU3 = (no lead + 10 mM thiourea), Pb2TU1 = (15 mM lead+ no thiourea), Pb2TU2 = (15 mM lead+ 2.5 mM thiourea), Pb2TU3 = (15 mM lead+ 10 mM thiourea). Error bars that share the same lettering for a parameter showed non-significant variation at a significance level of $p \leq 0.05$.

research, TU encourages the growth of plants subjected to lead poisoning, such as fenugreek[48] and maize.[49] Improved F_v/F_m and chlorophyll levels in Pb-stressed plants have been connected to the therapeutic effect of TU on plant growth under Pb stress,[50,51] which is similar to what was seen in the wheat plants used in this experiment. By enhancing the cellular water status, the TU treatment raised the concentrations of osmotic chemicals, which may improve the plants' ability to withstand stress.[51]

In plant metabolism, the generation and consumption of several ROS, including O^{2-}, $OH^{\bullet}$, and H_2O_2, are typical processes. Distortion of balance under circumstances has numerous detrimental effects on plant metabolism and overall output.[52] Peroxidative reactions can arise from the interaction of ROS species with various components of cells. Significant harm is done to lipids, nucleic acids, carbohydrates, and proteins, plant growth is reduced, and in the worst cases, even cell death is caused by this situation.[53] Under a variety of environmental stressors, thiourea has demonstrated its strength as an antioxidant and ROS scavenger.[54,55] For example, abiotic stressors lead to oxidative damage through an increased ROS production. By boosting antioxidant defense, soil application of TU scavenged the reactive oxygen species in stressed wheat.[56] Furthermore, lipid peroxidation (LPO) is a crucial process that can be interpreted as an indication of oxidative damage brought on by stressors in the environment. When chickpea plants were exposed to abiotic stress circumstances, TU treatment (13.6 mM) dramatically reduced LPO and increased the efficiency of the photosystem II quantum.[57]

It has been possible to successfully apply TU to reduce the harmful impacts of many abiotic stresses, including salinity, drought, and metal (loids).[58,59] The decrease in the heavy metal buildup and the overall alterations brought about by TU in the transcripts and metabolism of plants, as seen in rice, may also be connected to a boost in plant development.[47] Plant buildup of heavy metals, plant stress, and antioxidant responses

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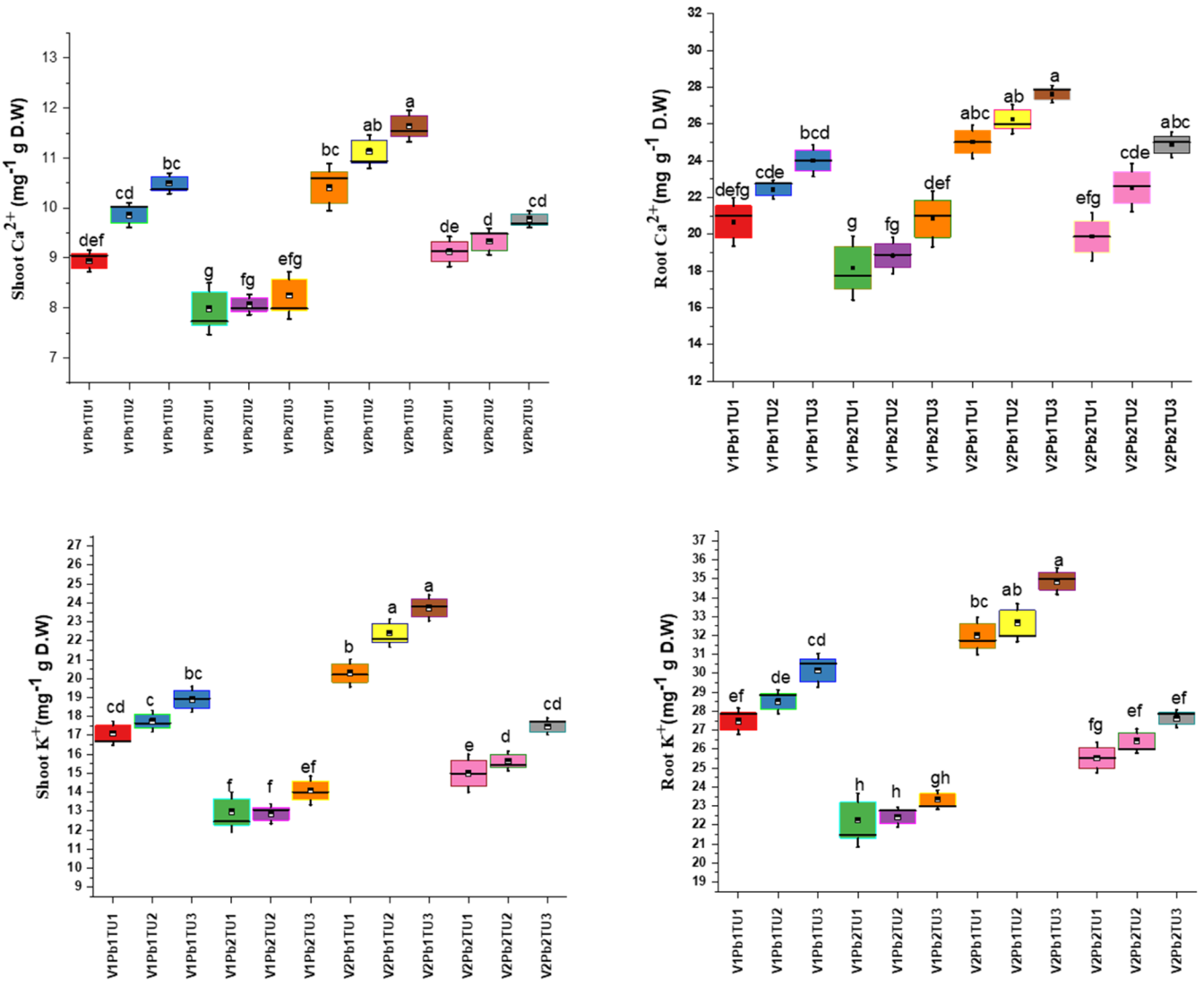

Figure 4. Effect of thiourea on shoot Ca^{2+} ($mg^{-1}g$ D.W), root Ca^{2+} ($mg^{-1}g$ D.W), shoot K^+ ($mg^{-1}g$ D.W) and root K^+ ($mg^{-1}g$ D.W) under lead (Pb) stress. V_1 = Akbar 19, V_2 = Ghazi 11, Pb1TU1 = Control (no Pb+ no thiourea), Pb1TU2 = (no lead+ 2.5 mM thiourea), Pb1TU3 = (no lead + 10 mM thiourea), Pb2TU1 = (15 mM lead+ no thiourea), Pb2TU2 = (15 mM lead+ 2.5 mM thiourea), Pb2TU3 = (15 mM lead+ 10 mM thiourea). Error bars that share the same lettering for a parameter showed non-significant variation at the significance level of $p \leq 0.05$.

were assessed to test this. When plants are stressed by heavy metals, ROS generation is observed. The alterations in totality point to oxidative stress because free oxidative species harm proteins, nucleic acids, and metabolic pathways, cause membrane lipids to peroxide, and enhance electrolyte leakage.[60,61] Thiourea is a recognized ROS scavenger and functions as a bioregulator for plant growth.

It has been suggested that the capacity of TU to control the plants' redox status and reduce ROS generation is the mechanism underlying its potential to mitigate stress.[23] In a previous study, supplementing wheat plants with TU led to the ameliorated activities of antioxidant enzymes alleviating oxidative stress.[62] The components of reactive oxygen species detoxification that help regulate the amount of ROS in plants include CAT, SOD, and POD among enzymatic antioxidants and photosynthetic content carotenoids among molecular antioxidants.[45] The studied three antioxidant enzymes SOD, CAT, and POD exhibited different reactions. But for the most part, the enzymes did not demonstrate a discernible shift or a decrease in TU-treated plants relative to control plants. A shift in the expression of genes of antioxidant enzymes may be the cause of this reaction.[47,63] This appears to be related to TU amendment-induced stress reduction through alternative redox-regulatory pathways, which lessens the requirement for increased antioxidant enzyme activity.[29,64] According to Mishra et al.,[65] the concentration of MDA demonstrated a noteworthy decrease, indicating a decrease in lipid peroxidation and a corresponding decrease in oxidative stress. For this reason, more research is required to define the other potential regulators of oxidative stress, including phenolics and molecular antioxidants, to better understand the mechanism of TU.

The study's findings showed that TU treatments and heavy metal stress had a good impact on wheat varieties' fresh weight-based nutrient absorption in the roots and shoots (Table 1). While K^+ and Ca^{2+} buildup declined, as reported in the earlier study,[66] the current data showed that Na^+ and Pb accumulation in roots and shoots was dramatically enhanced in both wheat varieties under heavy metal stress. In addition to preventing the entrance and binding of necessary ions like K^+,

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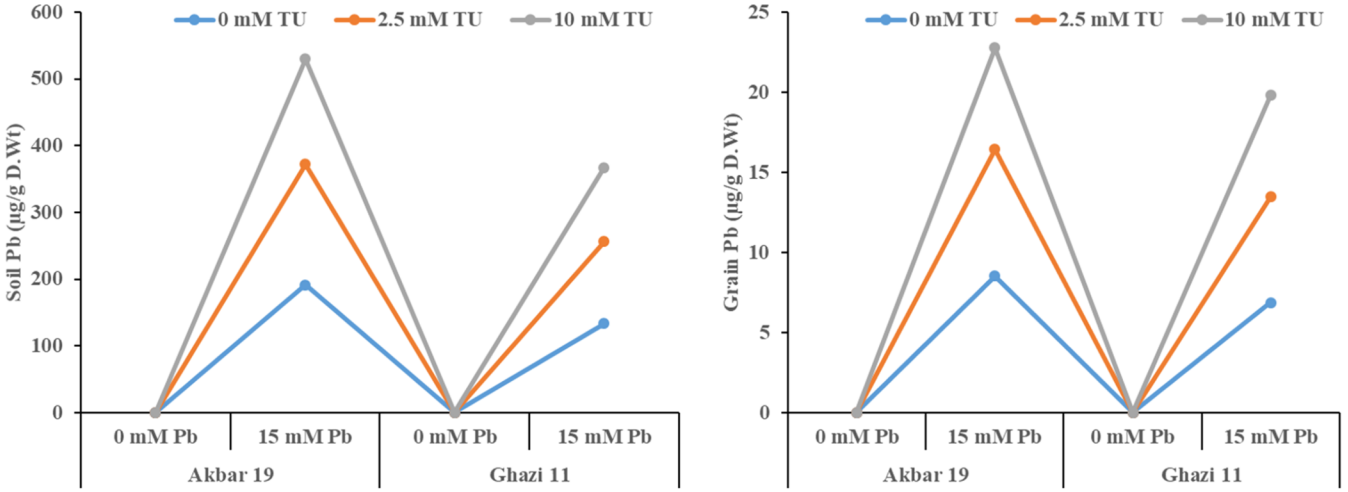

Figure 5. Effect of thiourea on soil Pb (μg^{-1}g D·W) and grain Pb (μg^{-1}g D·W) under lead (Pb) stress. V_1 = Akbar 19, V_2 = Ghazi 11, Pb1TU1 = Control (no Pb + no thiourea), Pb1TU2 = (no lead + 2.5 mM thiourea), Pb1TU3 = (no lead + 10 mM thiourea), Pb2TU1 = (15 mM lead + no thiourea), Pb2TU2 = (15 mM lead + 2.5 mM thiourea), and Pb2TU3 = (15 mM lead + 10 mM thiourea). Error bars that share the same lettering for a parameter showed nonsignificant variation at a significance level of $p \leq 0.05$.

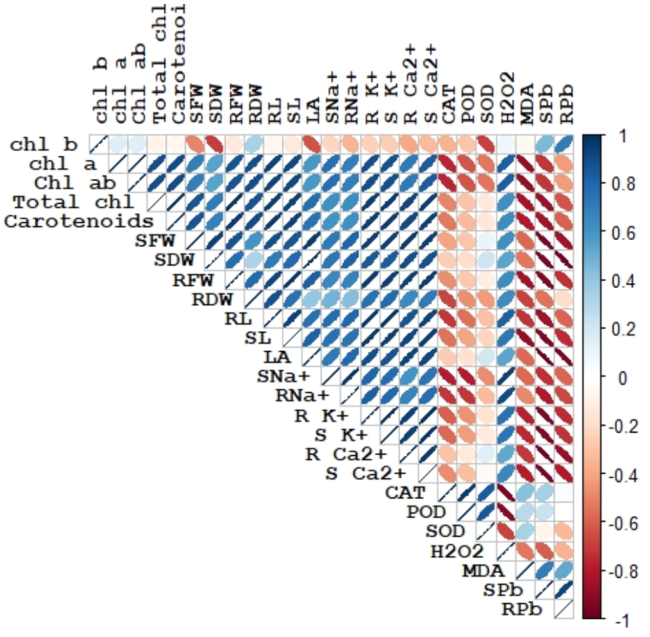

Figure 6. Correlation analysis between various morpho-physiological and ionic attributes of wheat varieties exposing the impact of soil incorporation of thiourea (TU) under lead stress.

Ca^{2+}, and Mg^{+}, the hazardous metals stop the mineral and water intake in plants and are closely associated with their extensive building up, especially in roots.[67,68] On the other hand, TU treatments greatly increased the buildup of K^{+} and Ca^{2+} in comparison to the control, while also playing an ameliorative role and reducing the concentrations of Pb and Na^{+} in wheat roots and shoots.

Plants that grow in Pb-rich soils easily absorb lead, which is primarily stored in the roots and to a lesser degree in the leaf parts, stem portions, and seeds.[69] The primary components that keep Pb from entering the cell are the cell wall and membrane.[70] Phytochelatins reduce the amount of lead that enters the cells.[71] However, in the current investigation, Pb toxicity caused greater Pb collection in plant tissues, especially in the roots, which greatly inhibited root growth; similar reductions in root growth have been previously documented for wheat cultivars.[72] By limiting the metal uptake, several techniques are being employed to lessen the harm that metals do to plant growth.[73] For example, the harmful effects of metals have been mitigated by the exogenous application of TU through several soil-induced endogenously generated compounds by plants.[74] The purpose of the ongoing study was to determine if supplementing wheat plants with TU alone or in combination can mitigate the negative effects of lead toxicity. Thiourea considerably decreased the amount of lead that accumulated in wheat plant roots. Moreover, TU decreased the Pb transfer from roots to above-ground portions. Blocked Pb uptake from the roots and its delivery to the above-ground plant sections were more successfully achieved by using TU. Additionally, *Trigonella foenum* graecum L. has been observed to have lower root and shoot Pb concentrations as a result of TU application under Pb toxicity. No data on the hindered effect of TU on Pb-stressed plants could be deciphered from the previous literature.[48]

Foliar application of Asp and Thi increased NR activity, total N, NO_3, and NO_2 levels and decreased NH_4^{+} levels as GS and GOGAT utilize ammonium for amino acid synthesis. This led to more N usage in the chlorophyll synthesis and improved growth of Pb-stressed plants. The NR enzyme adjusts the rate of limiting reactions in N metabolism, thereby getting involved in important metabolic events such as synthesis of secondary compounds containing N and amino acids.[75] It has been stated by other researchers that Thi increases the N concentration and NR activity in plants.[76]

CONCLUSIONS

Lead stress lowered the morpho-physiological and biochemical characteristics of wheat varieties, which decreased the plant's ability to absorb nutrients. However, by increasing the photosynthetic rate and reducing the detrimental effects of lead stress, thiourea treatment enhanced the characteristics of plant growth. Thiourea upregulated SOD, CAT, and POD in wheat plants to scavenge hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), and it also enhanced the activity of enzyme antioxidants under lead stress. Furthermore, via lowering Pb uptake, thiourea also regulated the uptake of

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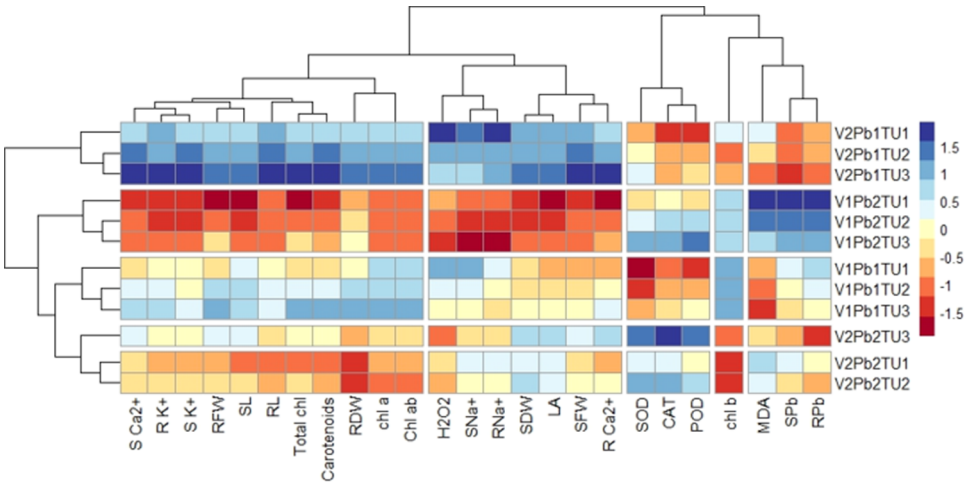

Figure 7. Heatmap with a dendrogram between various morpho-physiological and ionic attributes of wheat varieties exposing the impact of soil incorporation of thiourea (TU) under lead stress. V_1 = Akbar 19, V_2 = Ghazi 11, Pb1TU1 = Control (no Pb + no thiourea), Pb1TU2 = (no lead + 2.5 mM thiourea), Pb1TU3 = (no lead + 10 mM thiourea), Pb2TU1 = (15 mM lead + no thiourea), Pb2TU2 = (15 mM lead + 2.5 mM thiourea), and Pb2TU3 = (15 mM lead + 10 mM thiourea).

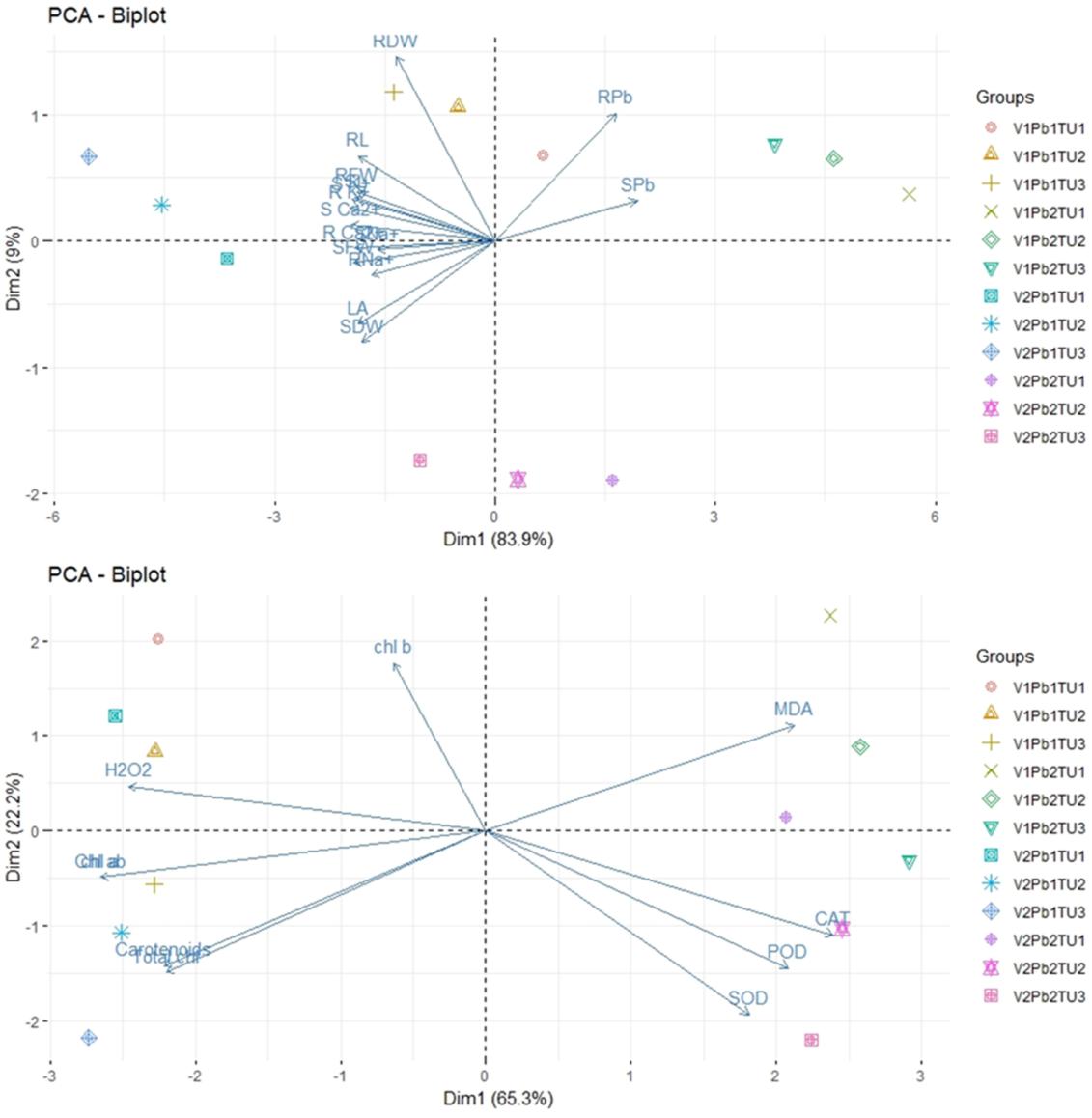

Figure 8. Principal component analysis (PCA) between morpho-physiological and ionic attributes of wheat varieties (Akbar 19 and Ghazi 11).

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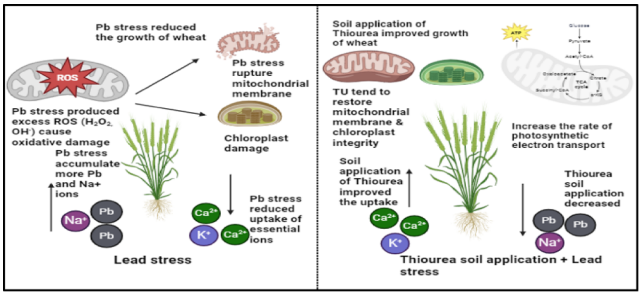
nutrients in wheat plants. Additionally, thiourea treatment increased nutrient uptake, decreased stress indicators, and increased Pb uptake in wheat roots and shoots, all of which contributed to improved plant growth. Furthermore, all morpho-physiological parameters exhibited that Ghazi 11 (V_2) outperformed Akbar 19 (V_1). Future roadmaps should be based on exogenous applications of thiourea-triggered changes in ovule development and pollen fertility. Additionally, more research needs to be done to examine the larger-scale application of thiourea in field settings, utilizing various techniques.

■ ENVIRONMENTAL IMPLICATIONS

Lead pollution in soil poses a threat to both food security and human health issues. The creation of Pb negatively impacts plant growth by altering mineral nutrient accumulation and harming the root architecture. A recent study suggests that thiourea can alleviate stress and reduce Pb toxicity in wheat Scheme 1.

Scheme 1. Schematic Diagram Showing the Mechanism of Thiourea Absorption under Lead Stress



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Author Contributions

S.M. and E.A.W.: methodology; A.H., M.H.S., S.A., and T.R.: software; A.H., T.R., M.H.S., and S.A.: formal analysis; A.H. and A.F.: investigation; E.A.W.: data curation; A.P.A.H. and H.U.R.: writing—original draft preparation; M.H.S., S.A., T.R., and A.H.: writing—review and editing; and M.H.S. and S.A.: resource and funding. All authors have read and agreed to the published version of the manuscript.

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Notes

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