Article

Phytoremediation Capability and Copper Uptake of Maize (*Zea mays* L.) in Copper Contaminated Soils

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Abstract: Copper is a frequently used heavy metal worldwide and known to be an essential micronutrient for most living organisms including plants. However, excessive levels of copper in soil may adversely affect plant growth and survival. The continuing introduction of copper to soil, e.g., through excessive utilization of agrochemicals has raised serious environmental concerns throughout the world. A variety of plants have the capability to eliminate pollutants from soil through different mechanisms; however, limited information is reported on phytoremediation potential of maize (*Zea mays* L.) and its uptake and the accumulation potential in copper-containing soils. The effects of additions of 0, 50, 100, 200 and 300 mg kg^{-1} of copper to soil on growth parameters of *Z. mays*, copper uptake from soil and accumulation in roots and shoots, and phytoremediation potential of *Z. mays* were investigated in this research. Copper content in soil and plant samples were determined using atomic absorption spectrophotometry. The addition of 50 mg kg^{-1} Cu stimulated *Z. mays* growth parameters, while higher content of Cu exhibited inhibitory effects of plant growth. Results indicated that roots accumulated significantly higher levels of Cu than shoots in all treatments, suggesting dominancy of phytostabilization mechanism in remediation of Cu-polluted soil by *Z. mays*. However, translocation of Cu from the roots to the aerial parts enhanced to some extent with copper level in soil. The greatest Cu accumulation capacity of 5210 µg per pot was gained in *Z. mays* cultivated in soil treated with 200 mg kg^{-1} copper. Results demonstrated that *Z. mays* can promisingly remediate low to moderately copper-contaminated soils.

Keywords: *Z. mays*; copper; phytoremediation

1. Introduction

Heavy metal contamination and their probable impacts on the food chain have been associated with a wide range of human health risks. The accumulation of heavy metals in a soil matrix can significantly endanger human health as well as other living organisms life [1]. Despite many organic contaminants that can be biodegraded or naturally attenuated in the environment, heavy metals may not be degraded through natural processes and therefore tend to be continuously accumulated in the environment [2,3]. The occurrence of heavy metals in agricultural soil, which can be caused by various activities such as fertilization with fertilizers containing heavy metals, may endanger human health due to the vulnerability of water resources and food chain. In other words, soil contamination with heavy metals disrupts natural ecosystem services and ultimately adversely affects the human health through food chain [4]. Heavy metals can also negatively affect central nervous system posing mental disorder and damage other vital organs such as liver and lungs, thereby promoting an array of diseases for humans. Several adverse impacts of heavy metals of human health have been reported in the literature [5,6].

Heavy metals have also been recognized to endanger food security due to the rapid rise in industrial and agricultural activities, over population as well as disruption of natural

ecosystems [3]. It is documented that millions of sites with the area of more than 20 million ha have been contaminated with various contaminants worldwide, with over fifty percent of them affected by heavy metals or metalloids [7,8]. Main causes of soil contamination with heavy metals include, but not limited to, metal plating and finishing operations, manure, biosolids, sewage sludge, industrial wastewater, pesticides, herbicides, fertilizers, and waste dumping [9]. Among them, agrochemicals play a significant role in the contamination of agricultural soils. Agrochemicals, which are mainly being used in organic agriculture contain remarkable levels of some heavy metals, e.g., copper sulfate [10,11]. Heavy metals may enter the agro-ecosystem through natural (e.g., soils having high background levels of such elements) and/or anthropogenic processes (e.g., application of phosphate fertilizers, sewage sludge, urban runoff, etc.) [12]. An excessive application of agrochemicals to crops contributes to the accumulation of chemicals in soil matrix and plant exposure [9]. Copper (Cu) is a frequently used metal worldwide and its small quantities are considered to be essential to promote survival of plant species. Copper is a vital micronutrient for all plant species and its deficiency can suppress plant growth. However, high levels of copper occurrence in soil due to excessive utilization of some agrochemicals may exhibit toxic effects. Numerous studies have reported the Cu-induced growth inhibition, oxidative damage and antioxidant response in agricultural food crops such as wheat, rice, maize, sunflower and cucumber [13]. Excessive Cu levels in soil can induce formation of reactive oxygen species (ROS) and interference with plant's respiration and photosynthesis [14].

Typically, the required copper level for healthy growth and development of plant species may vary with plant type [15]. Excessive concentrations of Cu in plants may reach crucial levels at which negative effects including molecular, physiological and morphological symptoms occur in plants at all growth stages. For instance, germination of wheat (*Triticum aestivum* L.) cultivated on filter papers in Petri dishes was reduced by 46% in presence of 500 ppm Cu after one day of incubation [16] and shoot height of rice declined by 48% in soil treated with 1000 mg kg^{-1} Cu compared to the control [17]. Accumulation potential and nonbiodegradability of copper in soil make it detrimental to plant life and human health at high concentrations [18–20]. Therefore, possibility of removal of excessive amounts of copper from soil using maize (*Z. mays*) was investigated in this research. *Z. mays* was selected because it is known as a tolerant and fast-growing plant species which has been successfully employed in various phytoremediation studies [21,22] and its seeds can be readily accessed and cultivated in most parts of the world with pretty low maintenance requirements.

Designing and applying various approaches to remove toxic contaminants from soil has been an area of interest over the last decades [23]. Various remediation methods, including physicochemical and biological techniques, have been introduced for removal of contaminants from soil; however, proper selection and application of a remediation strategy should be made based on factors such as technical/financial constraints and nature of pollutants in an affected site. In situ and ex situ decontamination approaches are being practiced to remediate heavy metal contaminated soils. The ex situ approaches such as pump-and-treatment, thermal desorption, and vitrification are mainly energy intensive and costly [24,25]. In addition, some hazardous chemicals may be used in such remediation methods; therefore, implying further potential risks to the environment [26]. Nowadays, there is an extensive need in removal of high levels of heavy metals from soil using ecologically sound and cost-effective remediation approaches. Phytoremediation is an ecologically acceptable technology through which synergistic effect of plants and their associated microorganisms promotes remediation of soil affected with different organic and inorganic contaminants, e.g., heavy metals [27]. It is also known as a green biotechnology, facilitating removal of a wide range of substances from different media. A wide range of brownfields such as fertilized farmlands, oil refineries, waste disposal sites, power plants and agricultural sites can be managed using phytoremediation [28].

This research aimed to investigate the remediation of Cu-contaminated soil by phytoremediation. The impact of different contents of copper on the biomass of *Z. mays*; uptake and

translocation of Cu in *Z. mays* cultivated in Cu-contaminated soil; accumulation capacity and remediation capability of *Z. mays* to remediate copper were evaluated in this study. High concentrations of Cu in soil may suppress *Z. mays* growth; however, if the plant can still establish sufficient biomass, extraction of enough Cu from soil can be expected. Therefore, the hypothesis of the present study was to ensure that, despite reduction in the growth of *Z. mays* by Cu toxicity, sufficient biomass is still produced to extract sufficiently high amounts of Cu from the soil.

2. Materials and Methods

2.1. Preparation and Characterization of Soil

The used soil was collected from a depth of 0–30 cm from a non-metal-contaminated region in Southern part of Tehran, Iran. The gravel and large debris were removed by passing the soil through a 2 mm sieve, before being air-dried at 22–25 °C for 7 days. Different levels of Cu(SO$_4$)·5H$_2$O were added to 150 mL distilled water (DW), then mixed with 1.5 kg soil to be placed in plastic pots. Control pots received the same amount of DW, without addition of Cu(SO$_4$)·5H$_2$O. The prepared pots were incubated for 14 days in a dark room and received tap water to maintain 75% of the field capacity. The test soil was analyzed for the selected chemical properties using standard methods prior to planting. For instance, phosphorous and total nitrogen were determined by the Olsen P extracting solution and Kjeldahl measurement method, respectively [29,30], and Walkley-Black method was used to analyze soil Organic Carbon (OC) [31]. Selected characteristics of the test soil are provided in Table 1.

Table 1. Selected characteristics of the test soils in different phytoremediation treatments.

Treatment	pH	EC (ds m^{-1})	OC (%)	Total N (%)	P (mg kg^{-1})	Total Cu (mg kg^{-1})
T1 (Control) [†]	7.81 ± 0.15 *	1.81 ± 0.04	0.78 ± 0.14	0.49 ± 0.06	8.18 ± 0.43	32.97 ± 3.41
T2 (50 mg kg^{-1} Cu)	7.58 ± 0.12	1.86 ± 0.08	0.69 ± 0.15	0.42 ± 0.07	7.52 ± 1.00	80.13 ± 8.38
T3 (100 mg kg^{-1} Cu)	7.19 ± 0.05	1.99 ± 0.11	0.62 ± 0.11	0.36 ± 0.05	6.80 ± 0.53	135.71 ± 11.30
T4 (200 mg kg^{-1} Cu)	6.87 ± 0.10	2.11 ± 0.10	0.57 ± 0.11	0.33 ± 0.06	5.62 ± 0.70	219.18 ± 19.75

* Values represent mean ± standard error (*n* = 3), [†] Soil Texture: Clay content (27%), Silt Content (38%), and Sand Content (35%); Clay Loam (CL). T1, T2, T3 and T4 represent Treatment 1, Treatment 2, Treatment 3 and Treatment 4, respectively.

Acid digestion (6 mL nitric acid and 2 mL chlorohydric acid) in a microwave reaction system was applied to determine copper content in soil. Extracts were finally analyzed for copper concentration using an atomic absorption spectrophotometry (Perkin Elmer 700, Boston, MA, USA) based on the standard methods [32].

2.2. Pot Experiments

Plant seeds were sown in plastic pots containing various levels of copper. Initially, five treatments were considered, namely, T1, T2, T3, T4 and T5 (*n* = 3) with copper contents of 0, 50, 100, 200 and 300 mg kg^{-1}, respectively. Applying 300 mg kg^{-1} copper almost completely prevented seedling emergence and plant growth in this treatment was negligible, therefore T5 was excluded from the reminder of the experiments and analyses. *Z. mays* was cultivated in a greenhouse for 42 days, under the natural sunlight. Pots were irrigated twice or thrice a week and monitored daily to evaluate germination rate and plant growth. After harvesting the plants, the roots and shoots were separated carefully, root length and shoot height were measured, then they were placed in an oven for 24 h at 70 °C to obtain dry biomass.

2.3. Analysis of Copper in Plant Roots and Shoots

Roots and shoots of *Z. mays* were initially air dried, then oven dried for 12 h at 60 °C. The dried plant materials were ground to pass a 200 mesh, then extracted using digestion method (4.0 mL HNO$_3$ and 1.0 mL HClO$_4$) at 100–230 °C. Filtration of the extracts was

performed by MILEXHA 0.45 µm diameter filter. The total concentration of copper was ultimately analyzed using a Perkin Elmer 700 atomic absorption spectrometer [33,34].

2.4. Translocation, Bioconcentration, and Bioaccumulation Factors and Phytoremediation Ratio

The translocation factor (TF) is an important index to evaluate capability of a given plant species to translocate a contaminant from roots to shoots was determined in this study. *Z. mays* potential to uptake copper from soil was assessed by calculation of bio concentration factor (BCF). Bioaccumulation Coefficient (BAC) was also determined for different treatments in this research. In addition, the phytoextraction efficiency of *Z. mays* was evaluated through determination of phytoremediation ratio (PR) index. *TF, BCF, BAC,* and *PR* values were determined using the following equations [35,36]:

$$TF = \frac{Concentration\ of\ metal\ in\ shoots}{Concentration\ of\ metal\ in\ roots}. \tag{1}$$

$$BCF = \frac{Concentration\ of\ metal\ in\ roots}{Concentration\ of\ metal\ in\ test\ soil} \tag{2}$$

$$BAC = \frac{Concentration\ of\ metal\ in\ shoots}{Concentration\ of\ metal\ in\ test\ soil} \tag{3}$$

$$PR\ (\%) = \frac{Copper\ concentration\ in\ plant \times dry\ biomass\ of\ plant}{Copper\ concentration\ in\ soil \times Soil\ weight\ in\ each\ pot} \times 100 \tag{4}$$

2.5. Statistical Analysis

In this study, all statistical analyses were performed using IBM SPSS Statistics 24. Standard errors for three replicates are presented (mean $\pm$ SE; n = 3). Significance of differences was determined using one-way analysis of variance (ANOVA). Significance level was considered at p = 0.05.

3. Results and Discussion

3.1. Z. mays Growth

3.1.1. Germination rate of *Z. mays* in Copper-Treated Soils

Applying copper concentration of 50 mg kg^{-1} to soil did not alter seedling emergence in comparision with the control treatment, while gradual increase of copper content in soil reduced seedling emergence. Addition of 100 and 200 mg kg^{-1} copper significantly lessened germination of *Z. mays* (Figure 1).

For instance, the presence of 200 mg kg^{-1} copper in soil decreased *Z. mays* germination by 28% in contrast to the non-contaminated treatment (p < 0.05). Ultimately, the application of 300 mg kg^{-1} copper prevented germination of *Z. mays* in this study. In a study by Amin et al. (2021), the addition of 200 mg/kg Cu to soil lessened germination of *A. sativa* by around 55%, while germination of *G. max* did not occur at the same content of Cu in soil [35]. Phytotoxicity of heavy metals to plant seeds varies among different plant species. Toxic metals may interfere with enzymes, e.g., protease, thereby accelerate breakdown of stored food materials in plant seeds, which can alter permeability properties of cell membranes [37]. Reduced germination of plant species in presence of excessive quantities of copper in soil has been reported [38]. In this study, delayed emergence of *Z. mays* seedlings was also occurred due to the application of 200 mg kg^{-1} Cu to soil. However, *Z. mays* exhibited promising toleration in presence of copper in soil in most cases.
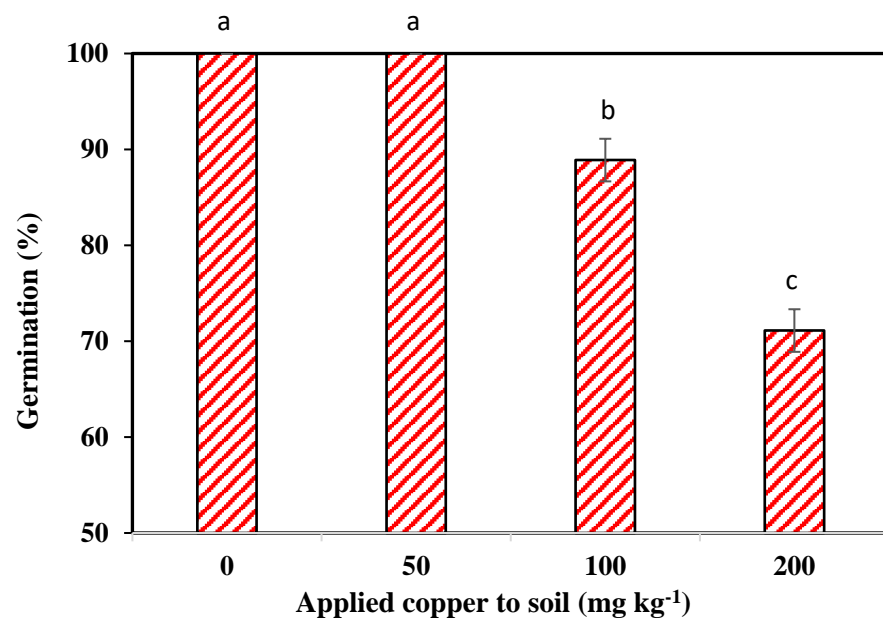
Figure 1. Ultimate germination of *Zea mays* in contaminated soil with 0, 50, 100 and 200 mg kg^{-1} copper. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).

3.1.2. Copper Toxic Impacts on Growth of *Z. mays*

The *Z. mays* biomass and length were used to evaluate the impacts of different levels of Cu in soil on plant growth (Figures 2 and 3). Results showed that addition of 50 mg kg^{-1} Cu slightly increased plant biomass, in contrast to the control treatment. This is because of the fact that copper is a beneficial micronutrient to plant growth, particularly at low concentrations. However, addition of higher concentrations of Cu reduced plant biomass in contrast to T1. Increased Cu content in soil caused further decrease of root biomass in this study. For instance, addition of 200 mg kg^{-1} Cu reduced both root biomass and shoot biomass significantly, in comparison with the control treatment ($p < 0.05$) (Figure 2).
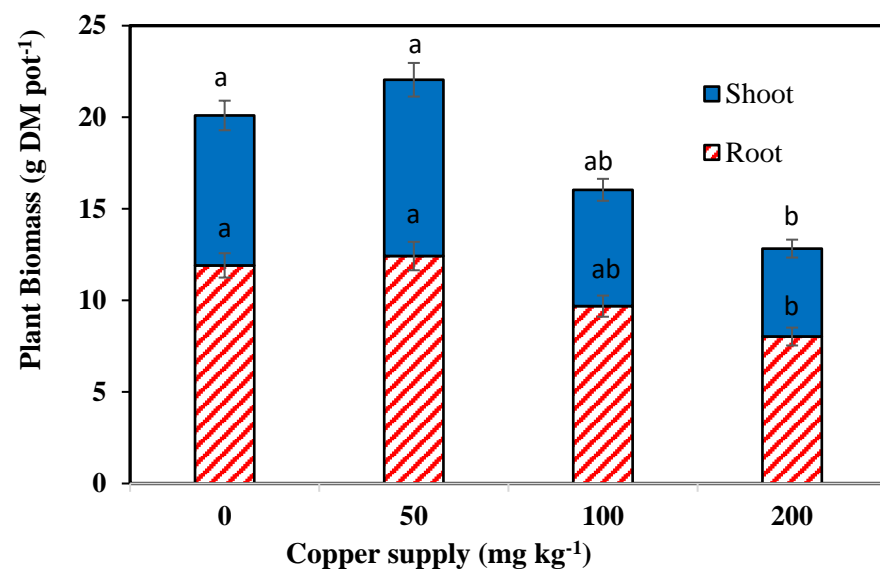

Figure 2. Dried biomass of *Zea mays* grown in contaminated soil with soil with 0, 50, 100 and 200 mg kg^{-1} copper after 42 days. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).
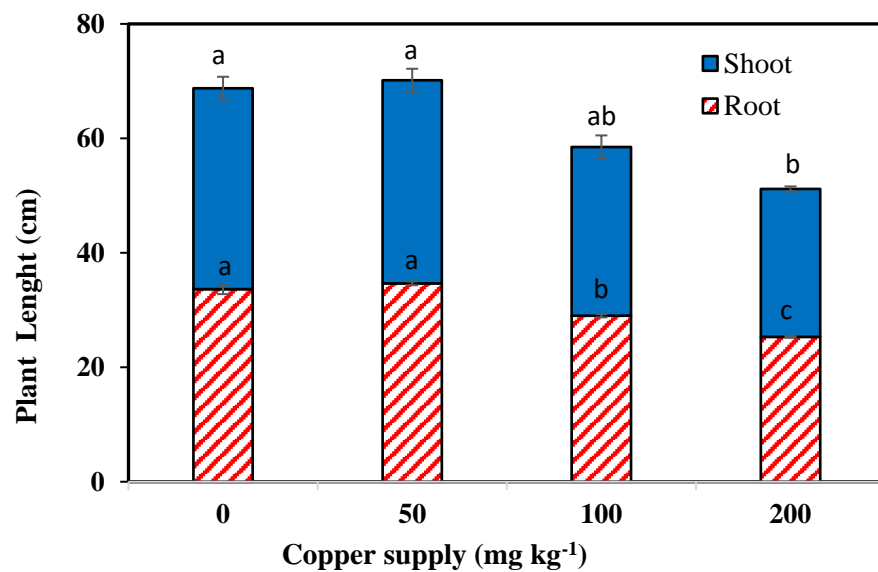
Figure 3. The length of *Zea mays* grown in contaminated soil with soil with 0, 50, 100 and 200 mg kg^{-1} copper after 42 days. Mean values that do not share a letter are significantly different at *p* = 0.05 (mean ± S.E.; *n* = 3).

　　　Biomass of the total plant became somewhat enhanced through the addition of 50 mg kg^{-1} Cu, whereas applying higher copper levels reduced *Z. mays* biomass. Presence of 100 and 200 mg kg^{-1} copper in soil reduced root biomass of *Z. mays* by 18.72% and 32.66%, respectively. Plant growth depression upon occurrence of high levels of heavy metals in soil was suggested to be associated with the plant type, metal speciation and concentration [39]. Heavy metal stress can disturb plant metabolic activities, reduce photosynthesis, and decrease uptake of essential nutrients from soil, which in turn diminish plant biomass establishment [35,40]. For instance, remarkable reduction in Fe, Zn and P uptake in maize cultivated in copper contaminated soil was reported in the literature [41]. Selected plants for phytoremediation are preferred to have fast growth, extensive root development capability, remarkable biomass establishment and high storage capacity for heavy metals [42]. Based on the obtained results, *Z. mays* seems to be a reasonably tolerant and rapid growing plant which is beneficial in terms of phytoremediation potential. Remarkable biomass establishment is also a crucial factor which can promote phytoremediation potential of plants [43]. Shoot height and root length of *Z. mays* in different treatments illustrated similar trend as biomass in response to applying different levels of copper to soil and lessened with rising copper content from 100 to 200 mg kg^{-1} (Figure 3).

　　　The extension of *Z. mays* in T4 with the root length and shoot height of, respectively, 25.3 ± 0.3 cm and 25.8 ± 0.8 cm, was significantly suppressed compared to the plant growth in T1 (*p* < 0.05). Phytotoxicity of excessive amounts of heavy metals in soil has been reported by other authors [44,45]. The sensitivity of *Z. mays* biomass and length to the addition of 200 mg kg^{-1} copper to soil was obtained in this study, while lower levels of copper did not inhibit plant growth significantly (*p* > 0.05). In other words, elongation of *Z. mays* root and shoot indicated a remarkable sensibility to the presence of excessive amounts of Cu in soil. Heavy metals can adversely affect uptake of nutrients and water, thereby reduce root extension in soil [46]. Migration of metals such as copper from roots to shoots can act on sensible parts of the plants, e.g., leaves and adversely affect photosynthesis process, which can pose plant height reduction [47]. Lower levels of Cu favoured plant establishment and growth, whereas higher Cu levels, i.e., 100 and 200 mg kg^{-1} in soil suppressed *Z. mays* growth. Additionally, onsite observations indicated that *Z. mays* leaves were withered more rapidly due to applying higher levels of copper.

3.2. Copper Uptake and Accumulation in Z. mays Tissues

Figure 4 shows the distribution of Cu in the roots and shoots of *Z. mays* for different treatments at the end of the phytoremediation experiment. The average concentrations of Cu in *Z. mays* roots were determined to be 56.0, 126.4, 237.4 and 435.8 mg kg^{-1} in amended soils with 0, 50, 100, and 200 mg kg^{-1} copper, respectively. Phytoextraction is the principal mechanism through which metals are eliminated from soil during a phytoremediation process [48]. The results showed that the concentration of Cu in *Z. mays* roots and areal parts became enhanced with copper content in soil. For instance, copper concentrations in *Z. mays* roots and shoots in T4 were 435.8 mg kg^{-1} and 831.5 mg kg^{-1} with a significant enhancement of 7.8 and 10.2 times, respectively, compared to T1 ($p < 0.05$). This is consistent with the literature in which an increase in copper content of soil from 20 mg kg^{-1} to 100 mg kg^{-1} caused a reduction in maize growth and an increase in copper uptake from soil [49]. This might be attributed to the higher availability of copper in soil to be up taken by plant roots. In other words, increase in bioavailability of Cu in soil treated with higher Cu levels could be a probable reason for that phenomenon. Metal speciation in soil is a crucial factor in controlling metal uptake by plants. In this study, soil pH values decreased with enhanced levels of Cu in soil (Table 1), which may alter Cu speciation in soil and make them more accessible to be up taken by roots. Contaminant bioavailability is also a critical factor affecting contaminant toxicity as well as the contaminant accessibility to both soil microbes and plants. For plants to be able to remediate pollutants, they must be accessible by the plant roots, such that plants can act on them [50]. Concentration of copper in *Z. mays* roots were greater than shoots in both treated and non-treated soils, suggesting that *Z. mays* roots are the preferential organ for copper storage. This is consistent with the results reported by Korzeniowska et al. (2011) who found higher concentrations of Cu and Zn in maize roots compared to the stems and leaves [51].
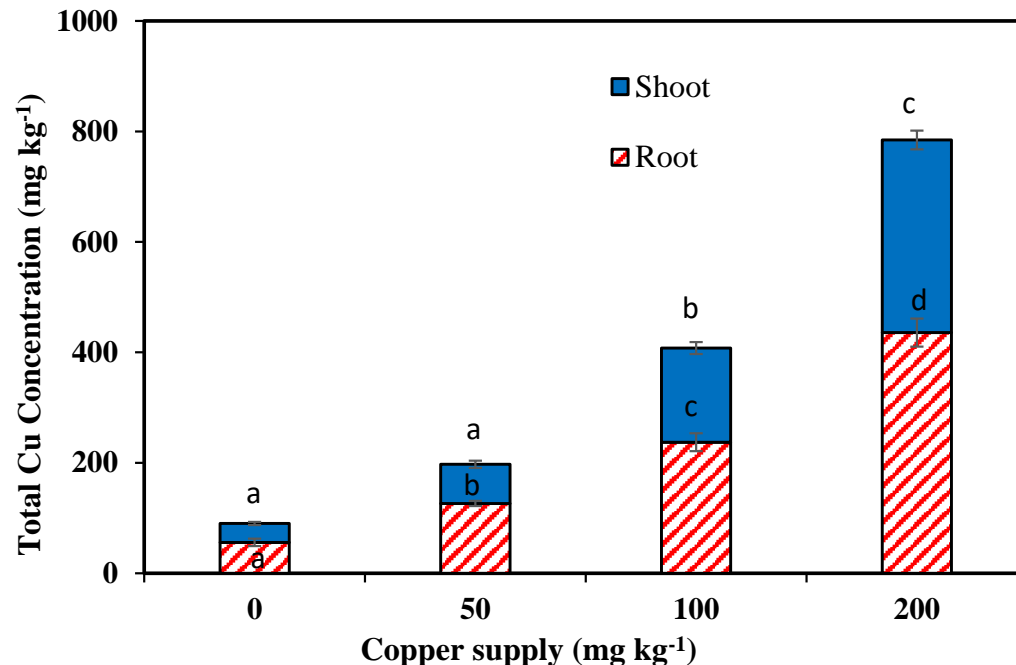

Figure 4. Copper concentration in roots and shoots of *Zea mays* grown in contaminated soil with soil with 0, 50, 100 and 200 mg kg^{-1} copper. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).

Along with concentrations, copper accumulation in *Z. mays* tissues is also an important factor that determines phytoremediation capability [52]. Copper accumulation capacity of *Z. mays* depends on both the copper concentration in tissues as well as biomass of a given plant species (Figure 5). Copper accumulation capacity of *Z. mays* in the control treatment

was 959.8 µg per pot, which was continually increased with Cu levels in soil. The highest total Cu accumulation capacity was obtained to be 5210.5 µg per pot (T4). Phytoremediation of metals can be promising when considerable concentrations of metals are up taken and accumulated in plant tissues [9]. Phytoremediation mechanisms in remediation of contaminants are mainly complex and not depends only on direct metabolism by plant species [28].
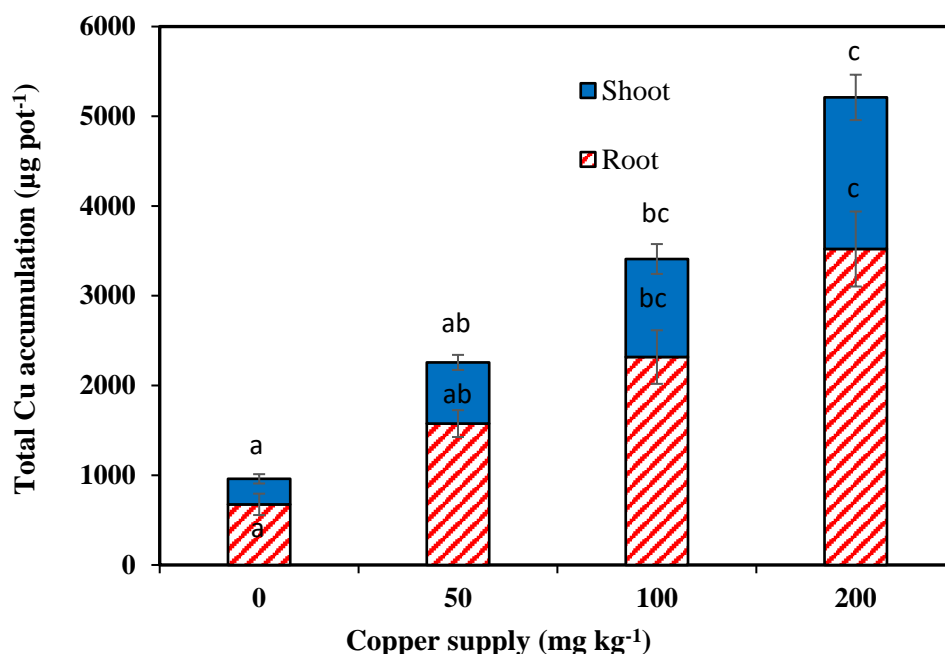

Figure 5. Copper accumulation capacity in *Zea mays* grown in contaminated soil with soil with 0, 50, 100 and 200 mg kg^{-1} copper. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).

The accumulation capacity of roots showed similar trend as the areal parts (Figure 5). However, higher accumulation capacity was found in roots rather than shoots, because of higher root biomass as well as greater Cu concentrations in root. Plant biomass reduction due to the addition of higher concentrations of Cu to soil did not reduce Cu accumulation capacity of *Z. mays*, because of the greater impact of enhanced Cu uptake in corresponding treatments. However, aaccumulation of heavy metals in plant tissues may cause oxidative stress as a result of evolution and accumulation of reactive oxygen species (ROS). Therefore, excessive amounts of copper in soil may bring about remarkable oxidative stress, and therefore remarkable damage to the antioxidant enzymes of plant species. On the other hand, some plant species, e.g., ramie (*Boehmeria nivea* L.) may activate a detoxification mechanism that partially neutralize the adverse effect of oxidative stress when exposed to copper [53].

Heavy metals at higher levels can also change water balance and assimilation of nutrients, which might ultimately lead to the death of the plant. Moreover, heavy metals in soil may compete with mineral nutrients for adsorption by plant roots. This phenomenon may result in deficiency of essential elements for plant survival, e.g., iron [54,55]. In a previous study, increase in applied concentration of lead in soil promoted its accumulation by plants [56]. To be brief, the obtained results suggested that *Z. mays* is a suitable plant species for copper extraction from soil and its accumulation in the plant tissues, particularly in presence of higher copper contents in soil, i.e., in T4.

3.3. Cu phytoextraction Efficiency

Translocation factors (TF), bio-concentration factors (BCF), and bioaccumulation coefficients (BCF) were determined and are presented in Figure 6 in order to better demonstrate

the effect of variation of Cu content on phytoextraction efficiency of *Z. mays*. BCF and BAC values for most treatments were found to be higher than 1, while TF values in all the examined treatments were less than 1, suggesting that phytostabilization is the principal mechanism by which Cu is remediated by *Z. mays*. However, Cu phytoextraction efficiency of *Z. mays* enhanced with Cu content in soil. In other words, TF values increased in presence of higher levels of Cu, with the greatest value of 0.8 in T4. Occurrence of heavy metals in soil may block the plant root apex, thereby reduce plant capability to transfer heavy metals from roots to the areal parts [57].
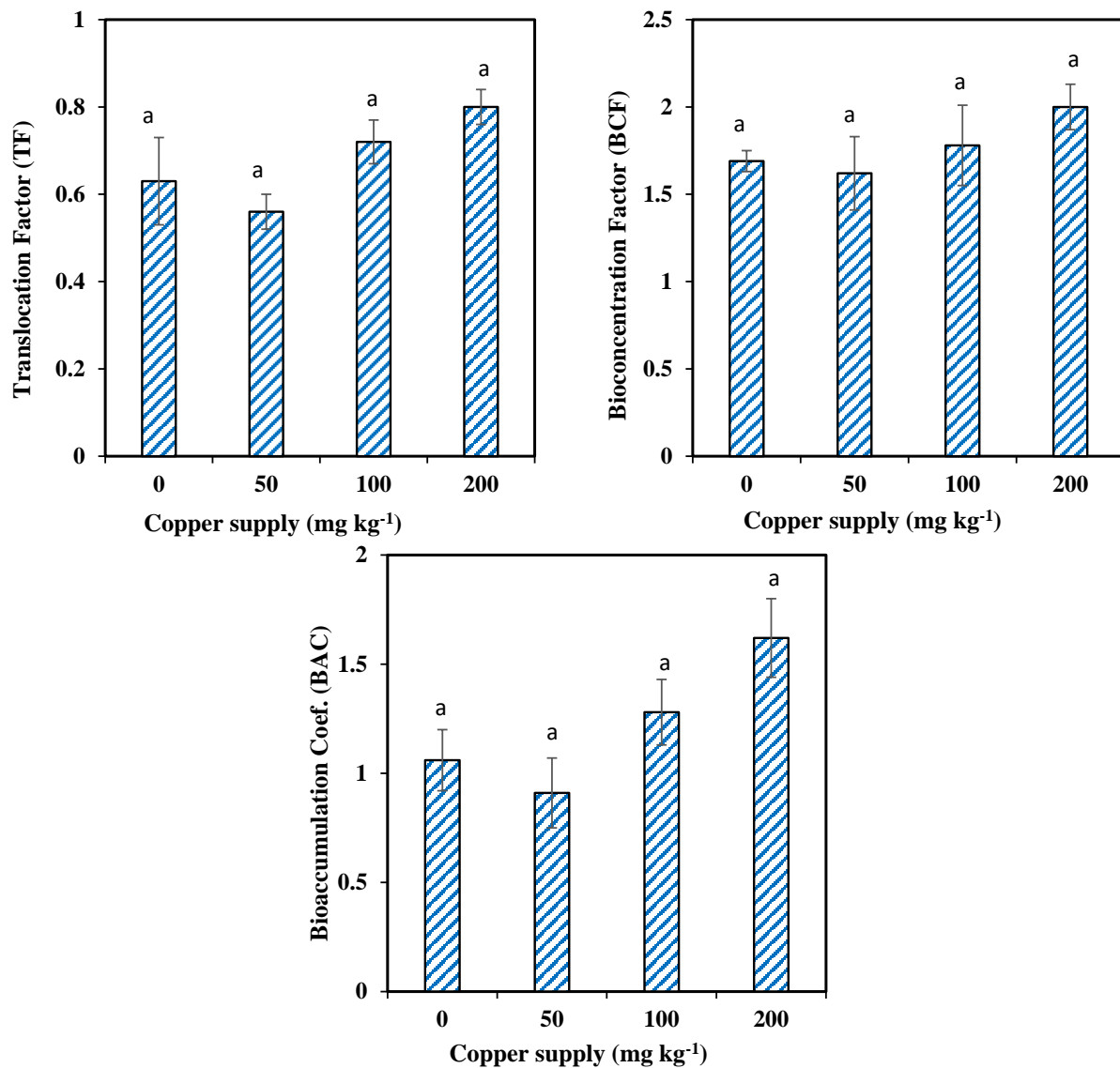

Figure 6. Translocation Factor (TF), Bioconcentration Factor (BCF) and Bioaccumulation Coefficient (BAC) for Cu in the studied treatments. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).

Moreover, apoplastic barriers might be formed proximate to the root apex in plant species cultivated in heavy metal contaminated soils, which reduces the translocation of sorbed metals from roots to shoots [58]. Using fast growing plant species to remove heavy metals from soil by phytoextraction mechanism has been encouraged in the literature [59]. When suitable plants are employed, heavy metal removal through phytoextraction can be up to ten times less expensive than the conventional remediation techniques, with comparable effectiveness [60]. The following five distinct phases have been recognized for

phytoextraction: (a) adsorption of some fractions of metals at root surface, (b) sorption of bioavailable fraction of metals via cellular membrane, (c) immobilization of small fraction of sorbed metals by roots in vacuole, (d) entry of mobile metals into the xylem, and (e) migration of metals from roots to the areal parts. Plant biomass enhancement as well as increase in bioavailability of heavy metals in soil matrix can promote phytoextraction efficiency for heavy metals [9,46].

BCF is known to be an important indicator of heavy metal accumulation capability because the ability of plants to extract heavy metals from soil is taken into consideration for its determination. Results indicate the increased ability of *Z. mays* to uptake copper from soil when higher concentrations of copper were applied. The highest value of BCF was obtained as be 2.0 in T4. Considerably high BCF values for Cu in *Z. mays* has been reported in the literature which is mainly due to the easy uptake of copper from soil by maize [61]. BAC is also a beneficial factor gaining insight into the ratio of heavy metals accumulated in the areal parts to that in soil. Plants exhibiting BCF values greater than one and TF values less than 1 are suggested to be more suitable candidates to remove metals from soil through phytostabilization [62]. Overall, results suggested that *Z. mays* can be categorized as Cu phytostabilizer plant species. Based on the obtained results, Cu phytoextraction as well as phytostabilization efficiency of *Z. mays* promoted with elevated levels of Cu in soil.

3.4. Phytoremediation Ratio (PR)

Phytoremediation capability of a given plant may be affected by several factors, e.g., pollutant bioavailability in soil, soil properties, plant type, root exudate composition, etc. The PR values for *Z. mays* grown under different Cu treatments were determined in this study and are presented in Figure 7. PR values for all the studied treatments were found to be greater than 1%. The highest PR values was found in T2 (3.7%) followed by T1 (3.7%). In other words, an addition of 100 and 200 mg kg^{-1} Cu to the soil slightly reduced PR for *Z. mays*, such that applying 200 mg kg^{-1} of copper reduced PR by 15.5%, compared to the control treatment. However, the differences found among the studied treatments were not statistically significant ($p > 0.05$). The capability of a given plant species in removing heavy metals from soil depends on biomass establishment as well as metal uptake and accumulation ability. Overall, the results indicated that *Z. mays* is a phytoremediator plant with a high capability to remove Cu from soil.
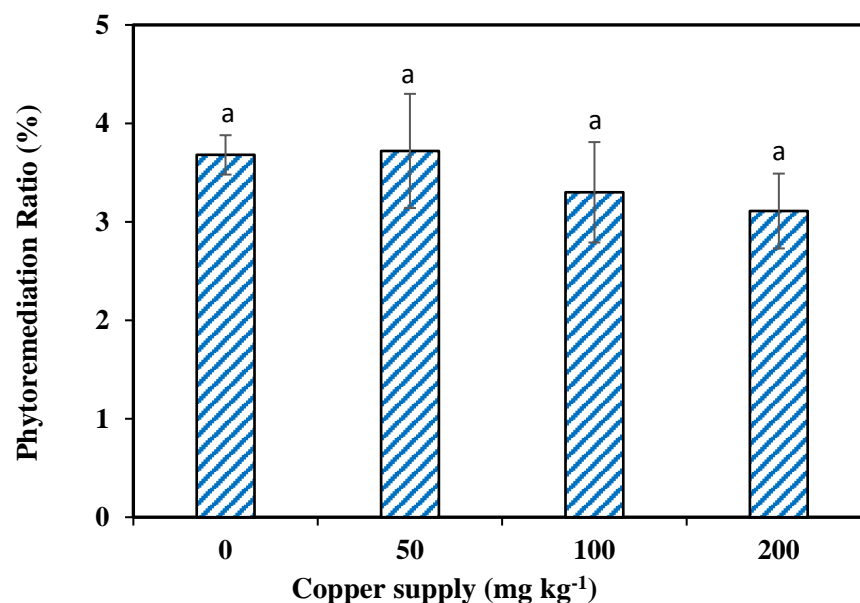

Figure 7. Phytoremediation Ratio (PR) for Cu in the studied treatments. Mean values that do not share a letter are significantly different at $p = 0.05$ (mean $\pm$ S.E.; $n = 3$).

4. Conclusions

The increasing occurrence of copper in soil can endanger food safety for the upcoming years, which necessitates the application of eco-friendly and effective approaches to remove excessive amounts of copper from soil. Copper uptake from soil by *Z. mays* and accumulation capacity of its roots and shoots have not been investigated extensively and have therefore been addressed in this research. This study investigated the capability of *Z. mays* to remediate soil contaminated with different levels of copper. The results indicate that *Z. mays* is a promising plant species to remediate Cu from soil contaminated with low to medium levels of Cu. Furthermore, we found that *Z. mays* is a tolerant plant and could establish considerable biomass over the period of the experiment. The addition of low concentrations of Cu, i.e., 50 mg kg^{-1} supported *Z. mays* growth compared to the control treatment. However, the greater levels of Cu in soil exhibited adverse effects on plant growth. An addition of Cu to soil (50–200 mg kg^{-1}) enhanced Cu concentration in *Z. mays* roots and shoots, compared to the control treatment. Higher concentrations of the applied Cu to soil effectively enhanced the capacity of *Z. mays* for copper. Greater accumulation of copper in *Z. mays* roots rather than the shoots for all treatments suggesting that phytostabilization might be the principal mechanism in phytoremediation of copper using *Z. mays*. Along with phytostabilization, Cu phytoextraction efficiency was also boosted by applying higher concentrations of Cu to soil. High values of BAC, BCF and PR suggested that *Z. mays* is a promising plant to uptake and accumulate Cu from soil. Overall, phytoremediation of copper contaminated soils by *Z. mays* was found to be promising approach to manage copper-contaminated soils, particularly at low to moderate Cu levels.

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