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RESEARCH ARTICLE

Foliar application of gibberellic acid endorsed phytoextraction of copper and alleviates oxidative stress in jute (*Corchorus capsularis* L.) plant grown in highly copper-contaminated soil of China

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Abstract

Copper (Cu) is an abundant essential micronutrient element in various rocks and minerals and is required for a variety of metabolic processes in both prokaryotes and eukaryotes. However, excess Cu can disturb normal development by adversely affecting biochemical reactions and physiological processes in plants. The present study was conducted to explore the potential of gibberellic acid (GA_3) on fibrous jute (*Corchorus capsularis* L.) seedlings grown on Cu mining soil obtained from Hubei Province China. Exogenous application of GA_3 (10, 50, and 100 mg/L) on 60-day-old seedlings of *C. capsularis* which was able to grow in highly Cu-contaminated soil (2221 mg/kg) to study different morphological, physiological, and Cu uptake and accumulation in different parts of *C. capsularis* seedlings. According to the results, increasing concentration of GA_3 (more likely 100 mg/L) alleviates Cu toxicity in *C. capsularis* seedlings by increasing plant growth, biomass, photosynthetic pigments, and gaseous exchange attributes. The results also showed that exogenous application of GA_3 reduced oxidative stress in *C. capsularis* seedlings by the generation of extra reactive oxygen species (ROS). The reduction in oxidative stress in *C. capsularis* seedlings is because that plant has strong enzymatic antioxidants [superoxidase dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT)], which ultimately increased their activities to overcome oxidative damage in the cells/tissues. In addition to the plant growth, biomass, and photosynthesis, foliar application of GA_3 also helps to increase metal (Cu) concentration in different parts of the plants when compared to 0 mg/L of application of GA_3. From these findings, we can conclude that foliar application of GA_3 plays a promising role in reducing ROS generation in the plant cells/tissues and increased phytoextraction of Cu in different plant parts. However, more investigation is needed on field experiments to find a combination of GA_3 with a very higher concentration of Cu using fibrous *C. capsularis*.

Keywords Antioxidants · Fibrous crop · Heavy metals · Plant hormone · Reactive oxygen species

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Introduction

Metal contamination issues are becoming increasingly common in India and elsewhere, with many documented cases of metal toxicity in mining industries, foundries, smelters, coal-burning power plants, and agriculture (Czymmek et al. 2020; Farid et al. 2018b; Ketterings et al. 2007; Kumar et al. 2019a, b; Parveen et al. 2020; Tangahu et al. 2011; Wuana and Okieimen 2011). Long-term mining is a major resource of heavy metal contamination and health risk to humans. Mining activities in China alone have generated about 3.0 million ha derelict land by the year 2000, and it is increasing at a rate of 46,700 ha per year (Farid et al. 2019; Saleem et al. 2020a; Yang et al. 2020). However, the depletion of arable land in China has significantly impeded food security. Thus, it is important that mining sites be restored ecologically and heavy metal emissions through phytoremediation to reduce health risks and to increase Chinese food safety (Ahmad et al. 2019; Farid et al. 2018a; Saleem et al. 2020a). Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food safety and marketability, crop growth due to phytotoxicity, and environmental health of soil organisms (Pajević et al. 2016; Vardhan et al. 2019). Nevertheless, remediation of metal-polluted soils by conventional physical and chemical approaches is not ideal as it needs large investments, time consuming, and environmentally destructive (Ullah et al. 2015; Vasavi et al. 2010). Recently, the emerging technology phytoremediation of polluted sites due to their cost effectiveness, esthetic advantages, scientific applicability, and can be done on sites should be considered for remediation (Afshan et al. 2015; Ashraf et al. 2017; Rehman et al. 2019c; Zaheer et al. 2015). Some plant roots can absorb and immobilize metal pollutants, while other plant species have the ability of metabolizing or accumulating organic and nutrient contaminants. Multifarious relationships and interactions between plants, microbes, soils, and contaminants make these numerous phytoremediation processes possible (Laghlimi et al. 2015; Niazy Abdou and Wahdan 2017; Tahmasbian and Sinegani 2016). Phytoremediation processes are most effective where contaminants are present at low to medium levels, as high contaminant levels can inhibit plant and microbial growth and activity (Daud et al. 2018; Elleuch et al. 2013; Saleem et al. 2020b; Vasavi et al. 2010).

For this purpose, fibrous species such as jute (*Corchorus capsularis* L.) plant has been exclusively, for the remediation of toxic pollutants from the soil in order to get a pacific environment (Ogunkunle et al. 2015; Saleem et al. 2020d, e; Uddin Nizam et al. 2016). Moreover, *C. capsularis* were specifically grown for the remediation of toxic soil contaminants in order due to its specific physiological properties to create a sustainable environment (Abubakari et al. 2017; Saleem et al. 2019a, 2020d). Unlike fibrous species, *C. capsularis* is also grown as a vegetative crop because some essential micronutrients such as calcium, potassium, and iron, as well as an abundance of important vitamins, are available (Ahmed and Slima 2018; Singh et al. 2018). In addition, *C. capsularis* tolerates stressful heavy metal environments due to its biological properties that help tolerate stress and scavenge reactive oxygen species (ROS) under heavy metals due to an active antioxidative defense system (Abubakari et al. 2017; Saleem et al. 2020c, 2020e). Stress conditions can disturb the dynamic equilibrium of ROS production and elimination under normal growth in plants which promotes ROS accumulation, membrane lipid peroxidation, and disrupt the structure and function of cell membrane system (Husak 2015; Kamran et al. 2019; Rana et al. 2020; Fahad and Bano 2012; Fahad et al. 2013; Fahad et al. 2014a, b; Fahad et al. 2015a, b; Fahad et al. 2016a, b, c, d; Fahad et al. 2017; Fahad et al. 2018; Fahad et al. 2019a, b; Akram et al. 2018a, b). Antioxidant enzymes such as superoxidase dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) are involved in the scavenging of ROS (Saud et al. 2013, 2014, 2016, 2017, 2020; Shah et al. 2013). Although the climatic conditions are very suitable for agriculture in China, their yield potential is still very low for the *C. capsularis* production in China. The total yield of *C. capsularis* per hectare in China is very low 12,002 kg/ha contrary to its potential yield (Saleem et al. 2019b, 2020d). In our previous literature review, we have discussed briefly that, however, *C. capsularis* plants are also hyperaccumulators for various heavy metals (especially Cu) and their physiological and morphological characteristics, which makes them an excellent candidate for phytoremediation of different heavy metals (Saleem et al. 2020d).

The extension and quality enhancement of *C. capsularis* fibers can be accomplished by exploiting such environmental controls together with the inclusion of plant growth regulators (Parveen et al. 2020; Saleem et al. 2020a; Zaheer et al. 2015). Phytohormones, in particular gibberellic acid (GA_3), are a key growth hormone for controlling different physiological mechanisms including plant growth and composition, flowering, leaf expansion stimulation, elongation, and osmoregulation stimulation in internodes, dry matter, and biomass composition, germination, and also increase sink space improvement (Fahad et al. 2014a, b; Saleem et al. 2015; Ullah et al. 2017). GA_3 is a plant growth regulator including auxins and cytokinesis that controls every aspect of plant growth from embryogenesis and regulates an antioxidant protection system that decreases oxidative stress when plants grow under stress (Hadi et al. 2010; Ji et al. 2015). In addition, GA_3 is a kind of diterpenoid composite widely used as phytohormones to increase heavy metal phytoextraction in many studies (Hadi et al. 2010; Ji et al. 2015; Sun et al. 2013; Uzal and Yasar 2017). Previously, we carried out experiments in Cu mining soil (Saleem et al. 2020a), natural soil which was artificially spiked with $CuSO_4 \cdot 5H_2O$ (Saleem et al. 2020e), mixing of Cu mining soil and natural soil (Saleem et al. 2020c), and also in

Petri plates (Saleem et al. 2019a) on *C. capsularis* plants at the toxic levels. However, foliar application of plant hormones such GA_3 has not been explored on jute plants, which not only improved plant growth and development in the stress environment but also increased the phytoremediation potential of a plant when grown under Cu-polluted soil. But the foliar application of plant hormones like GA_3 to *C. capsularis* plants was not explored, which not only improved the growth and development of plants in the stress environment, but also increased the plant's phytoremediation ability when grown in Cu-polluted soil. A lot of literature is, however, available on other plant species, such as *Zea mays* L., *Tagetes patula* L., and *Solanum nigrum* L., which were exogenously supplemented by GA_3 to enhance plant growth as well as plant growth and composition when grown on metal contaminated soil (Hadi et al. 2010; Sun et al. 2013; Uzal and Yasar 2017). The uniqueness of *C. capsularis* plants due to its high biomass production and tolerance towards Cu can be valuable traits for phytoremediation capability; however, sufficient information is not available regarding Cu tolerance, antioxidative defense system, and Cu accumulation, when grown as fiber under different foliar levels of GA_3. In the present study, anatomical and physiological variables of *C. capsularis* were determined to address the following hypotheses: (i) different applications of GA_3 can affect the plant growth and biomass of *C. capsularis* seedlings, (ii) oxidative stress and response of different activities of antioxidants of *C. capsularis* seedlings, and (iii) phytoremediation potential of *C. capsularis* seedlings when grown on Cu-contaminated soil. According to best of our knowledge, this study is among the few studies which focus on the metal tolerance and accumulation among fiber crops in order to investigate their suitability for metal-contaminated sites with the foliar spar of GA_3. Findings from the present study will add to our understanding the mechanism of Cu tolerance and accumulation in *C. capsularis.*

Materials and methods

Soil and seed preparation

The soil was collected from a Cu mining area of Baisha village, DaYe County, Hubei, China (115.20′E, 29.85′N) at depth of 0–20 cm. The soil was thoroughly mixed, air-dried under shade, ground, and sieved through a 5-mm sieve before a pot experiment. Physicochemical properties of soil used for pot experiment are presented in Table 1. The seeds of jute (*Corchorus capsularis* L.) type C-3 (released from Bangladesh) were subjected to sterilization using 1% (*w*/*v*) sodium hypochlorite for 15 min followed by washing with distilled water for the prevention of surface fungal/bacterial contamination. The seeds of *C. capsularis* used in the current study were collected from Bast and Fiber Research Center, Huazhong Agricultural University, Hubei Province, P.R. China. The same variety of *C. capsularis* (C-3) is a Cu hyperaccumulator species which has been demonstrated in our previous studies (Saleem et al. 2019a, 2020c, e). The experiment was conducted in the green house at the College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China (114.20′E, 30.28′N), during spring 2019. Pots were placed in a glasshouse, where plants received natural light, with day/night temperature of 25/30 °C and day/night humidity of 70/90%. Each treatment was arranged in a completely randomized design (CRD) with five replications. Weeding, irrigation with metal-free water, and other necessary intercultural operations were done when needed. No additional/external phytohormone or fertilizers were added during the whole experiment. Different levels of GA_3 used in this study were higher than (Uzal and Yasar 2017) under Cd stress in *Capsicum annuum* L., but lesser than (Ji et al. 2015) also under Cd stress in *Solanum nigrum* L. After 2 months of seed planting, all plants had been rooted and divided into roots, leaves, and stems to study different biological attributes. Roots were uprooted and immersed in 20 mM Na2EDTA for 15–20 min to remove Cu adhered to the surface of roots. Then, roots were washed thrice with distilled water and finally once with de-ionized water and dried for further analysis (Burd et al. 2000). All chemicals used were of analytical grade, procured from Sinopharm Chemical Reagent Co., Ltd.

Exogenous application of GA_3

All pots were divided into four groups based on exogenous GA_3 supplementation, i.e., 0, 10, 50, and 100 mg/L and sprayed with GA_3 on all plant seedlings after 14 days of soil seed planting. The treatments were applied by spraying GA_3, including whole plant seedlings, until the solution falls. From 9:00 until 10:00 a.m., all plants were sprayed with GA_3 solution exogenously and spray was only once during the whole experiment. The respected plants were applied with Cu-free

Table 1 Physiochemical properties of Cu-contaminated soil used in pot experiment

Characteristics	Units	Cu-contaminated soil
pH	–	7.4
EC	µS/cm	284
CEC	cmol/kg	18.2
Organic matter	g/kg	30.96
Exchangeable K	mg/kg	120.25
Exchangeable N	g/kg	16
Exchangeable P	g/kg	0.17
Total Cu	mg/kg	2221

water after 3 h to keep the substratum wet. In 95% ethanol (C_2H_5OH) and distilled water, liquid spray of 4% gibberellin was prepared with the required amount of GA_3. The preparation and supplementation of GA_3 to the *C. capsularis* seedlings followed the method presented by Sun et al. (2013).

Plant harvest and data collection

All plants were wrapped in the first week of May for different morphological traits. Every sample of *C. capsularis* (under the application of GA_3) was sampled at rapid growth stages, at 09:00–10:00, with functional leaf (the fourth or sixth from the top). The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen, and stored in a freezer at low temperature (− 80 °C) for further analysis. Morphological traits, such as plant height, fresh plant weight, and plant dry weight, were measured after harvesting at 60 DAS. Five uniform plants were randomly selected for trait measurement. Plant height, defined as the total length of the plant (i.e., from the tip of the roots to the uppermost part of the leaves), was measured by using a measuring scale. Plant fresh weight was measured by measuring the total weight of the plant, including root and shoot weight, using a digital balance. For measuring plant dry weight, plant samples were oven-dried at 105 °C for 1 h, followed by at 65 °C for 72 h until the weight was uniform.

Determination of chlorophyll contents and gaseous exchange attributes

Leaves were collected at 60 DAS for determination of chlorophyll content. For chlorophyll content analysis, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of (Arnon 1949).

At the same days, gaseous exchange was also measured. Net photosynthesis (P*n*), leaf stomatal conductance (g*s*), transpiration rate (T*s*), and intercellular carbon dioxide concentration (C*i*) were measured from three different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf P*n*, g*s*, T*s*, and C*i* were measured with an LI-COR gas exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 mmol/mol and LED light intensity were set at 1000 mmol/m^2/s, which is the average saturation intensity for photosynthesis in *C. capsularis* (Austin 1990).

Determination of oxidative stress indicators and antioxidant response

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) content. Briefly, 0.1 g of frozen leaves was ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethylene pyrrole. The homogenate was centrifuged at 10,000×*g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ microplate absorbance spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol/g using the following formula: 6.45 (A532-A600)-0.56 A450. Lipid peroxidation was measured using a method previously published by (Heath and Packer 1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (*v*/*v*) H_2SO_4 and centrifuged at 6000*g* for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by extinction coefficient of 0.28 mmol/cm.

Stress-induced electrolyte leakage (EL) of uppermost stretched leaves was determined by Dionisio-Sese and Tobita (1998) method. The leaves were cut into minor slices (5 mm length) and placed in test tubes having 8 mL distilled water. These tubes were incubated and transferred into water bath for 2 h prior to measuring the initial electrical conductivity (EC_1). The samples were autoclaved at 121 °C for 20 min, and then cooled down to 25 °C before measuring the final electrical conductivity (EC_2). Electrolyte leakage was measured using pH/conductivity meter (model 720, INCO-LAB Company, Kuwait) and calculated as

$$\mathrm{EL} = (\mathrm{EC1}/\mathrm{EC2}) = \times 100$$

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0) including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatant was used for measurement of SOD and POD activities. SOD activity was assayed in 3-mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ microplate absorbance spectrophotometer; Bio-Rad). Enzyme activity was measured using a method by Chen and Pan (1996) and expressed as U/g FW.

POD activity in the leaves was estimated using the method of Sakharov and Ardila (1999) using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation were recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

Catalase activity was analyzed according to Aebi (1984). The assay mixture (3.0 mL) was composed of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss (ε = 39.4 mM/cm).

Ascorbate peroxidase activity was measured according to Nakano and Asada (1981). The mixture containing 100 μL enzyme extract, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm (ε = 2.8 mM/cm).

Determination of Cu concentration

Dried plant part (roots and shoots) samples were ground in a stainless steel mill and passed through a 0.1-mm nylon sieve for Cu analysis. Briefly, 0.1 g of dried sample was digested in $HNO_3/HClO_4$ (4:1) solution. The digested solution was washed in 25-mL flasks and diluted in de-ionized water until reaching the final volume of 25 mL. The supernatant was passed through a 0.45-μm filter paper and determined using a Perkin-Elmer 3100 Atomic Absorption Spectrophotometer, which calibrated with standard solutions containing known concentrations of each element.

Statistical analysis

The normality of data was analyzed using the UNIVARIATE procedure of Statistix 8.1. All the data were subjected to a one-way analysis of variance (ANOVA) followed by the Tukey's honestly significant difference (LSD) method to avoid a type I error. The analysis showed that the data in this study were almost normally distributed. Thus, the mean difference between the treatments was deemed significant at $P \leq 0.05$ between the treatments. Graphical representation was conducted using Sigmaplot 12.5 and R_Studio.

Results and discussion

Plant growth and biomass

Excess Cu can affect important physiological processes in plants and cause problems in plant growth and development. Cu taken from the soil must be transported, distributed, and compartmentalized within different tissues and organelles for healthy plant growth and development (Adrees et al. 2015; Celis-Plá et al. 2018; Liu et al. 2018). On the other hand, excessive Cu is characterized by a reduced plant biomass, leaf chlorosis, inhibited root growth, bronzing, and necrosis (Ji et al. 2015; Sağlam et al. 2016). Furthermore, concentration of Cu within cellular components needs to be maintained at low level because toxic level of Cu can induce alterations in photosynthesis, respiration, enzyme activity, DNA, and membrane integrity leading towards inhibited growth and endangered survival of plants (Elleuch et al. 2013; Rehman et al. 2019c; Saleem et al. 2019a). In this study, minimum plant growth and biomass have been observed in the plants cultivated under high Cu concentration without exogenous GA_3 supplementation (Table 2). Foliar spray of GA_3, however, even under Cu stress, increased plant growth and biomass, while maximum plant growth and biomass were reported at the highest level (100 mg/L) of GA_3 (Table 2). The overall increased in plant height, fresh, and dry biomass, 31.3%, 30.6%, and 35.8%, respectively, at the highest level of GA_3 (100 mg/L) compared to plants cultivated without the application of GA_3 in Cu-contaminated soil. Ji et al. (2015) stated that, when grown in Cd-polluted soil, a substantial increase in plant growth and biomass was observed in *S. nigrum* under GA_3 application. The foliar application of GA_3 also noticed a twice increase in plant growth and biomass compared with untreated plants in *Carapichea ipecacuanha* (Brot.) L. Andersson (Isogai et al. 2008). In the present study, increase in plant growth and biomass in Cu-stressed plants with the application of GA_3 might be to increase nutrient uptake and/or GA_3 helps in decreasing free metal ions in plants as suggested by Shafigh et al. (2016). Improving goal accumulation of contaminants in existing high-yield plants without lowering their yields is the most reasonable strategy for phytoremediation (Mahar et al. 2016; Parmar and Singh 2015; Sun et al. 2013). Remediating polluted soils using GA_3 to induce plant growth and composition is therefore achievable.

Chlorophyll contents and gaseous exchange attributes

Chlorophyll content is an important parameter for the evaluation of plant stress (Habiba et al. 2015). Cu is an important micronutrient at low minute level; however, the minimum chlorophyll contents and gaseous exchange attributes were found in plants grown in the soil under high Cu concentration, without application of GA_3 (Table 2, Fig. 1). According to the results, foliar application in 60-day-old seedlings of *C. capsularis* increased total chlorophyll contents by 40% in 100-mg/L GA_3-treated plants compared without GA_3-treated plants (Table 2). Similarly, the plants treated with 100 mg/L of GA_3 increased P*n*, T*r*, G*s*, and C*i* by 19.1%, 27.5%, 166.6%, and 7.1%, respectively, compared with non-treated GA_3 plants (Fig. 1). The chlorophyll content and gaseous exchange attributes are likely to be

Table 2 Effect of different concentration of GA_3 on plant height (cm), plant fresh weight (g), plant dry weight (g), and chlorophyll contents (mg^{-1} FW) on *C. capsularis* seedlings grown on Cu-contaminated soil

Treatments	Plant height	Plant fresh weight	Plant dry weight	Chlorophyll contents
Cu	86 ± 2 c	88 ± 2 c	53 ± 2 c	1.5 ± 0.01 d
Cu + GA_{3-10}	101 ± 3 b	101 ± 2 b	63 ± 2 b	1.8 ± 0.01 c
Cu + GA_{3-50}	109 ± 3 a	111 ± 2 a	68 ± 2 a	1.9 ± 0.01 b
Cu + GA_{3-100}	113 ± 2 a	115 ± 2 a	72 ± 2 a	2.1 ± 0.01 a

Means sharing similar letter(s) within a column for each parameter do not differ significantly at $P < 0.05$. Data in the table are means of three repeats ($n = 3$) of just one harvest of *C. capsularis* seedlings ± standard deviation (SD). Relative radiance of plastic filter used: Cu (Cu contamination soil without the application of GA_3), Cu + GA_{3-10} (Cu contamination soil with the application of 10 mg/L GA_3), Cu + GA_{3-50} (Cu contamination soil with the application of 50 mg/L GA_3), and Cu + GA_{3-100} (Cu contamination soil with the application of 100 mg/L GA_3)

reduced due, during the development phase, to chloroplast damage following Cu exposure in the soil system (Rehman et al. 2019a; b; Saleem et al. 2020g). Habiba et al. (2015) have provided strong evidence for the reduction of chlorophyll biosynthesis that could be related to destruction of thylakoid membrane and also Cu's impediment with the structured chlorophyll method. Previously, we also noticed that toxic level of Cu in the soil destroys the ultra-structure of chloroplast and thus

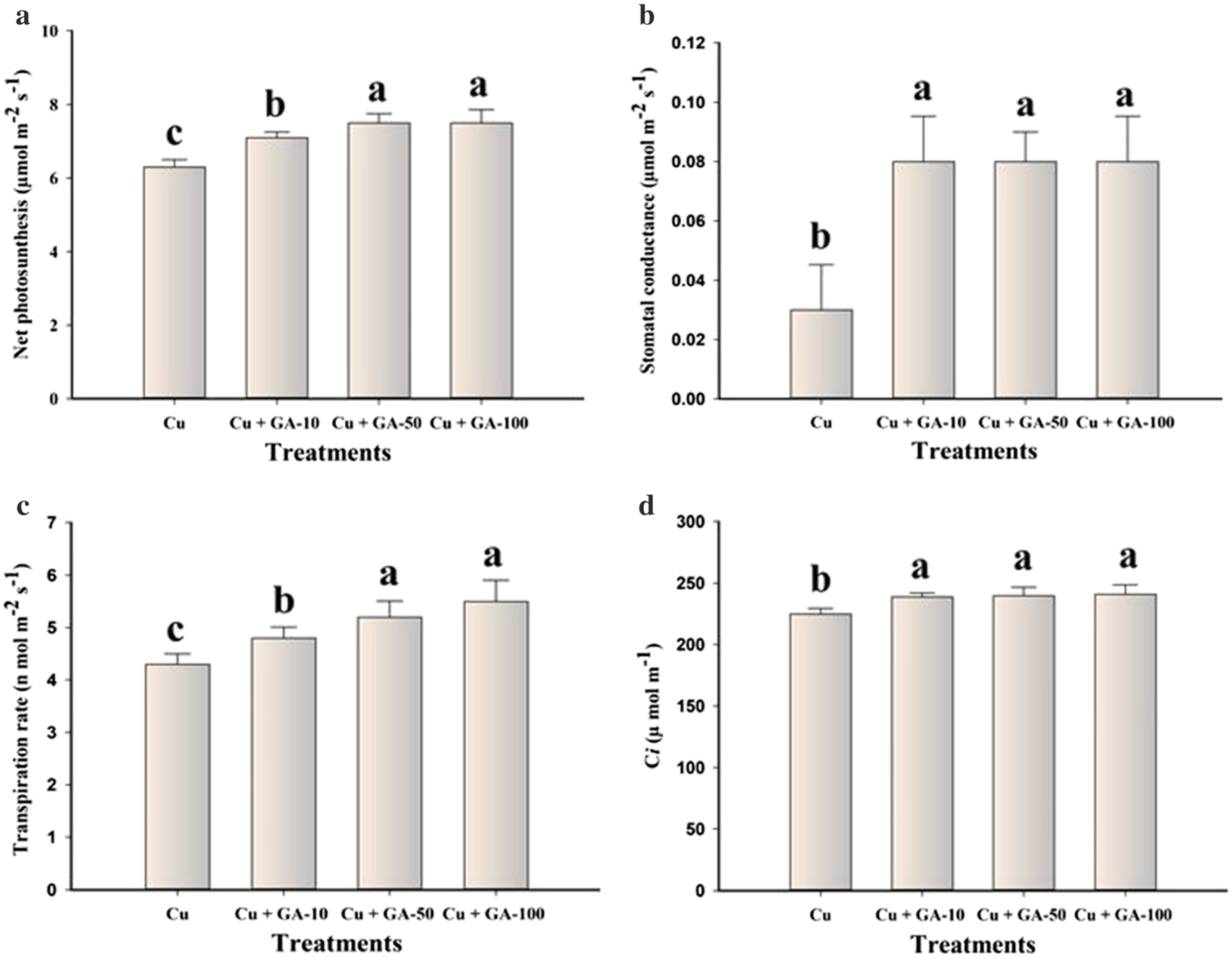

Fig. 1 Effect of different concentration of GA_3 on gaseous exchange attributes of *C. capsularis* seedlings grown under high concentration of Cu in the soil. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $P < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *C. capsularis* seedlings ± standard deviation (SD). Relative radiance of plastic filter used: Cu (Cu contamination soil without the application of GA_3), Cu + GA_{3-10} (Cu contamination soil with the application of 10 mg/L GA_3), Cu + GA_{3-50} (Cu contamination soil with the application of 50 mg/L GA_3), and Cu + GA_{3-100} (Cu contamination soil with the application of 100 mg/L GA_3)

affected the photosynthetic machinery in *C. capsularis* plants (Parveen et al. 2020; Saleem et al. 2020a; c). Nevertheless, the foliar application of GA_3 to Cu-stressed plants increased the contents of total chlorophyll and gaseous exchange attributes (Fig. 1). Many studies have already found in their findings that a protective role of GA_3 for the metal stressed plants (Falkowska et al. 2011; Masood and Khan 2013; Ouzounidou and Ilias 2005; Saleem et al. 2015) which improved photosynthetic machinery. The possible reason behind this mechanism is the reducing free metal ions and/or enhance the activities of antioxidant enzymes that reduced the oxidative damage (Iqbal and Ashraf 2013; Zaheer et al. 2015; Rehman et al. 2020; Saleem et al. 2020h, i).

Oxidative stress and antioxidant response

A direct effect of excess Cu in plants at the cellular level is oxidative stress caused by the increased concentration of reactive oxygen species (ROS) either directly or indirectly by affecting metabolic pathways (Liu et al. 2018; Quartacci et al. 2015; Thounaojam et al. 2012; Saleem et al. 2020j, k, l). Up-regulation of activity of various antioxidative enzymes shows the capacity of plants to scavenge excessive ROS in the cells (Chen et al. 2015; Imran et al. 2019; Kamran et al. 2019; Saleem et al. 2020f). Plant response to oxidative stress also depends upon plant species and cultivars. For instance, increasing Cu concentration in the soil increased the activities of various antioxidants in *Brassica napus* L. (Zaheer et al. 2015) and *Orzya sativa* L. (Thounaojam et al. 2012). The enhancement of antioxidant activity can be considered as an indication of increased generation and mitigation of ROS (Kanwal et al. 2014; Rehman et al. 2019a; Saleem et al. 2020g). Nevertheless, oxidative stress was minimized in this study by enhancing the activities of various antioxidants due to the foliar application of GA_3 (Figs. 2 and 3). According to the results, foliar

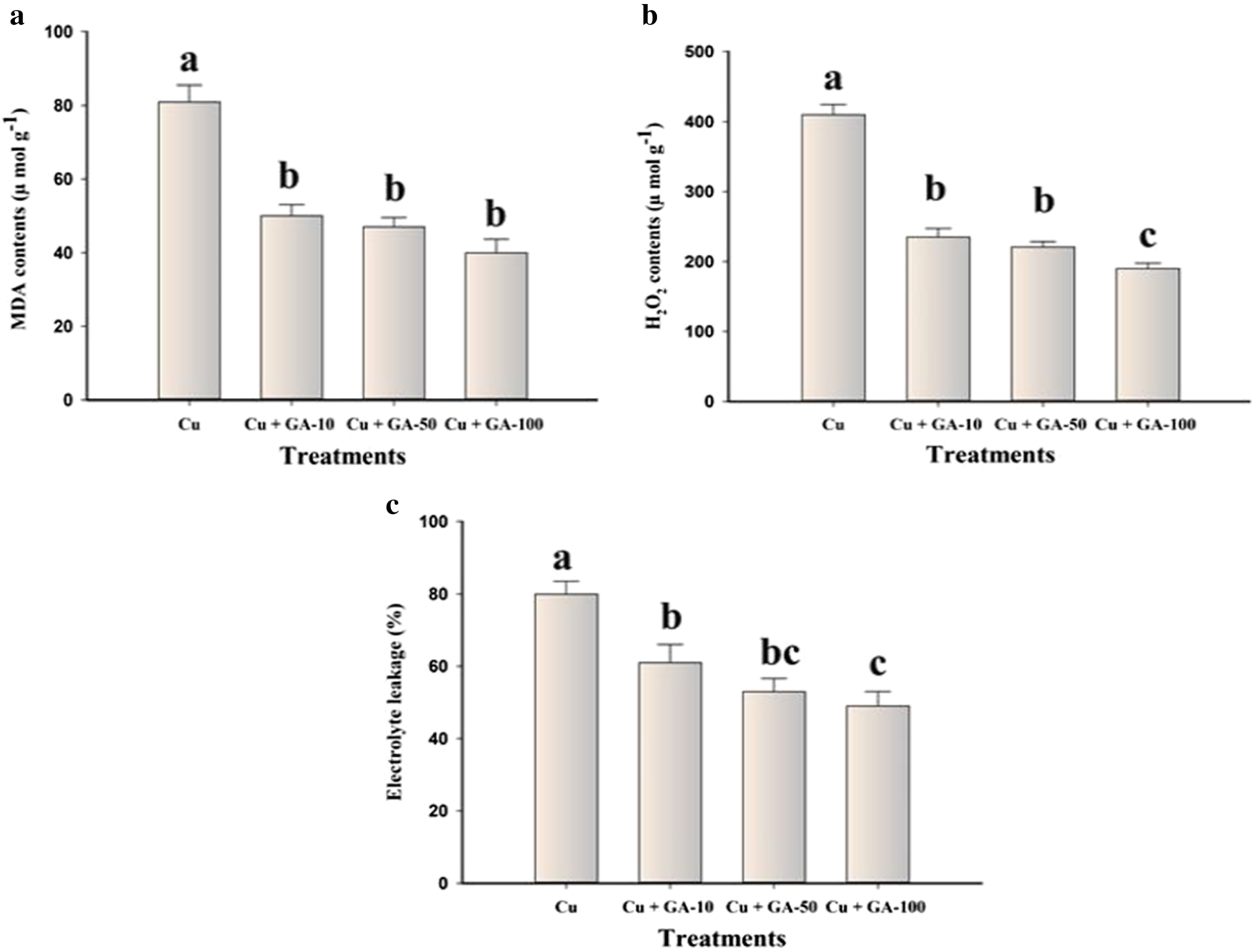

Fig. 2 Effect of different concentration of GA_3 on MDA contents (**a**), H_2O_2 contents (**b**), and electrolyte leakage (**c**) in the leaves of *C. capsularis* seedlings grown under high concentration of Cu in the soil. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $P < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *C. capsularis* seedlings ± standard deviation (SD). Relative radiance of plastic filter used: Cu (Cu contamination soil without the application of GA_3), Cu + GA_{3-10} (Cu contamination soil with the application of 10 mg/L GA_3), Cu + GA_{3-50} (Cu contamination soil with the application of 50 mg/L GA_3), and Cu + GA_{3-100} (Cu contamination soil with the application of 100 mg/L GA_3)

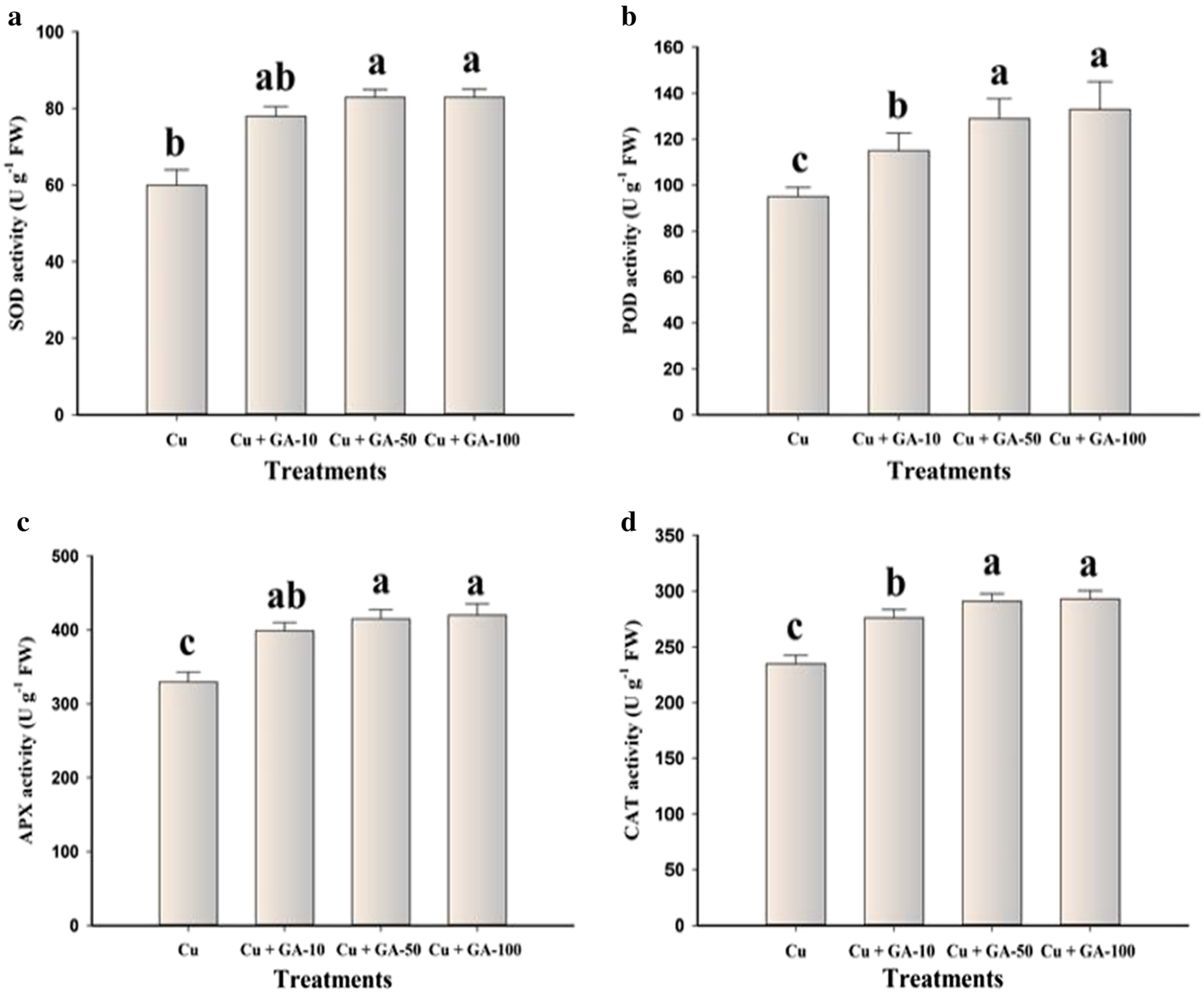

Fig. 3 Effect of different concentration of GA_3 on SOD (**a**), POD (**b**), CAT (**c**), and APX (**d**) in the leaves of *C. capsularis* seedlings grown under high concentration of Cu in the soil. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $P < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *C. capsularis* seedlings ± standard deviation (SD). Relative radiance of plastic filter used: Cu (Cu contamination soil without the application of GA_3), Cu + GA_{3-10} (Cu contamination soil with the application of 10 mg/L GA_3), Cu + GA_{3-50} (Cu contamination soil with the application of 50 mg/L GA_3), and Cu + GA_{3-100} (Cu contamination soil with the application of 100 mg/L GA_3)

application of GA_3 (100 mg/L) decreased the contents of MDA, H_2O_2, and EL by 50.6%, 53.6%, and 38.8%, respectively, compared without treated with GA_3 application. In contrast, application of GA_3 (100 mg/L) caused a significant increase in the activities of SOD, POD, CAT, and APX, which were increased by 53.5%, 40%, 24.7%, and 27.3%, respectively, compared with the plants without treated with GA_3 supplementation. These results coincide with the findings of Zaheer et al. (2015) who reported that addition of phytohormone (citric acid) in the nutrient solution caused a significant decrease in oxidative stress in the plants by up-regulation of the activities of various antioxidants in a Cu stress environment. In the current literature, better growth of *C. capsularis* seedlings under elevating levels of exogenously sprayed GA_3 under high contents of Cu contents in the soil might be associated with a better antioxidant system of the plants. Likewise, higher Cu uptake and accumulation by the plants were concomitant with an accumulation of Cu in the tissues of plants (Table 3).

Cu uptake and accumulation

Plant species vary in their capacity for Cu accumulation depending on growth stage and fertilizer application. Root system of plants plays an active role for uptake of Cu from the soil solution and after absorption by roots; Cu is transported to shoots via the xylem. The mechanism of Cu uptake is initiated by the adsorption of Cu on the root surface from where it dissociates from its complex forms before absorption by plants

Table 3 Effect of different concentration of GA_3 on Cu (mg/kg) uptake and accumulation in different parts (roots, leaves, and stems) of *C. capsularis* seedlings

Treatments	Roots	Leaves	Stems
Cu	63 ± 2 c	131 ± 8 c	110 ± 4 c
Cu + GA_{3-10}	79 ± 5 b	178 ± 5 b	148 ± 5 b
Cu + GA_{3-50}	86 ± 4 b	199 ± 5 a	175 ± 5 a
Cu + GA_{3-100}	90 ± 1 a	200 ± 8 a	185 ± 5 4 a

Means sharing similar letter(s) within a column for each parameter do not differ significantly at $P < 0.05$. Data in the table are means of three repeats ($n = 3$) of just one harvest of *C. capsularis* seedlings ± standard deviation (SD). Relative radiance of plastic filter used: Cu (Cu contamination soil without the application of GA_3), Cu + GA_{3-10} (Cu contamination soil with the application of 10 mg/L GA_3), Cu + GA_{3-50} (Cu contamination soil with the application of 50 mg/L GA_3), and Cu + GA_{3-100} (Cu contamination soil with the application of 100 mg/L GA_3)

(Adrees et al. 2015; Chen et al. 2015; Sağlam et al. 2016). *C. capsularis* includes fibrous crop which can sustain and accumulate in their harvestable parts (leaves and stems) from the metal-polluted soil significant amounts of heavy metals (Abubakari et al. 2017; Ahmed and Slima 2018; Ogunkunle et al. 2015; Saleem et al. 2019a). Though numerous heavy metals, *C. capsularis* plants not only thrive at high levels of pollutants, they can also revoke large amounts of pollutants from contaminated soils (Saleem et al. 2020a; Uddin Nizam et al. 2016). We discussed previously in our literature review the comprehensive characteristics of *C. capsularis* plants tolerating and accumulating different heavy metals (Saleem et al. 2020d). In the same Cu-polluted soil, after 120 days of seed sowing, the maximum uptake of Cu contents in the shoots was 214 mg/kg Cu without the application/fertilization of chelators/fertilizers (Saleem et al. 2020c). However, in this study, foliar application of GA_3 increased the metal uptake in both parts of the plants, i.e., aboveground parts and belowground parts of the *C. capsularis* seedlings (Table 3). At high level of GA_3 (100 mg/L) application, the maximum Cu was accumulated in the leaves (200 mg/kg), stems (185 mg/kg), and roots (90 mg/kg) compared to the plants which were not treated with any level of GA_3 exogenously (Table 3). This might be due to the increase in the transpiration rate (Fig. 1c) which help to increase Cu uptake to the aboveground parts through water movement (Habiba et al. 2015). Foliar application of GA_3 increased phytoextraction of a plant species which has been shown in number of plant species under different metal stress environment (Falkowska et al. 2011; Hadi et al. 2010; Ji et al. 2015). Niazy Abdou and Wahdan (2017) studied *C. capsularis* plants under lead-polluted soils and noticed that application of plant hormone (citric acid) increased not only plant growth and biomass under lead-contaminated soil, but also increased phytoextraction of lead using *C. capsularis* plants. Although there is no previous study, foliar application of GA_3 increased metal uptake in *C. capsularis* plants, but we have noticed, in a pot experiment, that external fertilization with phosphorus increased plant growth and biomass as well as metal uptake and accumulation in different parts of *C. capsularis* (Saleem et al. 2020a).

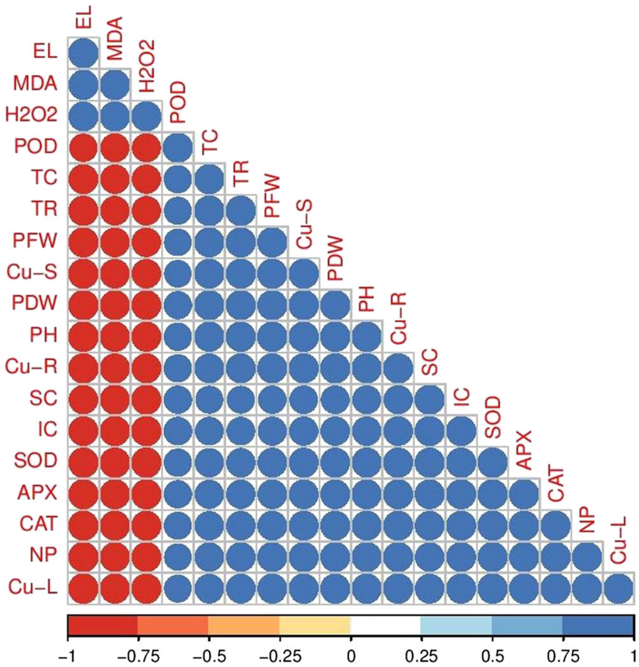

Fig. 4 Correlation between Cu uptake with different morpho-physiological traits of *C. capsularis* seedlings. EL (electrolyte leakage), MDA (MDA contents), H_2O_2 (H_2O_2 contents), POD (POD activity), TC (total chlorophyll contents), TR (transpiration rate), PFW (plant fresh weight), Cu-S (Cu concentration in stems), PDW (plant dry weight), PH (plant height), Cu-R (Cu concentration in roots), SC (stomatal conductance), IC (intercellular CO_2), SOD (SOD activity), APX (APX activity), CAT (CAT activity), NP (net photosynthesis), and Cu-L (Cu concentration in leaves)

Relationship

The Pearson correlation analysis was conducted to quantify the relationship between different parameters studied in this study (Fig. 4). Cu concentration in the roots was positively correlated with Cu concentration in the leaves and other morpho-physiological traits of the plants, but negatively correlated with oxidative stress indicators of *C. capsularis* seedlings. However, MDA contents in the leaves are positively correlated with H_2O_2 contents in the shoots while negatively correlated with other growth, gaseous exchange attributes, antioxidant enzymes, and Cu concentration of the *C. capsularis* seedlings. This correlation reflected the close connection between Cu uptake and growth in *C. capsularis* seedlings.

Conclusion

Based on the present study, it can be concluded that plant has strong antioxidant defense system to scavenge ROS production, which generated due to toxic contents of Cu in the soil. Although Cu toxicity was also overcome by the application of GA_3 which not only increased plant growth, biomass, chlorophyll contents, and gaseous exchange attributes but also increased phytoextraction of Cu in *C. capsularis* seedlings, hence, *C. capsularis* can be used as a tool for phytoremediation of Cu in Cu-polluted soil and foliar application of GA_3 increased plant growth and biomass and Cu accumulation capabilities and can be used as a bio-resource and fibrous crop to fulfil the market demand of the fiber.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abubakari M, Moomin A, Nyarko G, Dawuda M (2017) Heavy metals concentrations and risk assessment of roselle and jute mallow cultivated with three compost types. Ann Agric Sci 62:145–150

Adrees M, Ali S, Rizwan M, Ibrahim M, Abbas F, Farid M, Zia-ur-Rehman M, Irshad MK, Bharwana SA (2015) The effect of excess copper on growth and physiology of important food crops: a review. Environ Sci Pollut Res 22:8148–8162

Aebi H (1984) [13] Catalase in vitro, methods in enzymology. Elsevier, pp. 121-126

Afshan S, Ali S, Bharwana SA, Rizwan M, Farid M, Abbas F, Ibrahim M, Mehmood MA, Abbasi GH (2015) Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in *Brassica napus* L. Environ Sci Pollut Res 22:11679–11689

Ahmad R, Ali S, Rizwan M, Dawood M, Farid M, Hussain A, Wijaya L, Alyemeni MN, Ahmad P (2019) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. Physiol Plant

Ahmed DA, Slima DF (2018) Heavy metal accumulation by Corchorus olitorius L. irrigated with wastewater. Environ Sci Pollut Res 25: 14996–15005

Akram R, Turan V, Hammad HM, Ahmad S, Hussain S, Hasnain A, Maqbool MM, Rehmani MIA, Rasool A, Masood N, Mahmood F, Mubeen M, Sultana SR, Fahad S, Amanet K, Saleem M, Abbas Y, Akhtar HM, Waseem F, Murtaza R, Amin A, Zahoor SA, ul Din MS, Nasim W (2018a) Fate of organic and inorganic pollutants in paddy soils. In: Hashmi MZ, Varma A (eds) Environmental Pollution of Paddy Soils, Soil Biology. SPRINGER INTERNATIONAL PUBLISHING AG, GEWERBESTRASSE 11, CHAM, CH-6330, SWITZERLAND, pp 197–214

Akram R, Turan V, Wahid A, Ijaz M, Shahid MA, Kaleem S, Hafeez A, Maqbool MM, Chaudhary HJ, Munis MFH, Mubeen M, Sadiq N, Murtaza R, Kazmi DH, Ali S, Khan N, Sultana SR, Fahad S, Amin A, Nasim W (2018b) Paddy land pollutants and their role in climate change. In: Hashmi MZ, Varma A (eds) Environmental Pollution of Paddy Soils, Soil Biology. SPRINGER INTERNATIONAL PUBLISHING AG, GEWERBESTRASSE 11, CHAM, CH-6330, SWITZERLAND, pp 113–124

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris L. Plant Physiol 24:1

Ashraf MA, Hussain I, Rasheed R, Iqbal M, Riaz M, Arif MS (2017) Advances in microbe-assisted reclamation of heavy metal contaminated soils over the last decade: a review. J Environ Manag 198: 132–143

Austin RB (1990) Prospects for genetically increasing the photosynthetic capacity of crops

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46: 237–245

Celis-Plá PS, Brown MT, Santillán-Sarmiento A, Korbee N, Sáez CA, Figueroa FL (2018) Ecophysiological and metabolic responses to interactive exposure to nutrients and copper excess in the brown macroalga Cystoseira tamariscifolia. Mar Pollut Bull 128:214–222

Chen C-N, Pan S-M (1996) Assay of superoxide dismutase activity by combining electrophoresis and densitometry. Bot Bull Acad Sin 37

Chen J, Shafi M, Li S, Wang Y, Wu J, Ye Z, Peng D, Yan W, Liu D (2015) Copper induced oxidative stresses, antioxidant responses and phytoremediation potential of Moso bamboo (*Phyllostachys pubescens* J.Houz.). Sci Rep 5:13554

Czymmek K, Ketterings Q, Ros M, Battaglia M, Cela S, Crittenden S, Gates D, Walter T, Latessa S, Klaiber L, Albrecht G. (2020) The New York Phosphorus Index 2.0. Agronomy Fact Sheet Series. Fact Sheet #110. Nutrient Management Spear Program. Cornell University Cooperative Extension. At: http://nmsp.cals.cornell.edu/publications/factsheets/factsheet110.pdf (Accessed: 6/1/2020)

Daud M, Ali S, Abbas Z, Zaheer IE, Riaz MA, Malik A, Hussain A, Rizwan M, Zia-ur-Rehman M, Zhu SJ (2018) Potential of duckweed (Lemna minor) for the phytoremediation of landfill leachate. J Chem 2018

Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135:1–9

Elleuch A, Chaâbene Z, Grubb DC, Drira N, Mejdoub H, Khemakhem B (2013) Morphological and biochemical behavior of fenugreek (*Trigonella foenum-graecum* subsp. gladiata (M.Bieb.) P.Fourn.) under copper stress. Ecotoxicol Environ Saf 98:46–53

Fahad S, Bano A (2012) Effect of salicylic acid on physiological and biochemical characterization of maize grown in saline area. Pak J Bot 44:1433–1438

Fahad S, Chen Y, Saud S, Wang K, Xiong D, Chen C, Wu C, Shah F, Nie L, Huang J (2013) Ultraviolet radiation effect on photosynthetic pigments, biochemical attributes, antioxidant enzyme activity and hormonal contents of wheat. J Food, Agri Environ 11(3&4):1635–1641

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2014a) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res 22(7):4907–4921. https://doi.org/10.1007/s11356-014-3754-2

Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah N, Faiq M, Khan MR, Tareen AK, Khan A, Ullah A, Ullah N, Huang J (2014b) Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul 75(2):391–404. https://doi.org/10.1007/s10725-014-0013-y

Fahad S, Hussain S, Saud S, Tanveer M, Bajwa AA, Hassan S, Shah AN, Ullah A, Wu C, Khan FA, Shah F, Ullah S, Chen Y, Huang J (2015a) A biochar application protects rice pollen from high-temperature stress. Plant Physiol Biochem 96:281–287

Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang J (2015b) Crop plant hormones and environmental stress. Sustain Agric Rev 15:371–400

Fahad S, Hussain S, Saud S, Hassan S, Chauhan BS, Khan F et al (2016a) Responses of rapid viscoanalyzer profile and other rice grain qualities to exogenously applied plant growth regulators under high day and high night temperatures. PLoS One 11(7):e0159590. https://doi.org/10.1371/journal.pone.0159590

Fahad S, Hussain S, Saud S, Khan F, Hassan S, Amanullah Jr, Nasim W, Arif M, Wang F, Huang J (2016b) Exogenously applied plant growth regulators affect heat-stressed rice pollens. J Agron Crop Sci 202:139–150

Fahad S, Hussain S, Saud S, Hassan S, Ihsan Z, Shah AN, Wu C, Yousaf M, Nasim W, Alharby H, Alghabari F, Huang J (2016c) Exogenously applied plant growth regulators enhance the morphophysiological growth and yield of rice under high temperature. Front Plant Sci 7:1250. https://doi.org/10.3389/fpls.2016.01250

Fahad S, Hussain S, Saud S, Hassan S, Tanveer M, Ihsan MZ, Shah AN, Ullah A, Nasrullah KF, Ullah S, AlharbyH NW, Wu C, Huang J (2016d) A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. Plant Physiol Biochem 103:191–198

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147. https://doi.org/10.3389/fpls.2017.01147

Fahad S, Muhammad ZI, Abdul K, Ihsanullah D, Saud S, Saleh A, Wajid N, Muhammad A, Imtiaz AK, Chao W, Depeng W, Jianliang H (2018) Consequences of high temperature under changing climate optima for rice pollen characteristics-concepts and perspectives. Arch Agron Soil Sci. https://doi.org/10.1080/03650340.2018.1443213

Fahad S, Rehman A, Shahzad B, Tanveer M, Saud S, Kamran M, Ihtisham M, Khan SU, Turan V, Rahman MHU (2019a) Rice Responses And Tolerance To Metal/Metalloid Toxicity. In: Hasanuzzaman M, Fujita M, Nahar K, Biswas JK (eds) ADVANCES IN RICE RESEARCH FOR ABIOTIC STRESS TOLERANCE, WOODHEAD PUBL LTD, ABINGTON HALL ABINGTON, CAMBRIDGE CB1 6AH, CAMBS, ENGLAND, pp 299–312

Fahad, S., Adnan, M., Hassan, S., Saud, S., Hussain, S., Wu, C., Wang, D., Hakeem, K.R., Alharby, H.F., Turan, V., Khan, M.A., Huang, J., 2019b. Rice Responses And Tolerance To High Temperature, in: Hasanuzzaman, M and Fujita, M and Nahar, K and Biswas, JK (Ed.), ADVANCES IN RICE RESEARCH FOR ABIOTIC STRESS TOLERANCE. WOODHEAD PUBL LTD, ABINGTON HALL ABINGTON, CAMBRIDGE CB1 6AH, CAMBS, ENGLAND, PP. 201–224.

Falkowska M, Pietryczuk A, Piotrowska A, Bajguz A, Grygoruk A, Czerpak R (2011) The effect of gibberellic acid (GA3) on growth, metal biosorption and metabolism of the green algae Chlorella vulgaris (Chlorophyceae) Beijerinck exposed to cadmium and lead stress. Pol J Environ Stud 20:53–59

Farid M, Ali S, Rizwan M, Ali Q, Saeed R, Nasir T, Abbasi GH, Rehmani MIA, Ata-Ul-Karim ST, Bukhari SAH (2018a) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. Ecotoxicol Environ Saf 151: 255–265

Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018b) Glutamic acid assisted phyto-management of silver-contaminated soils through sunflower; physiological and biochemical response. Environ Sci Pollut Res 25: 25390–25400

Farid M, Ali S, Saeed R, Rizwan M, Bukhari SAH, Abbasi GH, Hussain A, Ali B, Zamir MSI, Ahmad I (2019) Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. Intl J Phytoremediat:1–8

Habiba U, Ali S, Farid M, Shakoor MB, Rizwan M, Ibrahim M, Abbasi GH, Hayat T, Ali B (2015) EDTA enhanced plant growth, antioxidant defense system, and phytoextraction of copper by *Brassica napus* L. Environ Sci Pollut Res 22:1534–1544

Hadi F, Bano A, Fuller MP (2010) The improved phytoextraction of lead (Pb) and the growth of maize (Zeamays L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere 80:457–462

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Husak V (2015) Copper and copper-containing pesticides: metabolism, toxicity and oxidative stress. J Vasyl Stefanyk Precarpathian Nat Univ:39–51

Imran M, Sun X, Hussain S, Ali U, Rana MS, Rasul F, Saleem MH, Moussa MG, Bhantana P, Afzal J (2019) Molybdenum-induced effects on nitrogen metabolism enzymes and elemental profile of winter wheat (Triticum aestivum L.) under different nitrogen sources. Int J Mol Sci 20:3009

Iqbal M, Ashraf M (2013) Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. Environ Exp Bot 86:76–85

Isogai S, Touno K, Shimomura K (2008) Gibberellic acid improved shoot multiplication in Cephaelis ipecacuanha. In Vitro Cell Dev Biol-Plant 44:216–220

Ji P, Tang X, Jiang Y, Ya T, Gao P, Han W (2015) Potential of gibberellic acid 3 (GA3) for enhancing the phytoremediation efficiency of *Solanum nigrum* L. Bull Environ Contam Toxicol 95:810–814

Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS, Saleem MH, Adil M, Heidari P, Chen J-T (2019) An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. Int J Mol Sci 21:148

Kanwal U, Ali S, Shakoor MB, Farid M, Hussain S, Yasmeen T, Adrees M, Bharwana SA, Abbas F (2014) EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress. Environ Sci Pollut Res 21:9899–9910

Ketterings Q, Czymmek K, Battaglia M. (2007) Revised 2020. Removal of phosphorus by field crops. Agronomy Fact Sheet Series. Fact Sheet #28. Nutrient Management Spear Program. Cornell University Cooperative Extension. At: http://nmsp.cals.cornell.edu/publications/factsheets/factsheet28.pdf (Accessed: 6/1/2020)

Kumar S, Lai L, Kumar P, Feliciano YMV, Battaglia ML, Hong CO, Owens VN, Fike J, Farris R, Galbraith J (2019a) Impacts of nitrogen rate and landscape position on soils and switchgrass root growth parameters. Agron J 111(3):1046–1059

Kumar P, Lai L, Battaglia ML, Kumar S, Owens V, Fike J, Galbraith J, Hong CO, Faris R, Crawford R, Crawford J, Hansen J, Mayton H, Viands D (2019b) Impacts of nitrogen fertilization rate and landscape position on select soil properties in switchgrass field at four sites in the USA. CATENA 180:183–193

Laghlimi M, Baghdad B, El Hadi H, Bouabdli A (2015) Phytoremediation mechanisms of heavy metal contaminated soils: a review. Open J Ecol 5:375

Liu J, Wang J, Lee S, Wen R (2018) Copper-caused oxidative stress triggers the activation of antioxidant enzymes via ZmMPK3 in maize leaves. PLoS One 13:e0203612

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121

Masood A, Khan N (2013) Ethylene and gibberellic acid interplay in regulation of photosynthetic capacity inhibition by cadmium. J Plant Biochem Physiol 1:2

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Niazy Abdou M, Wahdan M (2017) citric acid-Enhanced phytoremediation of lead using *Corchorus capsularis* L, and *Eucalyptus camaldulensis* Dehnh.

Ogunkunle CO, Ziyath AM, Adewumi FE, Fatoba PO (2015) Bioaccumulation and associated dietary risks of Pb, Cd, and Zn in amaranth (*Amaranthus cruentus* L.) and jute mallow (*Corchorus olitorius* L.) grown on soil irrigated using polluted water from Asa River, Nigeria. Environ Monit Assess 187:281

Ouzounidou G, Ilias I (2005) Hormone-induced protection of sunflower photosynthetic apparatus against copper toxicity. Biol Plant 49:223

Pajević S, Borišev M, Nikolić N, Arsenov DD, Orlović S, Župunski M (2016) Phytoextraction of heavy metals by fast-growing trees: a review, Phytoremediation. Springer, pp. 29-64

Parmar S, Singh V (2015) Phytoremediation approaches for heavy metal pollution: a review. J Plant Sci Res 2:135

Parveen A, Saleem MH, Kamran M, Haider MZ, Chen J-T, Malik Z, Rana MS, Hassan A, Hur G, Javed MT (2020) Effect of citric acid on growth, ecophysiology, chloroplast ultrastructure, and phytoremediation potential of jute (*Corchorus capsularis* L.) seedlings exposed to copper stress. Biomolecules 10:592

Quartacci M, Ranieri A, Sgherri C (2015) Antioxidative defence mechanisms in two grapevine (Vitis vinifera L.) cultivars grown under boron excess in the irrigation water. VITIS-J Grapevine Res 54: 51–58

Rana M, Bhantana P, Sun X-C, Imran M, Shaaban M, Moussa M, Hamzah Saleem M, Elyamine A, Binyamin R, Alam M, Afzal J, Khan I, Din I, Ahmad I, Younas M, Kamran M, Hu C (2020) Molybdenum as an essential element for crops: an overview. Int J Sci Res Growth 24:18535–18535

Rehman M, Liu L, Bashir S, Saleem MH, Chen C, Peng D, Siddique KH (2019a) Influence of rice straw biochar on growth, antioxidant capacity and copper uptake in ramie (*Boehmeria nivea* L.) grown as forage in aged copper-contaminated soil. Plant Physiol Biochem 138:121–129

Rehman M, Liu L, Wang Q, Saleem MH, Bashir S, Ullah S, Peng D (2019b) Copper environmental toxicology, recent advances, and future outlook: a review. Environ Sci Pollut Res:1–14

Rehman M, Maqbool Z, Peng D, Liu L (2019c) Morpho-physiological traits, antioxidant capacity and phytoextraction of copper by ramie (*Boehmeria nivea* L.) grown as fodder in copper-contaminated soil. Environ Sci Pollut Res 26:5851–5861

Rehman M, Yang M, Fahad S, Saleem MH, Liu L, Liu F, Deng G (2020) Morpho-physiological traits, antioxidant capacity and nitrogen metabolism in Boehmeria nivea L. under nitrogen fertilizer. Agron J 1–10

Sağlam A, Yetişsin F, Demiralay M, Terzi R (2016) Copper stress and responses in plants, Plant metal interaction. Elsevier, pp. 21-40

Sakharov IY, Ardila GB (1999) Variations of peroxidase activity in cocoa (*Theobroma cacao* L.) beans during their ripening, fermentation and drying. Food Chem 65:51–54

Saleem M, Asghar HN, Khan MY, Zahir ZA (2015) Gibberellic acid in combination with pressmud enhances the growth of sunflower and stabilizes chromium (VI)-contaminated soil. Environ Sci Pollut Res 22:10610–10617

Saleem MH, Ali S, Seleiman MF, Rizwan M, Rehman M, Akram NA, Liu L, Alotaibi M, Al-Ashkar I, Mubushar M (2019a) Assessing the correlations between different traits in copper-sensitive and copper-resistant varieties of jute (*Corchorus capsularis* L.). Plants 8:545

Saleem MH, Rehman M, Zahid M, Imran M, Xiang W, Liu L (2019b) Morphological changes and antioxidative capacity of jute (*Corchorus capsularis* L. Malvaceae) under different color light-emitting diodes. Braz J Bot

Saleem M, Ali S, Rehman M, Rana M, Rizwan M, Kamran M, Imran M, Riaz M, Hussein M, Elkelish A, Lijun L (2020a) Influence of phosphorus on copper phytoextraction via modulating cellular organelles in two jute (*Corchorus capsularis* L**.)** varieties grown in a copper mining soil of Hubei Province, China. Chemosphere

Saleem MH, Ali S, Hussain S, Kamran M, Chattha MS, Ahmad S, Aqeel M, Rizwan M, Aljarba NH, Alkahtani S (2020b) Flax (*Linum usitatissimum* L.): A potential candidate for phytoremediation? Biological and economical points of view. Plants 9:496

Saleem MH, Ali S, Irshad S, Hussaan M, Rizwan M, Rana MS, Hashem A, Abd_Allah EF, Ahmad P (2020c) Copper uptake and accumulation, ultra-structural alteration, and bast fibre yield and quality of fibrous jute (*Corchorus capsularis* L**.)** Plants grown under two different soils of China. Plants 9:404

Saleem MH, Ali S, Rehman M, Hasanuzzaman M, Rizwan M, Irshad S, Shafiq F, Iqbal M, Alharbi BM, Alnusaire TS (2020d) Jute: a potential candidate for phytoremediation of metals—a review. Plants 9:258

Saleem MH, Fahad S, Khan SU, Ahmar S, Khan MHU, Rehman M, Maqbool Z, Liu L (2020e) Morpho-physiological traits, gaseous exchange attributes, and phytoremediation potential of jute (*Corchorus capsularis* L.) grown in different concentrations of copper-contaminated soil. Ecotoxicol Environ Saf 189:109915

Saleem MH, Fahad S, Khan SU, Din M, Ullah A, Sabagh AEL, Hossain A, Llanes A, Liu L (2020f) Copper-induced oxidative stress, initiation of antioxidants and phytoremediation potential of flax (*Linum usitatissimum* L.) seedlings grown under the mixing of two different soils of China. Environ Sci Pollut Res 27:5211–5221

Saleem MH, Fahad S, Rehman M, Saud S, Jamal Y, Khan S, Liu L (2020g) Morpho-physiological traits, biochemical response and phytoextraction potential of short-term copper stress on kenaf (*Hibiscus cannabinus* L.) seedlings. PeerJ 8:e8321

Saleem MH, Rehman M, Kamran M, Afzal J, Noushahi HA, Liu L (2020h) Investigating the potential of different jute varieties for phytoremediation of copper-contaminated soil. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-020-09232-y

Saleem MH, Ali S, Rehman M, Rizwan M, Kamran M, Mohamed IA, Bamagoos AA, Alharby HF, Hakeem KR, Liu L (2020i) Individual and combined application of EDTA and citric acid assisted phytoextraction of copper using jute (Corchorus capsularis L.) seedlings. Environ Tech Innov. https://doi.org/10.1016/j.eti.2020.100895

Saleem MH, Kamran M, Zhou Y, Parveen A, Rehman M, Ahmar S, Malik Z, Mustafa A, Anjum RMA, Wang B (2020j) Appraising growth, oxidative stress and copper phytoextraction potential of flax (Linum usitatissimum L.) grown in soil differentially spiked with copper. J Environ Manag 257, 109994

Saleem MH, Rehman M, Fahad S, Tung Sa, Iqbal N, Hassan A, Ayub A, Wahid Ma, Shaukat S, Liu L, Deng G (2020k) Leaf gas exchange, oxidative stress, and physiological attributes of rapeseed (Brassica napus L.) grown under different light-emitting diodes. Photosynthetica 58(3):836–845

Saleem MH, Ali S, Kamran M, Iqbal N, Azeem M, Tariq Javed M, Ali Q, Zulqurnain Haider M, Irshad S, Rizwan M (2020l) Ethylenediaminetetraacetic Acid (EDTA) Mitigates the Toxic Effect of Excessive Copper Concentrations on Growth, Gaseous Exchange and Chloroplast Ultrastructure of Corchorus capsularis L. and Improves Copper Accumulation Capabilities. Plants 9(6):756

Saud S, Chen Y, Long B, Fahad S, Sadiq A (2013) The different impact on the growth of cool season turf grass under the various conditions on salinity and drought stress. Int J Agric Sci Res 3:77–84

Saud S, Li X, Chen Y, Zhang L, Fahad S, Hussain S, Sadiq A, Chen Y (2014) Silicon application increases drought tolerance of Kentucky bluegrass by improving plant water relations and morph physiological functions. Sci World J 2014:1–10. https://doi.org/10.1155/2014/368694

Saud S, Chen Y, Fahad S, Hussain S, Na L, Xin L, Alhussien SA (2016) Silicate application increases the photosynthesis and its associated metabolic activities in Kentucky bluegrass under drought stress and post-drought recovery. Environ Sci Pollut Res 23(17):17647–17655. https://doi.org/10.1007/s11356-016-6957-x

Saud S, Fahad S, Yajun C, Ihsan MZ, Hammad HM, Nasim W, Amanullah Jr, Arif M, Alharby H (2017) Effects of nitrogen supply on water stress and recovery mechanisms in Kentucky bluegrass plants. Front Plant Sci 8:983. https://doi.org/10.3389/fpls.2017.00983

Saud S, Fahad S, Cui G, Chen Y, Anwar S (2020) Determining nitrogen isotopes discrimination under drought stress on enzymatic activities, nitrogen isotope abundance and water contents of Kentucky bluegrass. Sci Rep 10:6415. https://doi.org/10.1038/s41598-020-63548-w

Shafigh M, Ghasemi-Fasaei R, Ronaghi A (2016) Influence of plant growth regulators and humic acid on the phytoremediation of lead by maize in a Pb-polluted calcareous soil. Arch Agron Soil Sci 62: 1733–1740

Shah F, Lixiao N, Kehui C, Tariq S, Wei W, Chang C, Liyang Z, Farhan A, Fahad S, Huang J (2013) Rice grain yield and component responses to near 2°C of warming. Field Crop Res 157:98–110

Singh H, Singh JIP, Singh S, Dhawan V, Tiwari SK (2018) A brief review of jute fibre and its composites. Mater Today: Pro 5: 28427–28437

Sun Y, Xu Y, Zhou Q, Wang L, Lin D, Liang X (2013) The potential of gibberellic acid 3 (GA3) and Tween-80 induced phytoremediation of co-contamination of Cd and Benzo [a] pyrene (B [a] P) using Tagetes patula. J Environ Manag 114:202–208

Tahmasbian I, Sinegani AAS (2016) Improving the efficiency of phytoremediation using electrically charged plant and chelating agents. Environ Sci Pollut Res 23:2479–2486

Tangahu BV, Abdullah S, Rozaimah S, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma G, Sahoo L, Sanjib P (2012) Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Biochem 53:33–39

Uddin Nizam M, Mokhlesur Rahman M, Kim J-E (2016) Phytoremediation Potential of Kenaf (*Hibiscus cannabinus* L.), Mesta (*Hibiscus sabdariffa* L.), and Jute (*Corchorus capsularis* L**.)** in Arsenic-contaminated Soil. Korean J Environ Agric 35:111–120

Ullah A, Heng S, Munis MFH, Fahad S, Yang X (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40

Ullah S, Anwar S, Rehman M, Khan S, Zafar S, Liu L, Peng D (2017) Interactive effect of gibberellic acid and NPK fertilizer combinations on ramie yield and bast fibre quality. Sci Rep 7:10647

Uzal O, Yasar F (2017) Effects of GA3 hormone treatments on ion uptake and growth of pepper plants under cadmium stress. Appl Ecol Environ Res 15:1347–1357

Vardhan KH, Kumar PS, Panda RC (2019) A review on heavy metal pollution, toxicity and remedial measures: current trends and future perspectives. J Molec Liquids 111197

Vasavi A, Usha R, Swamy PM (2010) Phytoremediation- an overview review. J Ind Pollut Control 26:83–88

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Isrn Ecology 2011

Yang C, Qiu W, Chen Z, Chen W, Li Y, Zhu J, Rahman SU, Han Z, Jiang Y, Yang G (2020) Phosphorus influence Cd phytoextraction in Populus stems via modulating xylem development, cell wall Cd storage and antioxidant defense. Chemosphere 242:125154

Zaheer IE, Ali S, Rizwan M, Farid M, Shakoor MB, Gill RA, Najeeb U, Iqbal N, Ahmad R (2015) Citric acid assisted phytoremediation of copper by Brassica napus L. Ecotoxicol Environ Saf 120:310–317