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Morpho-physiological traits, gaseous exchange attributes, and phytoremediation potential of jute (*Corchorus capsularis* L.) grown in different concentrations of copper-contaminated soil

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ABSTRACT

Jute (*Corchorus capsularis* L.) is the most commonly used natural fiber as reinforcement in green composites and, due to its huge biomass, deep rooting system, and metal tolerance in stressed environments, it is an excellent candidate for the phytoremediation of different heavy metals. Therefore, the present study was carried out to examine the growth, antioxidant capacity, gaseous exchange attributes, and phytoremediation potential of *C. capsularis* grown at different concentrations of Cu (0, 100, 200, 300, and 400 mg kg^{-1}) in a glass house environment. The results illustrate that *C. capsularis* can tolerate Cu concentrations of up to 300 mg kg^{-1} without significant decreases in growth or biomass, but further increases in Cu concentration (i.e., 400 mg kg^{-1}) lead to significant reductions in plant growth and biomass. The photosynthetic pigments and gaseous exchange attributes in the leaves of *C. capsularis* decreased as the Cu concentration in the soil increased. Furthermore, high concentrations of Cu in the soil caused lipid peroxidation by increasing the malondialdehyde content in the leaves. This implies that elevated Cu levels cause oxidative damage in *C. capsularis*. Antioxidants, such as superoxidase dismutase and peroxidase, come into play to scavenge the reactive oxygen species which are generated as a result of oxidative stress. In the present study, the concentrations of Cu in different parts of the plant (the roots, leaves, stem core, and fibers) were also investigated at four different stages of the life cycle of *C. capsularis,* i.e., 30, 60, 90, and 120 days after sowing (DAS). The results of this investigation reveal that, in the earlier stages of the growth, Cu was highly accumulated in the belowground parts of the plant while little was transported to the aboveground parts. Contrastingly, at a fully mature stage of the growth (120 DAS), it was observed that the majority of Cu was transported to the aboveground parts of the plant and very little accumulated in the belowground parts. The results also show a progressive increase in Cu uptake in response to increasing Cu concentrations in the soil, suggesting that *C. capsularis* is a potential bio-resource for the phytoremediation of Cu in Cu-contaminated soil.

1. Introduction

In recent decades, rapid increases in urbanization and industrialization have caused the excessive release of heavy metals in farmlands with damaging effects on ecosystems (Bashir et al., 2018; Nguyen et al., 2017; Rehman et al., 2019). Heavy metal accumulation in soils is of great concern in agricultural production due to its adverse effects on food safety and marketability, crop growth due to phytotoxicity, and the environmental health of soil organisms (Chen et al., 2017; Muszyńska and Hanus-Fajerska, 2015; Nagajyoti et al., 2010; Rehman et al., 2019; Singh et al., 2010 Wang and Zhou, 2005). In China, more than 16.1% of agricultural land is being contaminated with various heavy metals and about 2.1% is contaminated by Cu (Chen et al., 2015). Soil is being polluted by high concentrations of Cu because Cu-based agrochemical products, such as fertilizers, pesticides, insecticides, herbicides, fungicides, miticides, and nematicides, are used

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; POD, peroxidase

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(Adrees et al., 2015; Latorre et al., 2019; Mostofa and Fujita, 2013; Rehman et al., 2019). Cu is an essential nutrient required for the normal growth and development of the plant body and also plays a part in oxidation, photosynthesis, and carbohydrate, protein, and cell wall metabolism as well as in symbiotic N_2 fixation (Kulhari et al., 2013; Mostofa and Fujita, 2013; Nguyen et al., 2017; Singh et al., 2010). However, when the Cu concentration in the plant exceeds a critical value, toxicity symptoms appear, including reduced yield, poor seed germination, stunted leaf and root growth, and ultrastructural and anatomical alterations leading to the formation of reactive oxygen species (ROS) (Adrees et al., 2015; Latorre et al., 2019; Meng et al., 2007). A direct effect of excess Cu in the soil is the lipid peroxidation of cellular organelles that promotes ROS accumulation and impairs the functioning of the cell membrane system (Chen et al., 2014, 2015; Habiba et al., 2015; Nguyen et al., 2017; Zaheer et al., 2015; Zhou et al., 2015). Antioxidants such as superoxide dismutase (SOD) and peroxidase (POD) come into play to scavenge ROS; for example, SOD facilitates the conversion of superoxide radicals (O^{-1}) to hydrogen peroxide (H_2O_2) while POD decomposes H_2O_2 into water (H_2O) and molecular O_2 (Bouazizi et al., 2010; Kamran et al., 2019a; Tie et al., 2014; Wu et al., 2017a). The phytotoxicity of Cu increased the ROS scavenging activities of antioxidants in *Zea mays*, *Boehmeria nivea*, and *Phyllostachys pubescens* (Chen et al., 2015; Rehman et al., 2019; Tie et al., 2014; Zhao et al., 2010). Furthermore, when plants were exposed to excess Cu they accumulated high concentrations of proline in their tissues to overcome the phytotoxicity generated by Cu stress (Monteoliva et al., 2014; Rehman et al., 2019).

Recently, phytoextraction has been gaining interest due to its relatively low costs and simple techniques that are generally acceptable to the public and can often be carried out on site (Ashraf et al., 2019; Rehman et al., 2019; Zaheer et al., 2015). This approach exploits the potential of fast-growing plants for the removal of heavy metals and can also be used to remove extra nutrients from soil or water (Khalid et al., 2017). Hyperaccumulator species (that accumulate larger amounts of pollutants in their aboveground parts than in their belowground parts) can play a key role in the fate of the pollutants in contaminated matrixes via their root systems and their capacity to accumulate extraordinarily high amounts of metal. They demonstrate that plants have the genetic potential to clean up contaminated soil (Chen et al., 2015). Furthermore, plant species with a bioaccumulation factor (BAF) and translocation factor (TF) greater than 1 are considered good hyperaccumulator species for a particular heavy metal (Ashraf et al., 2019; Muszyńska and Hanus-Fajerska, 2015). Currently, more than 500 plant species are considered hyperaccumulators for different heavy metals (Muszyńska and Hanus-Fajerska, 2015) and about 25 plant species have been studied as Cu hyperaccumulators while many others are used as indicators and excluders (Chen et al., 2015; Rehman et al., 2019). Some of these Cu hyperaccumulator species are presented in Table 1S. It has also been observed that, in earlier stages of growth, Cu is highly accumulated in the belowground parts of the plants while little is transported to the aboveground parts, as showed by Rehman et al. (2019) in *Boehmeria nivea*, Thounaojam et al. (2012) in *Oryza sativa*, and Chandrasekhar and Ray (2017) in *Eclipta alba*. Previously, many fibrous crops such as *Boehmeria nivea*, *Linum usitatissimum*, and *Hibiscus cannabinus* have been used as hyperaccumulators for different heavy metals (Cu, Cd, and As) (Rehman et al., 2019; Nizam et al., 2016; Vrbova et al., 2012). Among the various fibrous crops tested, *C. capsularis* has been found to be more tolerant to heavily metal-stressed environments, possibly due to its specific physiological and biochemical activities (Ahmed and Slima, 2018; Ogoko, 2015). *C. capsularis* is a bast fiber crop (natural fiber) commonly used for bags, sacks, packs, and wrappings and more than 99% of global *C. capsularis* cultivation is carried out in Bangladesh, India, and China (Ahmed and Slima, 2018; Faruk et al., 2012). Production in China is far below the average yield for various reasons such as industrial pollution (heavy metal stress), environmental stress (biotic and abiotic), and a lack of awareness about modern agriculture (Liu et al., 2010).

Due to globalization, the average concentration of heavy metals (especially Cu) in agricultural land has increased to a tremendous amount and overpopulation has increased the market demand for the fiber of *C. capsularis*. Therefore, the use of fibrous crops such as *C. capsularis* for the hyperaccumulation of Cu is an innovative approach for the remediation of Cu-contaminated soils. Although very few studies have explored the phytoremediation and tolerance capacity of *C. capsularis* grown in different concentrations of heavy metal-contaminated soil (Abubakaria et al., 2017; Ahmed and Slima, 2018; Ogoko, 2015; Uddin et al., 2016), it could be a potential phytoremediation bio-resource due to its huge biomass, deep rooting system, and tolerance in metal-contaminated sites. This is the first study to examine the time-dependent responses of Cu accumulation in the different parts of *C. capsularis*. The results from this study will add to our knowledge about (i) the morpho-physiological responses of *C. capsularis* to Cu, (ii) the Cu-induced oxidative responses of *C. capsularis* and (iii) the time-dependent Cu accumulation potential of *C. capsularis* grown in various concentrations of Cu-polluted soil.

2. Material and method

2.1. Plant material and experimental treatments

A pot experiment was conducted in a glass house, under controlled conditions at the experimental station of Huazhong Agricultural University, Wuhan, China (114.20′ E, 30.28′ N). The plants received natural light with day/night temperatures of 35/30 °C and day/night humidities of 70/90%. Uncontaminated soil, obtained from the research fields at Huazhong Agricultural University, was air dried and passed through a 5-mm sieve. The physicochemical properties of the soil used for the pot experiment were as follows: pH 5.8, EC 2 dS cm^{-1}, 23.16 g kg^{-1} organic matter, 60 mg kg^{-1} exchangeable K, 0.18 g kg^{-1} total P, 40 g kg^{-1} total N, and total Cu 38.25 mg kg^{-1}. The soil was artificially treated with Cu at various concentrations, i.e., 0 (control), 100, 200, 300, and 400 mg kg^{-1}, using $CuSO_4 \cdot 5H_2O$ (99% pure) (Fig. 1S). After adding the Cu, the pots were equilibrated for 2 months with four cycles of saturation with distilled water and air drying. The plants were watered with Cu-free deionized water and maintained at 70% (w/w) of their water holding capacity. Weeding and other necessary intercultural operations were carried out when necessary. A complete randomized design with twelve replications was used. Each pot contained 15 kg of soil and three plants. Nitrogen (N), phosphorus (P), and potassium (K) were applied as recommended by Islam and Rahman (2008). The seeds were washed with 95% ethanol and ten seeds were sown in each pot. After two weeks of germination, thinning was performed and three plants were left in each pot. The seeds of C-3 (a type of white jute released from Bangladesh) were used in the pot experiment and sow in 40-cm-tall × 30-cm-wide pots on 15th April 2018. Seed germination was measured 14 days after sowing (DAS); when the seedling reached 2 cm they were considered germinated (Ahsan et al., 2007). All plants were harvested 120 DAS and their growth parameters were measured. All chemicals used were of analytical grade, procured from Sinopharm Chemical Reagent Co., Ltd.

2.2. Morphological traits and data collection

The plants were harvested on 15th August (120 DAS) for the analysis of various morphological parameters. Leaves from each treatment group were picked 30 DAS for chlorophyll, carotenoid, and antioxidant analysis. A fully functional leaf (fifth from the top) was harvested for the various enzymatic and pigment studies. The leaves were washed with distilled water, placed in liquid nitrogen, and stored at −80 °C for further analysis. The plants from each treatment were washed with tap water to remove debris and waste, and then with distilled water. They were separated into the roots, stem core, leaves, and fibers. Plant height was measured from the top of the leaf tips to the bottom of the roots.

The stem core and bark diameter (mm) was measured using digital vernier calipers (ST22302SG Tools, Hangzhou, China) 20 cm above the soil. Plant fresh weight was determined by measuring the weight of plant with a digital weighting balance and, after removing the leaves, the stem fresh weight was similarly measured. The different parts of the plant (the root, leaves, stem core, and fibers) were oven-dehydrated at 65 °C for 72 h for Cu determination and the total plant dry weight was also measured (Allen et al., 1986).

2.3. Determination of the chlorophyll concentration and gas exchange parameters of C. capsularis

Chlorophyll determination followed the methods of Arnon (1949) and was expressed in mg g^{-1} FW. On 35 DAS, the uppermost fully expanded leaves were used to measure gaseous exchange parameters, i.e., P*n*, T*r*, G*s*, and C*i*, between 9:00–11:00 a.m. using a portable photosynthesis system Li-6400 (Li-COR, Lincoln, NE, USA).

2.4. Determination of the lipid peroxidation, proline content, and antioxidant enzymatic activities of C. capsularis

The method described by Heath and Packer (1968) was adopted to measure the content of lipid peroxidation (MDA), expressed as μmol g^{-1} FW. Proline content was determined by the method described by Bates et al. (1973) and expressed as μg g^{-1} FW. The method described by Chen and Pan (1996) was followed to measure the activity of SOD and expressed as Ug^{-1} FW. The activity of POD was measured as described by Sakharov and Aridilla (1999) and expressed as Ug^{-1} FW.

2.5. Determination of Cu concentration in the soil and plant

For the analysis of Cu in the soil and different plant parts (the roots, leaves, stem core, and fibers) dry samples (0.1 g) were digested by mixed-acid digestion (Rehman et al., 2019). The plant parts were ground in a stainless-steel mill, passed through a 0.1-mm nylon sieve, and digested in $HNO_3/HClO_4$ (4:1) solution. The digested solution was washed in a 25-ml flask and the final volume was achieved by adding de-ionized water. The supernatant was passed through 0.45-μm filter paper and the Cu concentration analyzed using an atomic absorption spectrophotometer (Agilent 240FS-AA).

Soil samples (before and after the experiment) were also collected, spread across paper sheets, air dried, and passed through a 2-mm sieve to remove gravel and debris. The soil samples (0.1 g) were digested by mixed-acid digestion in a mixture of H_3PO_4:$HCLO_4$ (3:2) on a hot plate at 150–180 °C until a clear liquid was formed. Finally, the Cu content was determined using an atomic absorption spectrophotometer (Agilent 240FS-AA)

2.6. Bioaccumulation, translocation, and uptake of Cu

The bioaccumulation factor (BAF) was calculated as the ratio of the Cu concentration in the tissues and the Cu concentration in the soil using the following formula:

$$BAF = \frac{Cu\ concentration\ in\ plant\ tissues}{Cu\ concentration\ in\ soil}$$

The TF was calculated by evaluating the concentration of Cu in each part of plant with respect to the roots, as follow:

$$TF = \frac{Cu\ concentration\ in\ plant\ tissues}{Cu\ concentration\ in\ roots}$$

The uptake of Cu (%) from the soil was calculated using the formula previously used by Tanhan et al. (2007):

$$Uptake\ of\ Cu\ (\%) = \frac{C0 - C1}{C0\ \times\ 100}$$

where C0 is the initial concentration of Cu in the soil while C1 is the finial uptake of Cu by the plant.

2.7. Statistical analysis

All the results presented in this study are the arithmetic means of three replications with the standard deviation, apart from those otherwise explicitly described. One-way analysis of variance (ANOVA) was used to assess the significance of the variations of Cu among the different plant parts, followed by LSD tests using Statistix 8.1. Testing showed that all plant− or soil−related data were approximately normally distributed. Therefore, the differences between treatments were determined using ANOVA, and the least significant difference test ($P \leq 0.05$) was used for multiple comparisons between treatment means. Graphical representation was carried out using Sigma Plot 12 and R Studio software.

3. Results

3.1. Seed germination, growth, and biomass

The germination rates of *C. capsularis* seeds at different concentrations of Cu are presented in Table 1. The highest seed germination was found at 0 mg kg^{-1} Cu which was statistically similar to that at 100 mg kg^{-1} Cu. The *C. capsularis* seed germination rates at different levels of Cu ranged from 86 to 100%. Further, as the Cu concentration increased in the soil the seed germination rate decreased significantly, suggesting Cu toxicity to the germinating seedlings. The lowest germination rate was found at 400 mg kg^{-1} Cu (86%).

In the present study, the effects of different levels of Cu (0, 100, 200, 300, and 400 mg kg^{-1}) on plant height, plant fresh weight, stem core fresh weight, bark fresh weight, plant dry weight, plant diameter, and bark diameter were also measured (Table 1). According to the results *C. capsularis* showed considerable tolerance of up to 300 mg kg^{-1} Cu without significant decreases in plant growth or biomass. However, further increases in Cu concentration, i.e. 400 mg kg^{-1}, showed consequential declines in plant height, plant fresh weight, stem core fresh weight, bark fresh weight, plant dry weight, plant diameter, and bark diameter. Our results indicate that plant height was reduced by 19.3% at 400 mg kg^{-1} Cu compared to that at 0 mg kg^{-1} Cu. The results also indicate that plant

Table 1
Effect of different concentrations of Cu on plant height (cm), plant diameter (mm), bark diameter (mm), plant fresh weight (g), stem core fresh weight (g), bark fresh weight (g), plant dry weight (g), total chlorophyll contents (mg g^{-1}) and germination rate (%) of *C. capsularis*.

Cu treatments	Plant height	Plant diameter	Bark diameter	Plant fresh weight	Stem core fresh Weight	Bark fresh weight	Plant dry weight	Total chlorophyll	Germination Percentage
0	240 ± 5.6a	8.2 ± 0.2b	1.4 ± 0.2b	152 ± 4a	89 ± 7a	3.5 ± 0.2a	56 ± 3a	2.5 ± 0.07a	100
100	239 ± 5.3a	8.3 ± 0.2b	1.5 ± 0.2b	151 ± 4a	86 ± 5a	3.5 ± 0.3a	56 ± 3a	2.5 ± 0.02a	100
200	237 ± 7.3a	8.5 ± 0.3b	1.3 ± 0.2b	146 ± 6a	84 ± 6a	3.4 ± 0.4a	55 ± 2a	1.7 ± 0.04b	96
300	239 ± 7.2a	8.4 ± 0.2b	1.7 ± 0.3b	146 ± 6a	86 ± 3a	3.5 ± 0.2a	56 ± 3a	1.7 ± 0.05b	93
400	196 ± 9.6b	10 ± 0.4a	2 ± 0.2a	117 ± 3b	65 ± 4b	2.4 ± 0.3b	43 ± 2b	1.3 ± 0.06c	86

Values in the table is just one harvest. Mean ± SD (n = 5). Different letters within a column indicate significant difference between the treatments ($P < 0.05$). Relative radiance of plastic filter used: 0, 100, 200, 300 and 400 mg kg^{-1} Cu.

fresh weight, stem core fresh weight, bark fresh weight, and plant dry weight were decreased by 23.0%, 26.9%, 31.4%, and 23.2%, respectively, at 400 mg kg^{-1} Cu compared to their values at 0 mg kg^{-1} Cu. In contrast, the plant diameter and bark diameter increased as the Cu level in the soil increased, with maximum increases of 21.9% and 42.8%, respectively, at 400 mg kg^{-1} Cu compared to at 0 mg kg^{-1} Cu.

3.2. Chlorophyll content and gaseous exchange attributes

The effects of different concentrations of Cu on total chlorophyll content are presented in Table 1. Results from the present study reveal that high concentrations of Cu in the soil significantly affected the chlorophyll content of *C. capsularis*. The maximum reduction in chlorophyll content was observed at 400 mg kg^{-1} Cu (48% lower than the control). However, non-significant differences in total chlorophyll contents were also observed in the present study. The maximum chlorophyll content (2.5 mg g^{-1} FW) was observed at 0 mg kg^{-1} Cu while the minimum chlorophyll content (1.3 mg g^{-1} FW) was observed at 400 mg kg^{-1} Cu.

In the present study, the effects of different levels of Cu (0, 100, 200, 300, and 400 mg kg^{-1}) on the gas exchange attributes of the plants are presented in Fig. 1. P*n*, *Tr*, and G*s* decreased steadily with increasing Cu concentration in all treatments. In contrast, C*i* showed non-significant results up to a concentration of 300 mg kg^{-1} Cu, while further increases in Cu, i.e. to 400 mg kg^{-1}, significantly reduced it. P*n* was highest in the treatment without Cu and was reduced by 44.3% at 400 mg kg^{-1} Cu (Fig. 1a). The maximum reductions in *Tr* and G*s* were found at 400 mg kg^{-1} Cu (23.5% and 70.5%, respectively) when compared to the values in the groups without Cu (Fig. 1b and c). However, C*i* exhibited non-significant values up to 300 mg kg^{-1} Cu but was reduced by 7.9% compared to the control at 400 mg kg^{-1} Cu (Fig. 1d).

3.3. Lipid peroxidation, proline content and antioxidant enzymatic activity

In this study, the effects of different concentrations of Cu on lipid peroxidation, proline content, and the activities of antioxidant enzymes in *C. capsularis* were also investigated (Fig. 2). The effects of different concentrations of Cu on lipid peroxidation and the proline content in *C. capsularis* are shown in Fig. 2. High concentrations of Cu in the soil caused increases in the MDA content which indicates that Cu-induced oxidative damage occurred. Elevating the levels of Cu in the soil increased the MDA content and the maximum MDA content was observed at 400 mg kg^{-1} Cu, which is a 697.5% increase compared to the control. The minimum MDA content was observed at 0 mg kg^{-1} Cu (4 μmol g^{-1} FW) while the maximum MDA content was observed at 400 mg kg^{-1} Cu (31.9 μmol g^{-1} FW). Increasing levels of Cu in the soil caused more accumulation of proline in the leaves of *C. capsularis* (Fig. 2b). Our results indicate that the maximum content of proline was observed at 400 mg kg^{-1} Cu (73.8 μg g^{-1} FW) while the minimum content of proline was observed at 0 mg kg^{-1} Cu (12.2 μg g^{-1} FW).

The results revealed that the activities of SOD and POD increased with increasing Cu concentration, as shown in Fig. 2c and d. The activity of SOD increased by 37.6%, 47.6%, 58.2%, and 78.9% at 100, 200, 300, and 400 mg kg^{-1} Cu, respectively, when compared with the treatment without Cu (Fig. 2c). In the same way, POD activity also increased with increasing Cu concentration in the soil and the maximum increase in POD activity was found at 400 mg kg^{-1} Cu (345.5% higher than in the 0 mg kg^{-1} Cu treatment) (Fig. 2d).

3.4. Bioaccumulation of Cu from different parts of plants and soil

The results regarding the accumulation of Cu in different parts of *C. capsularis* (the roots, stem core, leaves, and fibers) are shown in Fig. 3. In this study, Cu accumulation in the different parts of *C. capsularis* was determined at different growth stages, i.e., 30, 60, 90, and 120 DAS. The accumulation of Cu in different parts of *C. capsularis* increased as the concentration of Cu in the soil increased. The maximum accumulation of Cu was recorded at 400 mg kg^{-1} Cu in all growth stages. In the earlier stages of growth, i.e., 30 DAS, most of the Cu was accumulated in the roots with little transported to the aboveground parts. The maximum concentration of Cu in the roots was 470 mg kg^{-1} while the minimum concentration of Cu was observed in the fibers (98 mg kg^{-1}) at 400 mg kg^{-1} Cu. At 120 DAS most of the Cu was transported to the aboveground parts and the maximum Cu concentration was observed in leaves (534 mg kg^{-1}), followed by the stem core (465 mg kg^{-1}), fibers (390 mg kg^{-1}), and then the roots (260 mg kg^{-1}) at 400 mg kg^{-1} Cu. It was also observed that Cu accumulation was higher in the roots, followed by the leaves, stem core, and fiber at 30 DAS, while Cu was highly accumulated in the leaves, followed by the stem core, fibers, and roots at 120 DAS.

The bioaccumulation factor (BAF) and TF were estimated to evaluate the concentration of Cu absorbed by different parts of *C. capsularis* (Fig. 4). All the values for BAF and TF in the earlier stage of growth, i.e. 30 DAS, were less than 1, except in the roots. This indicates that *C. capsularis* accumulated high concentrations of Cu in their roots while little was transported to the aboveground parts of the plant. The maximum value of BAF was recorded in the roots (1.17) followed by the leaves (0.36), stem core (0.29) and fibers (0.24) at 400 mg kg^{-1} Cu while the maximum value of TF was recorded in the leaves (0.30) followed by the stem core (0.24) and fibers (0.20) at 400 mg kg^{-1} Cu in the earlier stage of the growth (i.e., 30 DAS). In the later stage of growth the maximum value of BAF was recorded in the leaves (1.33), followed by the stem core (1.16), fibers (0.97), and roots (0.65) at 400 mg kg^{-1} Cu. However, at 120 DAS the maximum value of TF was recorded in the leaves (2.05), followed by the stem core (1.78) and fibers (1.50) at 400 mg kg^{-1} Cu. These results indicate that Cu accumulation was higher in the roots in earlier stages of growth while transport to the aboveground parts increased in the later stages of growth.

The higher the concentration of Cu in the initial soil (before planting), the higher it was in the soil after harvesting (Table 2). It was clear that *C. capsularis* can remove a large amounts of Cu from the soil, ranging 72-60% of the Cu in Cu-contaminated soil. The maximum Cu uptake ability of *C. capsularis* was 251 mg kg^{-1} Cu at 400 mg kg^{-1} Cu. The maximum recovery of Cu observed was 100 mg kg^{-1} Cu (72%), followed by 200 mg kg^{-1} Cu (71%), 300 mg kg^{-1} Cu (65%) and 400 mg kg^{-1} Cu (60%).

3.5. Correlation between Cu uptake and growth

Pearson correlation analysis was carried out to quantify the relationship between Cu uptake, gases exchange, and growth parameters (Fig. 5). The Cu concentration in the roots was positively correlated with the Cu concentration in the fibers, stem core, and leaves while it was negatively correlated with all other growth parameters (i.e., plant height, plant fresh weight, stem fresh weight, bark fresh weight, and plant dry weight) and gases exchange parameters, except plant and bark diameter. Similarly, the Cu concentration in the fibers, stem core, and leaves were also negatively correlated with all other growth and gases exchange parameters except plant and bark diameter. This correlation demonstrates a close relationship between Cu uptake, growth, and gases exchange parameters in *C. capsularis*.

A Heatmap-histogram correlation analysis was also carried out to study the relationship between the different growth attributes and Cu uptake (Fig. 2S). Only non-significant differences in the growth attributes were observed up to a Cu concentration of 300 mg kg^{-1} while significant decreases occurred at 400 mg kg^{-1} Cu. However, the Cu accumulation in different parts of the plants showed continuously significant differences at all concentrations of Cu in the soil. This histogram depicted a clear difference between the Cu uptake abilities and growth attributes of *C. capsularis*.

4. Discussion

Metal contamination issues are becoming increasingly common in China and elsewhere, with many documented cases of metal toxicity in

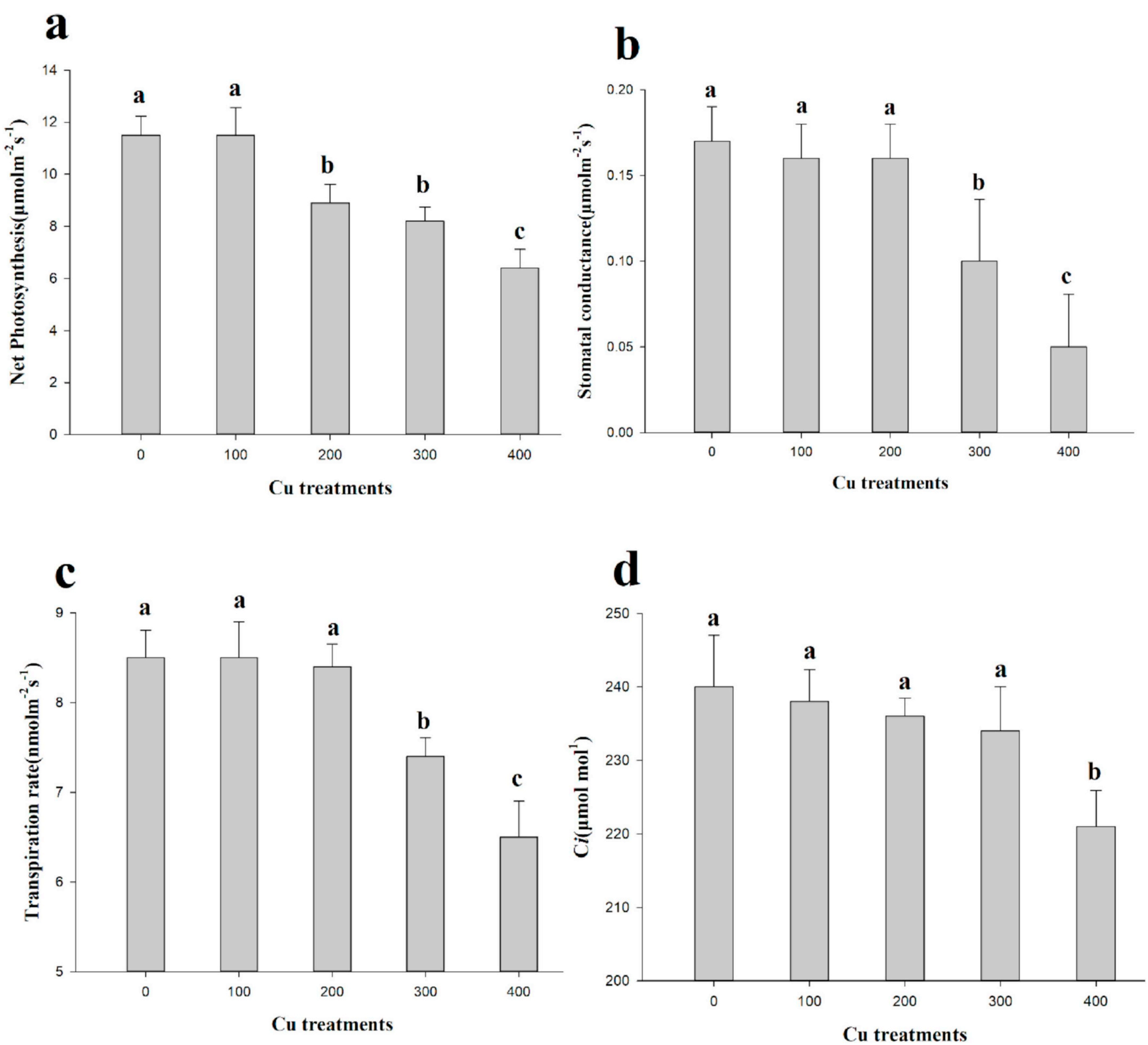

Fig. 1. Effect of different levels of Cu on gas exchange parameters of *C. capsularis*. Letters indicate statistical differences ($P \leq 0.05$) according to an LSD test. Data is the average of 3 replications (n = 3). Error bars represent standard deviation (SD) of three replicates.

mining industries, foundries, smelters, coal-burning power plants, and agriculture (Bashir et al., 2018; Chen et al., 2015; Rehman et al., 2019; Sun et al., 2018; Wang and Zhou, 2005; Wu et al., 2017b). Among the various heavy metals, Cu is among the most toxic to living organisms and can reduce crop yield and productivity (Adrees et al., 2015; Mostofa and Fujita, 2013; Nagajyoti et al., 2010; Nguyen et al., 2017; Singh et al., 2010). Cu has been used as a heavy metal for many kinds of morphological and physiological assays due to its having two types of effects on plants, i.e., essential and toxic effects (Brahim and Mohamed, 2011; Kulhari et al., 2013; Zhou et al., 2019). In the last few years, there has been a gradual increase in Cu concentration in the soil due to anthropogenic activities (Adrees et al., 2015). The average concentration of Cu in agricultural land ranges between 20 and 30 mg kg^{-1} (Chen et al., 2015; Rehman et al., 2019).

Germination assays are an important biological parameter to determine the effect of metal stress on plant seedlings (Khan et al., 2019; Nizam et al., 2016). In the present study, the germination rate of *C. capsularis* decreased as the Cu concentration in the soil increased, when compared with the plants germinated at 0 mg kg^{-1} Cu. This suggests that Cu partitioning occurs during the germinating stage (Ahsan et al., 2007). Similar results were found by Ahsan et al. (2007) who noticed that high concentrations of Cu reduced seed germination in rice seedlings. Moreover, the seed germination rates of different fibrous plants, i.e., jute, kenaf, and mesta, in metal-stressed environments were studied by Nizam et al. (2016) who reported that plants were could germinate under low concentrations of As, but that very high concentrations of As caused reductions in the germination percentage. This reduction in seed germination success is due to metal toxicity, as shown by Wang and Zhou (2005). The reductions in seed germination under high Cu concentrations, i.e. 300 and 400 mg kg^{-1} Cu, in *C. capsularis* might be due to the very slow decay of endosperm material. These finding were consistent with the findings of Islam et al. (2013).

The high concentration of Cu in the soil affects physiological processes in the plants which ultimately causes reductions in plant growth and development (Adrees et al., 2015; Meng et al., 2007; Mostofa and Fujita, 2013; Nguyen et al., 2017). When plants absorb Cu from the soil

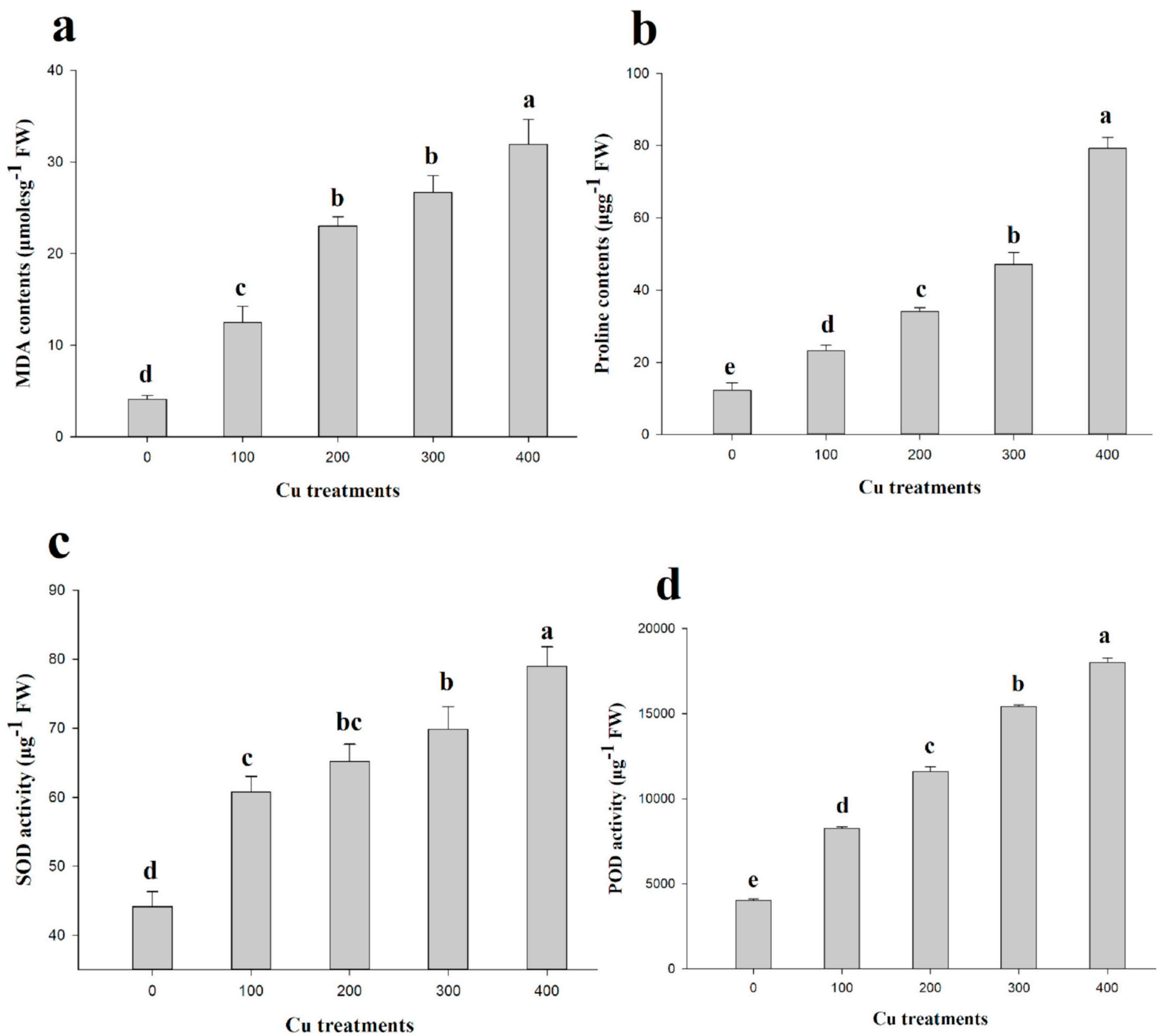

Fig. 2. Effect of different concentrations of Cu on MDA (a), Proline (b), SOD (c) and POD (d) in *C. Capsularis*. Letters indicate statistical differences ($P \leq 0.05$) according to an LSD test. Data is the average of 3 replications (n = 3). Error bars represent standard deviation (SD) of three replicates.

it should be transported to the other tissues of plant and utilized for better growth and development (Chen et al., 2014; Habiba et al., 2015; Rehman et al., 2019). Furthermore, the reductions in plant growth and development due to Cu toxicity might be due to less water absorption, nutritional deficiency, and a decrease in metabolic activities (Singh et al., 2010; Wang and Zhou, 2005; Zaheer et al., 2015). In the present study, the morphological traits tested were greatest in soil without Cu, although there were no significant differences between these results and those recorded at 100, 200, and 300 mg kg^{-1} Cu. However, further increases in Cu concentration, i.e., to 400 mg kg^{-1} Cu, caused significant reductions in growth and biomass. These findings demonstrate that *C. capsularis* shows no significant changes in growth and biomass up to a concentration of 300 mg kg^{-1} Cu while further increases in Cu cause reductions in both parameters. The reduction in the growth and biomass of *C. capsularis* as the Cu concentration increases in the soil shows that Cu causes toxicity at elevated concentrations. Several other researchers found similar results and reported that high concentrations of Cu in the soil triggered negative impacts on plant growth and biomass and noticed that the plants could not survive in soil containing high concentrations of Cu (Bouazizi et al., 2010; Meng et al., 2007; Rehman et al., 2019). Some plants discovered in Cu mining territory exhibited more tolerance than normal plants. *Elsholtzia splendens* is considered a highly tolerant plant in Cu-contaminated soil and can survive up to 600 mg kg^{-1} Cu (Jiang et al., 2003). Furthermore, some plants like *Sedum sediforme* can survive even higher levels of Cu contamination, i.e., 5000–16,800 mg kg^{-1} Cu, without significant decreases in plant growth or biomass (Poschenrieder et al., 2001). Obliviously *C. capsularis* is less tolerant than *S. sediforme* as the rise in Cu concentration > 300 mg kg^{-1} Cu triggered a breakthrough decline in its growth and biomass. Hence, these findings show that *C. capsularis* can survive Cu ≤ 400 mg kg^{-1} in Cu-contaminated soil without significant reductions to its growth and biomass. Similar results were also demonstrated by Liu et al. (2014) and Kolbert et al. (2012) when they studied *Zea mays* and *Arabidopsis thaliana*, respectively, under various Cu toxicity conditions and found that high Cu toxicity caused reductions in the growth and biomass of these plants. Some studies showed

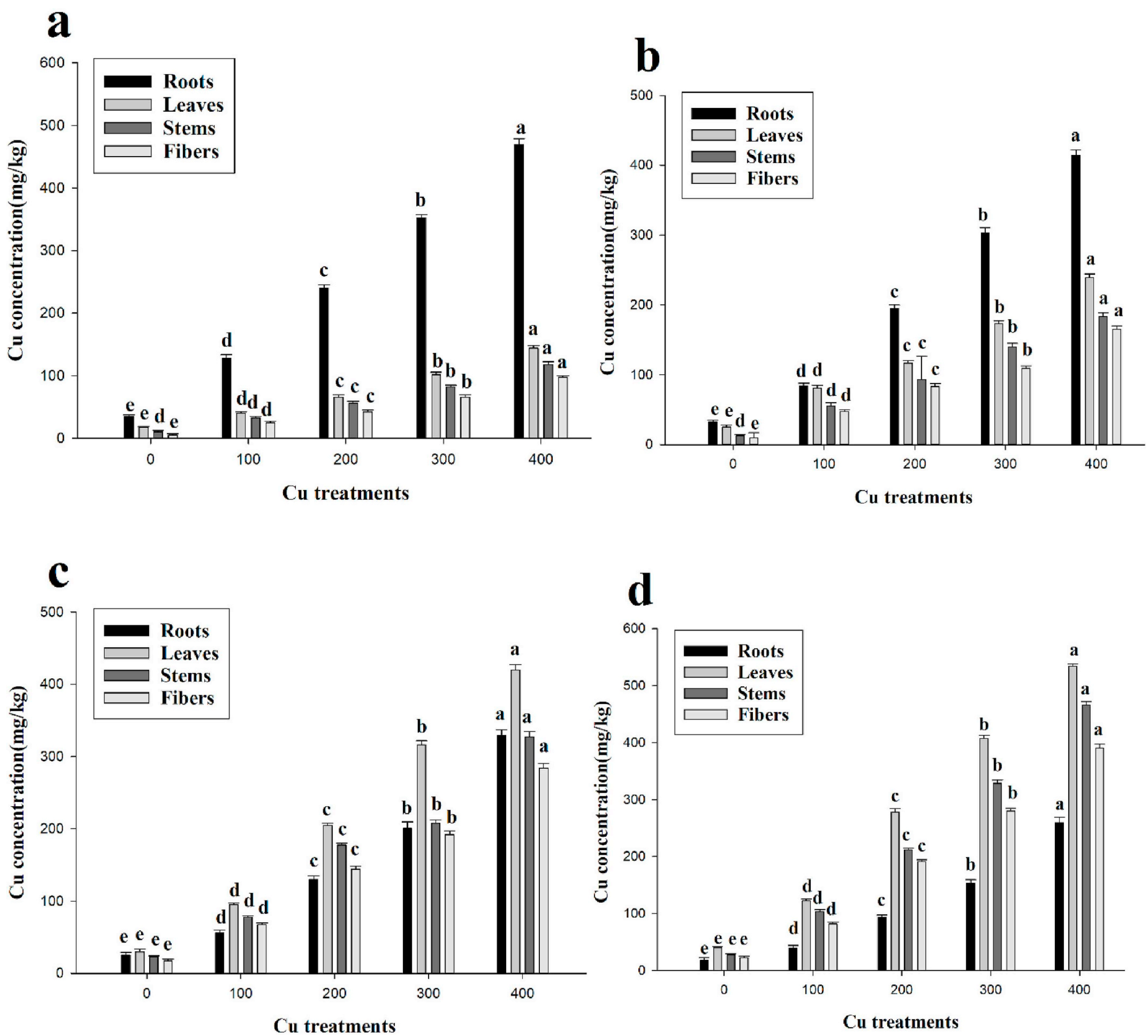

Fig. 3. Cu accumulation on different time interval by *C. Capsularis* at 30 DAS (a), 60 DAS (b), 90 DAS (c) and 120 DAS (d). Letters indicate statistical differences ($P \leq 0.05$) according to an LSD test. Data is the average of 3 replications (n = 3). Error bars represent standard deviation (SD) of three replicates.

that slow growth under metal-stressed conditions could be an adaptive feature for plant survival because it allows the plants to divert the energy otherwise used for shoot growth into protective molecules to fight stress (Adrees et al., 2015; Chen et al., 2015; Liu et al., 2014).

Photosynthetic pigments and gas exchange parameters are considered chief parameters when studying plant stress (Rehman et al., 2019; Zaheer et al., 2015). The main effect of high concentrations of Cu on the photosynthetic pigments and gas exchange parameters are related to decreases in the net photosynthesis rate, reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) efficiency, and the inhibition of electron transport and PSII activities (Brahim and Mohamed, 2011). It was also observed that gas exchange parameters and photosynthetic pigments play important roles in increasing the dry biomass (up to a certain level) of plants, as shown by Bashir et al. (2018) when they studied Chinese cabbages under different soil Cu concentrations. In the present study, the gas exchange parameters and photosynthetic pigments declined due to Cu toxicity. Previous studies suggest that, in higher plants, chloroplasts are the major site of Cu accumulation and this may affect the metabolic processes of photosynthesis and respiration at a genetic level (Habiba et al., 2015; Rehman et al., 2019). The damage to the integrity of the thylakoid membrane due to the accumulation of Cu can change the composition of fatty acid molecules and also stop various enzymes binding with chlorophyll (Adrees et al., 2015; Rehman et al., 2019; Zaheer et al., 2015). Moreover, Cu toxicity affects photosynthetic pigments and their composition, causing decreases in the photochemical activity of PSII, which was previously illustrated by Chen et al. (2015) and Habiba et al. (2015).

Heavy metals are considered a primary source of injury to the cell membrane, frequently attributed to lipid peroxidation. As a result of metal accumulation, a large number of active free oxygen radicals are formed which may be the main cause of cell membrane lipid peroxidation, and also harm the functioning and structure of the cell membrane (Bouazizi et al., 2010; Chandrasekhar and Ray, 2017; Thounaojam et al., 2012; Tie et al., 2014). MDA, an oxidizing product of the cell membrane, is considered a universal demonstrator of lipid peroxidation along with stress level (Meng et al., 2007; Kamran et al.,

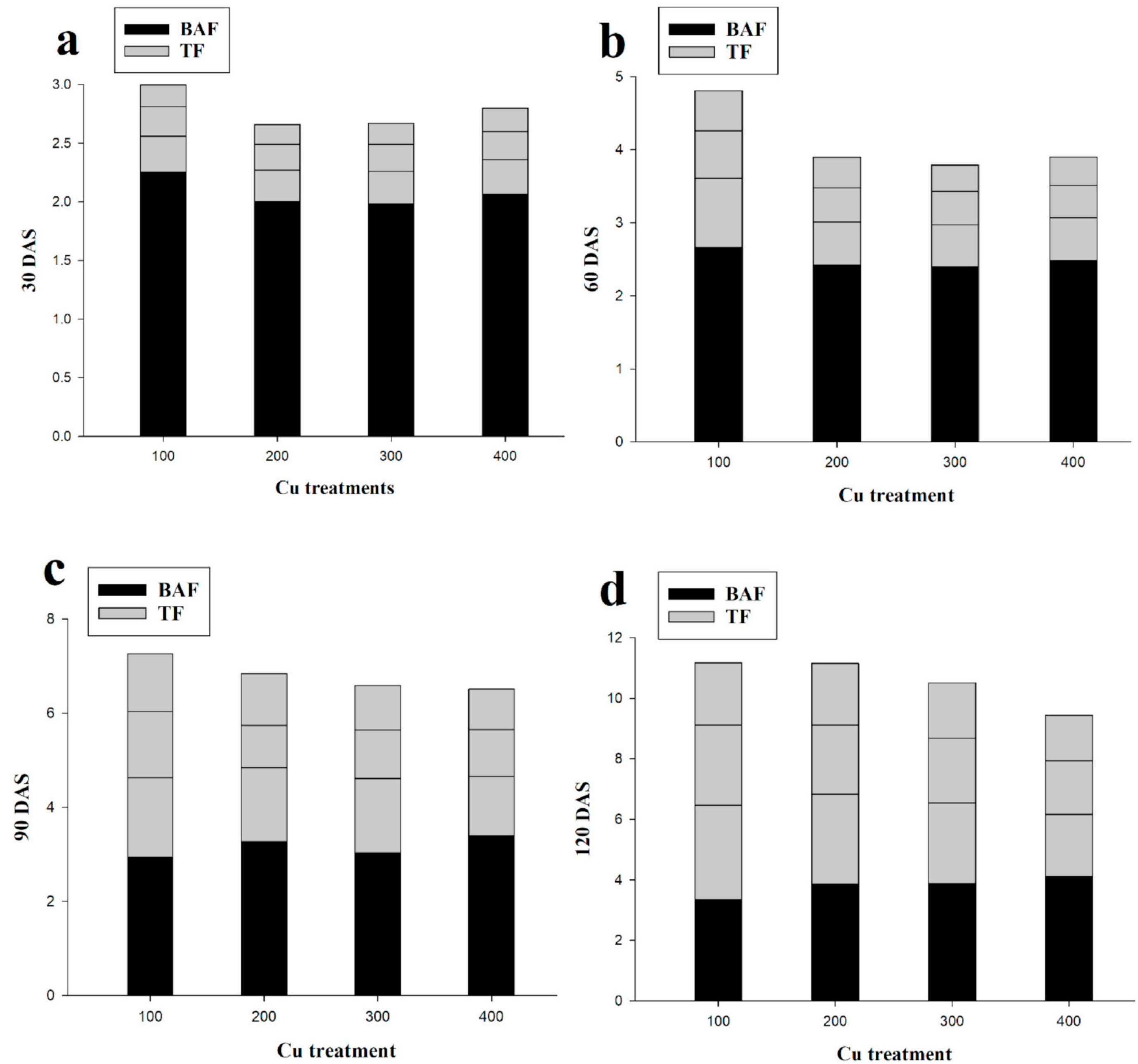

Fig. 4. Bioaccumulation factor (BAF) and translocation factor (TF) by *C. Capsularis* at 30 DAS (a), 60 DAS (b), 90 DAS (c) and 120 DAS (d).

Table 2
The efficiency of *C. capsularis* in extracting Cu (mg kg^{-1}) from soil at post-harvesting stage.

Cu concentrations	Initial concentration	(Final conc.) After uptake	Uptake	Removal %
0	–	–	–	–
100	103	29	74	72
200	206	59	147	71
300	310	107	203	65
400	414	163	251	60

Different letters within a column indicate significant difference between the treatments ($P < 0.05$). Relative radiance of plastic filter used: 0, 100, 200, 300 and 400 mg kg^{-1} Cu.

2019b). Plants have active antioxidative defense systems to defend against ROS production (Chen et al., 2014; Habiba et al., 2015; Liu et al., 2018; Singh et al., 2010; Zhao et al., 2010). In the present study, the MDA content of Cu-contaminated plants was significantly different from that of control plants (Fig. 2a). It was reported that excess Cu can increase lipid peroxidation and MDA, an oxidized product of membrane lipids, indicating the prevalence of oxidative stress and membrane damage (Bouazizi et al., 2010; Brahim and Mohamed, 2011; Chen et al., 2015). Similar results were reported by Sun et al. (2010) when they studied Cu toxicity in radishes and noticed that high Cu concentrations caused lipid peroxidation and induced oxidative damage in the leaves of radish plants. When plants undergo some environmental stress they secrete proline as an osmoregulatory solute to decrease any harmful effects (Monteoliva et al., 2014). Plant cells provide a space under conditions of oxidative stress where signal transduction can be carried out by the formation of proline, which is a vital response by plants against Cu toxicity (Mostofa and Fujita, 2013; Rehman et al., 2019; Tie et al., 2014). In the present study, the high proline accumulation in the leaves of *C. capsularis* shows that the Cu toxicity disrupted the equilibrium of osmoregulatory solutes but had no actual influence on the

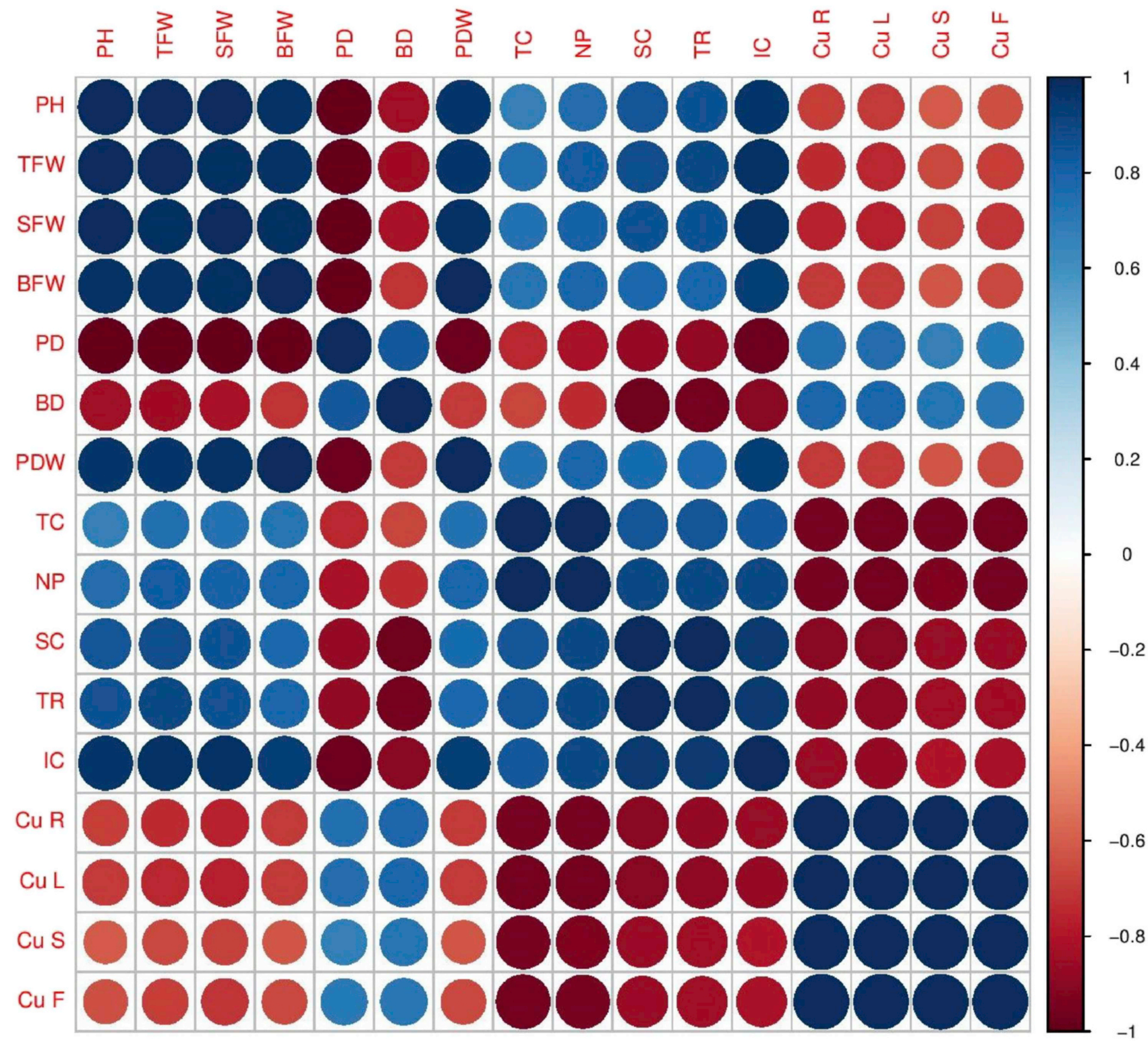

Fig. 5. Correlation of Cu uptake with chlorophyll contents, gases exchange and growth parameters in *C. Capsularis*. Cu R (Cu concentration in roots), Cu L (Cu concentration in leaves), Cu S (Cu concentration in stem), Cu F (Cu concentration in fiber), PH (plant height), TFW (total fresh weight), SFW (stem core fresh weight), BFW (bark fresh weight), PD (plant diameter), BD (bark diameter), PDW (plant dry weight), TC (total chlorophyll), NP (net photosynthesis), SC (stomatal conductance), TR (transpiration rate) and IC (intercellular CO_2).

plants, and that the decayed osmoregulatory solutes stimulated proline composition (Bouazizi et al., 2010; Monteoliva et al., 2014). Excess Cu caused the generation of ROS, which was scavenged through the activities of antioxidants such as SOD and POD. The formation of ROS oxidizes water (H_2O) into molecular oxygen (O_2) with the help of antioxidative enzymes, i.e., SOD and POD (Habiba et al., 2015; Liu et al., 2018; Meng et al., 2007). Zhao et al. (2010) studied SOD activity in the roots of *Festuca arundinacea* and *Lolium perenne* plants and noticed that SOD activity increased as the concentration of Cu in the soil increased. POD activity was studied by Liu et al. (2018) in the leaves of maize seedlings and they noticed that, with increases in Cu concentration, the activity of POD increased up to a certain limit. The tolerance mechanisms of a plant depend on the enzymatic activity of the plant and higher activities of antioxidants is an indication of a plant suffering from metal stress. Similar results were also found by Meng et al. (2007) and Brahim and Mohamed (2011) when they studied garlic and Mediterranean saltbush plants, respectively, in soil with high concentrations of Cu.

The uptake of heavy metals from the soil and its transport to different parts of the plant mainly depends on the Cu supply and growth conditions. The uptake of heavy metals from the soil involves its transport across the root-cell membrane, its transport and detoxification throughout the plant, and its cellular concentrations (Adrees et al., 2015; Jiang et al., 2003; Rehman et al., 2019; Rizwan et al., 2016). In the present study, the metal concentrations in different parts of the plant was determined at different intervals of time, i.e., 30, 60, 90, and 120 DAS (Fig. 3). This is the first report on the time-dependent decreases in Cu bioaccumulation in different parts of *C. capsularis* plants. The results revealed that Cu is highly accumulated in the roots in the earlier stages of growth and little is transport to the aboveground parts, while in the last stage of growth Cu was highly accumulated in the aboveground parts of the plant and very little accumulated in the roots of *C. capsularis*. This might be due to the presence of an oxidized zone around the root zone which helps to form an iron plaque. The iron plaque can bind Cu in the roots and can reduce its translocation to the aboveground parts of the plant (the leaves, stems, and fibers) (Jiang et al., 2003; Norra et al., 2005). Another possible reason for the high concentrations of Cu in the roots in the earlier stages of growth is that the microbial communities of the rhizosphere can dissolve ferric iron in the rhizosphere by exuding siderophores into the root-plaque interface (Jiang et al., 2003; Kraemer, 2004). The concentration of Cu in the roots reached up to 470 mg kg^{-1} Cu at 30 DAS, due to the formation of iron plaques. A similar experiment was also designed and performed by Bhattacharya et al. (2014) when they studied *C. capsularis* and *Oryza sativa* in As-contaminated soil and noticed that As was highly accumulated in the roots in the earlier stages of growth while it was transported to the aboveground parts (straw, husk, and grain) in the last stage of the growth. Liu et al. (2004) studied *Oryza sativa* plants and noticed that heavy metal was highly accumulated in the roots in the earlier stages of growth while very little was transported to the shoots. Rehman et al., 2019 studied *Boehmeria nivea* under different concentrations of Cu, i.e., 0 (control), 50, 100, 200, 300, and 400 mg kg^{-1} Cu, and noticed that the maximum concentration of Cu was accumulated in the roots (52 mg kg^{-1} DW) while very little was transported to the leaf (28 mg kg^{-1} Cu) and stem (20 mg kg^{-1} Cu) during the initial stage of growth. The higher concentration of Cu in the root tissues in the earlier stages of the growth was associated with the low transportation of Cu in the Cu-tolerant species (Rehman et al., 2019). The increasing concentration of Cu in the aboveground parts of *C. capsularis* with aging seems to indicate that there must be some physiological mechanism working in *C. capsularis* that is associated with the bioaccumulation of Cu in different parts of plant.

The most important parameters for determining the phytoremediation potential of plants are TF and BCF. Therefore, for a better understanding of the phytoremediation potential of plants, it is necessary to evaluate the BAF and TF (Ahmed and Slima, 2018; Bhattacharya et al., 2014; Chen et al., 2015). The capacity of plants to conduct metals or micronutrients from the roots to the shoots is known as the TF and the capacity of plants to obtain metals or micronutrients from the soil into the tissues is known as the BCF (Muszyńska and Hanus-Fajerska, 2015: Ogoko, 2015). On this basis the tolerance mechanisms of plants species can be divided into two categories according to their BAF and TF. (1) Metal excluder species: They can accumulate a very large amount of heavy metals in their roots but are unable to translocate it to their aboveground parts and their TF is less than 1 (Rehman et al., 2019). (2) Hyperaccumulator species: These plants can translocate a large amount of heavy metals to their aboveground parts, hence their BAF and TC are always greater than 1 (Muszyńska and Hanus-Fajerska, 2015). Plants which accumulate a large amount of metals in their roots and translocate only a small amount of this metal to their shoots are considered phytostabilizers for heavy metals (Chen et al., 2015). The maximum value of TF was recorded in the stem of nutsedge, i.e. 2.8 (Yoon et al., 2006), while the lowest value was recorded in Elsholtzia, i.e. 0.006 (Jiang et al., 2003). In earlier stages of growth most BAF and TF values are less than 1 except in the roots but at later stages of growth most of the BAF and TF values are greater than 1. This means that *C. capsularis* can be classified as a hyperaccumulator species for Cu-contaminated soil. These findings are similar to the finding of Nizam et al. (2016) and Uddin et al. (2016) who already reported that *C. capsularis* is a hyperaccumulator species for arsenic and lead when grown in lead-contaminated soil. Furthermore, Ahmed and Slima (2018) studied *C. capsularis* in wastewater irrigated soil and noticed that *C. capsularis* showed BAF values of 3.1 and TF values of 1.06 for Cu at the fully mature stage of the plant.

To study the remediation of Cu from Cu-contaminated soil the concentration of Cu in pre-planting and post-harvesting soil was also measured. After harvesting *C. capsularis* from Cu-contaminated soil the concentration of Cu in the soil was less than the initial concentration. The initial concentration of Cu was slightly higher than the Cu treatments (Nizam et al., 2016). The maximum Cu accumulation from the soil was 251 mg kg^{-1} Cu from 400 mg kg^{-1} Cu and the removal percentage ranged between 72 and 60%. This shows that *C. capsularis* can remove a large amount of Cu from Cu-contaminated soil and can be used as a phytoremediation material. In some previous studies, it was noticed that *Elsholtzia splendens* can remove up to 57% of Cu from Cu-contaminated soil (Chen et al., 2015). *Elsholtzia splendens* is considered a good candidate for the phytoremediation of heavy metal but, in the present study, the maximum removal of Cu from the soil was 72%. Thus it can be said that *C. capsularis* has the potential to remove a large amount of contaminants from the soil. Thus *C. capsularis* is a hyperaccumulator species for Cu-contaminated soil and a good candidate for phytoremediation. Ogoko (2015) found similar results that *C. capsularis* has the potential to revoke a large amount of heavy metal from the soil.

5. Conclusions

On the basis of these findings it can be concluded that *C. capsularis* has considerable potential to cope with high concentrations of Cu, due to its antioxidative defense mechanisms. Up to a concentration of 300 mg kg^{-1} Cu it showed no significant decreases in growth or biomass. Further increasing the concentration of Cu in the soil caused oxidative damage which was overcome by the activities of antioxidants. Moreover, the bioaccumulation of Cu in the roots of *C. capsularis* in the earlier stages of growth was high due to the formation of iron plaques in the roots that prevented the transportation of Cu to the aboveground parts of the *C. capsularis* plant. In the last stage of growth, the Cu concentration in the aboveground parts of *C. capsularis* is higher than that in the roots. In the same way, the BAF and TF values were lower than 1 in the earlier stages of growth while most BAF and TF values became greater than 1 in the last stage of growth. This shows that *C. capsularis* is a hyperaccumulator species for Cu in Cu-contaminated soil. Thus *C. capsularis* can grow in Cu-contaminated soil and can remove a large amount of Cu. However, future research is needed to study the effects of Cu on the quality of the fiber from *C. capsularis* to assess the viability of its potential application in the phytoremediation of Cu-contaminated soil. Moreover, the potential of *C. capsularis* in the remediation of soils polluted with various heavy metals should be tested under field conditions.

Author contributions compliance with ethical standards

Conflict of interest: The authors declare that there are no conflicts of interests.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109915.

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