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RESEARCH ARTICLE

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

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Abstract

Flax (*Linum usitatissimum* L.), one of the oldest cultivated crops, continues to be widely grown for oil, fiber and food. Furthermore, the plants show a metal tolerance dependent on species so is ideal for research. Present study was conducted to find out the influence of copper (Cu) toxicity on plant biomass, growth, chlorophyll content, malondialdehyde (MDA) contents, proline production, antioxidative enzymes and metal up taken by *L. usitatissimum* from the soil grown under mixing of Cu-contaminated soil with natural soil by 0:1 (control), 1:0, 1:1, 1:2 and 1:4. Results revealed that, high concentration of Cu in the soil affected plant growth and development by reducing plant height, plant diameter and plant fresh and dry biomass and chlorophyll contents in the leaves compared with the control. Furthermore, Cu in excess causes generation of reactive oxygen species (ROS) such as superoxide radical (O^{-}) and hydroxyl radicals (OH), which is manifested by high malondialdehyde (MDA) and proline contents also. The increasing activities of superoxidase dismutase (SOD) and peroxidase (POD) in the roots and leaves of *L. usitatissimum* are involved in the scavenging of ROS. Results also showed that *L. usitatissimum* also has capability to revoke large amount of Cu from the contaminated soil. As Cu concentration in the soil increases, the final uptake of Cu concentration by *L. usitatissimum* increases. Furthermore, the soil chemical parameters (pH, electrical conductivity and cation exchange capacity) were increasing to highest levels as the ratio of Cu concentration to the natural soil increases. Thus, Cu-contaminated soil is amended with the addition of natural soil significantly reduced plant growth and biomass, while *L. usitatissimum* is able to revoke large amount of Cu from the soil and could be grown as flaxseed and a potential candidate for phytoremediation of Cu.

Keywords Flax (*Linum usitatissimum*) · Phytoextraction · Flaxseed crop · Cu-contaminated soil · Natural soil · Cu uptake · Reactive oxygen species · Growth · Proline

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Introduction

Heavy metal contamination have become a severe issue in all over the world especially in China due to industrialization and globalization and many other documented cases such as smelters, foundries and agriculture (Yang et al. 2018; Rehman et al. 2019a). High concentration of heavy metals in the agricultural land may cause adverse effect on plants, causes reduction in crop yield and plant productivity and also affect soil organisms (Khan et al. 2016; Singh et al. 2018). Furthermore, high concentration of heavy metals affect not only plants but their metabolic activities and their biological redistribution through pollution of water, air and soil (Wuana and Okieimen 2011; Volland et al. 2014; ul Hassan et al. 2017). Cadmium (Cd), copper (Cu), lead (Pb) and mercury (Hg) are considered as major heavy metal pollutants, which mostly occur in the soil with high anthropogenic pressure (Nagajyoti et al. 2010; Song et al. 2017). Among these major heavy metal pollutants, Cu is an important element, which is also an essential element required in many physiological processes such as photosynthesis, respiration and N_2 fixation (Sun et al. 2014; Chen et al. 2015; Husak 2015). Moreover, Cu is considered as a trace element, essential for the plants but in minute quantity, while lack of Cu concentration causes many disorders in the plants (Verma and Bhatia 2014; Zlobin et al. 2015). Uptake and translocation of Cu in plant species mainly depend on levels of Cu supply and growth conditions Saleem et al. (2019a). However, Cu in cells need to be kept at low levels because excessive Cu can induce alterations in the DNA, cell membrane integrity, respiration, photosynthesis, and enzyme activity which may lead to growth inhibition and endangered survival of plants (Aggarwal et al. 2012; Nair and Chung 2015; Saleem et al. 2019b). The reduction in plant biomass is a common response in plants when exposed to excess Cu. The major causes of high concentration of Cu in the soil are the use of Cu-containing herbicides, pesticides, fungicides and industrial effluents, sewage sludge and mining (Mahmud et al. 2013; Yang et al. 2014; Rizwan et al. 2016). Cu in excess causes the generation of reactive oxygen species, which are toxic for the plant tissues, and these species scavenge by the activities of antioxidants such as superoxide dismutase (SOD) and peroxidase (POD), which also play a significant role in reducing Cu toxicity in *Boehmeria nivea* L. and *Zea mays* L. (Murakami and Ae 2009; Rehman et al. 2019a). Oxidative stress in terms of lipid peroxidation causes disturbance to many metabolic pathways and damage to membrane-bounded organelles in the cell (Halliwell and Gutteridge 2015; Liu et al. 2015). Proline is an important amino acid and believed to be a single molecule, which helps in activating many physiological and molecular responses in the plants even in stress conditions. It was observed that, when plants undergo Cu stress condition, they accumulate high contents of proline in their tissue to overcome metal stress (Szabados and Savouré 2010; Ku et al. 2012).

Recently, there are many physical-chemical technologies for the remediation of toxic pollutants and petroleum hydrocarbons from the contaminated soil. These technologies include soil washing, thermal desorption and incineration (Zhu et al. 2016; Yang et al. 2018). Most of these techniques are expensive to implement at full scale and require continuous monitoring and control for optimum performance. Some of the techniques simply move the contamination elsewhere and may create significant risks in the excavation, handling and transport of hazardous materials. But phytoremediation (use of green plants for remediation of contaminated soil) is an option to perform such operations using natural biological activity (Ashraf et al. 2019). Furthermore, this technique is relatively cheap, eco-friendly and scientifically approved, have a high public acceptance and can be carried out in vast fields (Zaheer et al. 2015). This technique is successful not only to accumulate heavy metals from the soil but extra nutrients and organic matter can also remediate with the technique. There are many types of phytoremediation (phytotransformation, rhizosphere bioremediation, phytostabilization, phytoextraction and rhizofiltration), but phytoextraction is most common as it is the use of green plants sown under contaminated soil and able to accumulate plants in their above ground parts of the plant (Vangronsveld et al. 2009; Habiba et al. 2015). This technique is relatively cheap than soil excavation and treatment or disposal (Tahmasbian and Sinegani 2016; Yahaghi et al. 2018). Recently, many plant species including flax (*Linum usitatissimum* L.) has been used for phytoextraction of different heavy metals such as Zn, Cu, Hg and As (Smolinska 2015; Pajević et al. 2016; Sidhu et al. 2018; Lajayer et al. 2019). Many previous studies also suggested that *L. usitatissimum* is an ideal candidate for the phytoremediation of different heavy metals due to many unique characteristics such as huge biomass and many biochemical activities (Griga et al. 2003a, b; Hancock et al. 2012). However, *L. usitatissimum* is used as flaxseed or fibrous crop and has many industrial applications like solid yarns, twine, cordage, hoses, wood shives and tires (Najmanova et al. 2012; Wróbel-Kwiatkowska et al. 2012). Flax seed is used as food, pharmaceutical, as a component in compound feed and its oil for the production of varnishes and paints. Linseed provides more options for use than most other crops. The main product of linseed is seed. Linseed is used whole or slightly crushed for the production of bread and rolls, adding to the dough, making bakery products. (Vrbová et al. 2013; Yurkevich et al. 2017).

A lot of investigations have been reported about the potential of *L. usitatissimum* for phytoremediation of toxic heavy metals (Belkhadi et al. 2010; Kaplan et al. 2015; Praczyk et al. 2015), but very limited studies have explored on Cu toxicity on *L. usitatissimum*. Furthermore, this unique study also provide the information regarding growth and biochemical response of *L. usitatissimum* under mixing of Cu-contaminated soil with natural soil collected from two different sites of China. To the best of our knowledge, this study is among the few studies that focus on the metal tolerance and accumulation among fibrous crops in order to investigate their suitability for metal-contaminated sites. Therefore, the important goals of our study are (1) to determine the effect of Cu toxicity on seedlings growth of *L. usitatissimum* and (2) to evaluate the phytoremediation potential of *L. usitatissimum* under Cu-contaminated soil.

Materials and methods

Plant material and experimental treatments

Cu-contaminated 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, while natural soil was collected from experimental stations of Huazhong Agricultural University, Wuhan, China (114.20′E, 30.28′N). The following are the main agrochemical properties of the tested loam soil: pH 6.87, electrical conductivity (EC) of 269 μs cm^{-1}, cation exchange capacity (CEC) of 14.99 cmol kg^{-1}, organic matter of 3.96 g kg^{-1}, total nitrogen of 0.16 g kg^{-1}, total phosphorus of 1.97 g kg^{-1}, total potassium of 12.25 g kg^{-1} and total Cu of 2153 mg kg^{-1}, while the physicochemical properties of natural soil were as follows: pH 6, EC 2 dS cm^{-1}, 22 g kg^{-1} organic matter, 23.16 mg kg^{-1} exchangeable K, 0.23 g kg^{-1} total P, 20 g kg^{-1} total N and total Cu 38 mg kg^{-1}.These soils were mixed in the ratio of Cu-contaminated soil to natural soil, i.e., 0:1 (control), 1:0, 1:1, 1:2 and 1:4, dried under the shade and passed through the sieves of 5 mm before starting a pot experiment. After the mixing of two different soils, the pots were equilibrated for 2 weeks by one cycle of saturation with distilled water and air-drying. The seeds of flax Longya 10 were sown in 16 kg of pots (30-cm-tall × 40-cm-wide) on 15 November 2018 (Fig. 1). Each treatment was arranged in a completely randomized design (CRD) with six replications and three plants in each pot. Weeding, irrigation with Cu-free water and other necessary intercultural operations were done when needed. Pots were placed in a glasshouse, where plants received natural light, with day/night temperature of 10/2 °C and day/night humidity of 80/90%. At the time of harvest, shoot and root samples of each variety were collected separately and cleaned thoroughly with tap water and rinsed with 0.1 M HCl solution followed by several rinses with deionized water. Shoot and root samples were processed after oven dried at 65 °C for 72 h, and some samples were stored in refrigerator at − 80 °C. Post-harvest soil samples were collected separately from each pot. All chemicals used were of analytical grade, procured from Sinopharm Chemical Reagent Co., Ltd.

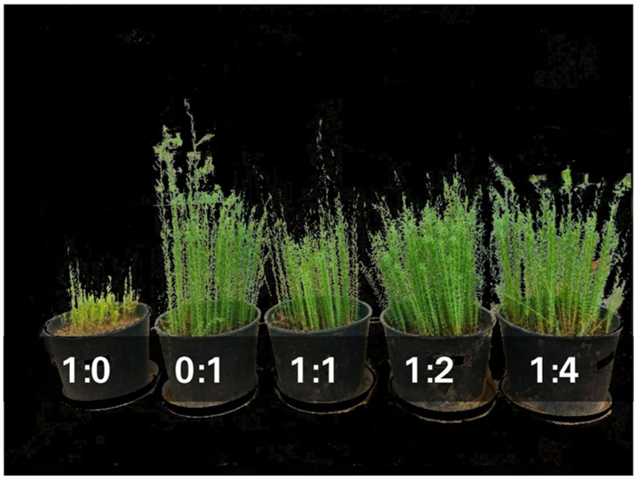

Fig. 1 Growth of *L. usitatissimum* under different levels of Cu-contaminated soil mixed with natural soil

Sampling and data collection

All plants were wrapped for different biological traits on mid of the January 2019 (60 days after seed sowing). On that day, leaves and roots samples were picked during 09:00–10:30 a.m. The samples were washed with distilled water, immediately placed in liquid nitrogen and stored in a freezer at low temperature (− 80 °C) for further analysis. Five plants per each treatment were selected for morphological traits, which were uprooted with the roots. Plant height was measured by measuring root length and shoot length of the plant. Plant diameter (mm) was measured at 10 cm above from the surface of soil using digital vernier caliper (ST22302 SG Tools, Hangzhou, China). Plant fresh weight was measured by measuring shoot weight and root weight together with the help of digital weighting balance. Later, plant samples (roots together with shoots) were dried in an oven at 105 °C for 1 h, then at 65 °C for 72 h to determine their dry weight. Roots were immersed in 20 mM Na_2EDTA 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.

Determination of chlorophyll contents

Chlorophyll content from fresh plant leaves was determined according to Lichtenthaler (1987) and expressed in mgg^{-1} FW.

Determination of MDA, proline content and antioxidant enzymes

The liquid nitrogen–chilled 500-mg leaf samples were used to obtain enzyme extract. The leaf samples were normalized with 4 mL of chilled 50 mM potassium-phosphate (K-P buffer), having pH 7, in a pre-cooled mortar and pester. The enzyme extract was transferred into Eppendorf tubes after centrifuging at 11,500×g (4 °C) for 20 min. The method given by Chen and

Pan (1996) was performed to measure activity of superoxide dismutase (SOD) and expressed as Ug^{-1} FW. The activity of peroxidase enzyme (POD) was measured as described by Sakharov and Ardila (1999) and expressed as Ug^{-1} FW. The method described by Heath and Packer (1968) was adopted to measure the concentration of lipid peroxidation (MDA). Proline contents were measured by the procedure of Bates et al. (1973) and expressed as μgg^{-1} FW. The MDA and proline contents were expressed as $\mu molesg^{-1}$ FW.

Soil analysis

Soil samples were collected from each pot at post-harvest stage, air dried and passed through 0.1-mm nylon for further analysis. Soil pH, EC and CEC were measured according to Lu (2000). The bio-available Cu was measured according to Houben et al. (2013). The digestion of soil samples were performed in a microwave oven operating system (Milestone, ETHOS D) with an energy output 0–400 W (0–100% potency, respectively). Approximately 0.100 g of dry soil samples were placed into the Teflon microwave digestion vessels, and then 3 ml of H_3PO_4 and 2 ml of $HClO_4$ were added to each sample. Plant samples were digested using the optimized microwave programs. After cooling to room temperature, the digested samples were diluted to a final volume of 25 ml with deionized water. Blank samples were prepared simultaneously. These solutions were stored in a refrigerator at 4 °C until the analysis was carried out. The total contents of Cu in the digests were determined by graphite furnace atomic absorption spectrophotometer (AAS) model Agilent 240 FS-AA equipped with deuterium background correctors, a graphite furnace GF95 and an autosampler (Rehman et al. 2019c). The replicates were taken for each sample. The determination of Cu removal (%) was measured by using the formula of Tanhan et al. (2007):

$$\text{Percentage }(\%)\text{ Efficiency} = C_0 - C_1/C_0 \times 100$$

where C_0 refers to the initial concentration of a heavy metal and C_1 refers to its final concentration.

Statistical analysis

The data recorded were statistically analyzed using Statistix 8.1 Analytical Software, Tallahassee, FL, USA. Testing showed that all plant- or soil-related data were approximately normally distributed. Thus, the differences between treatments were determined using analysis of variance, and the least significant difference test ($P \leq 0.05$) was used for multiple comparisons between treatment means. Pearson's correlation analysis was performed to quantify relationships between various analyzed variables. Graphical presentation was carried out using SigmaPlot 12.5. The R Studio was used to calculate Pearson's correlation.

Results

Growth, biomass and chlorophyll contents of *L. usitatissimum*

Results regarding plant height, plant diameter, fresh and dry biomass of *L. usitatissimum* were presented in the Table 1. These results showed that addition of Cu concentration to natural soil causes significant ($P \leq 0.05$) decrease in plant growth and development when compared with control. It was observed that maximum plant height was observed in the plants, which grown without any addition of Cu in the soil (control), while addition of 1:0, 1:1, 1:2 and 1:4 Cu concentration to natural soil causes reduction by 60, 52, 40 and 26%, respectively. Similarly, plant diameter was decreased by 56, 48, 36 and 20% by adding of Cu-contaminated soil to natural soil in the ratio of 1:0, 1:1, 1:2 and 1:4, respectively, compared with control, i.e., 0:1. Fresh and dry biomasses of the plant significantly ($P \leq 0.05$) decreased by adding of Cu-contaminated soil into the natural soil. Compared with the control, plant fresh weight was decreased by 67, 56, 42 and 21%, while plant dry weight was decreased by 80, 52, 40 and 20% by mixing of Cu-contaminated soil to natural soil at 1:0, 1:1, 1:2 and 1:4, respectively. Total chlorophyll contents in the leaves were also affected when Cu-contaminated soil were added to the natural soil (Table 1). The results were showed that addition of Cu-contaminated soil to natural soil by 1:0, 1:1, 1:2 and 1:4 decreased chlorophyll contents in the leaves by 61, 40, 21 and 7%, respectively, compared with the plants grown in natural soil without concentration of Cu.

Lipid peroxidation, proline and antioxidant enzymes

Lipid peroxidation in term of malondialdehyde (MDA) contents was also determined from the roots and leaves of *L. usitatissimum* (Fig. 2a). It was noticed that addition of Cu-contaminated soil to the natural soil significantly ($P \leq 0.05$) increased MDA contents in the roots and leaves of *L. usitatissimum*, which is the indication of oxidative damage occur in the plants due to phytotoxicity of Cu concentration. Compared with the control, the contents of MDA were increased by 508, 421, 284 and 163% in the roots and 386, 235, 136 and 60% in the leaves by mixing of Cu-contaminated soil to natural soil at 1:0, 1:1, 1:2 and 1:4, respectively. Similarly, proline contents were also increased in the plants as concentration of Cu increased in the soil (Fig. 2b). The contents of proline increased by 23, 14, 10 and 5% in the roots while increased by 16, 10, 6 and 3% in the leaves when Cu-contaminated soil was mixed with natural soil at:0, 1:1, 1:2 and 1:4, respectively, compared with control, i.e., 0:1.

In the present study, the activity of superoxidase dismutase (SOD) and peroxidase (POD) were also investigated under the mixing of two different soils (Fig. 2c, d). These results

Table 1 Effect of different levels of Cu-contaminated soil mixed with natural soil on plant height (cm), plant diameter (mm), plant fresh weight (g), plant dry weigh (g) and total chlorophyll contents (mg g^{-1} FW) in *L. usitatissimum*

Treatments	Plant height	Plant diameter	Plant fresh weight	Plant dry weigh	Chlorophyll contents
Ck	25 ± 1.31 a	2.5 ± 0.25 a	4.8 ± 0.20 a	2.5 ± 0.27 a	2.8 ± 0.08 a
T1	9.8 ± 0.62 e	1.1 ± 0.2 d	1.6 ± 0.27 e	0.5 ± 0.19 d	1.1 ± 0.06 e
T2	12.1 ± 0.52 d	1.3 ± 0.16 d	2.1 ± 0.27 d	1.2 ± 0.15 c	1.7 ± 0.1 d
T3	14.9 ± 0.38 c	1.6 ± 0.15 c	2.8 ± 0.15 c	1.5 ± 0.08 c	2.2 ± 0.07 c
T4	18.4 ± 0.46 b	2 ± 0.23 b	3.8 ± 0.18 b	2 ± 0.15 b	2.6 ± 0.1 b

Values in the table are 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: Ck control (soil without Cu concentration), T1 (Cu-contaminated soil is mixed with natural soil by 1:0), T2 (Cu-contaminated soil is mixed with natural soil by 1:1), T3 (Cu-contaminated soil is mixed with natural soil by 1:2) and T4 (Cu-contaminated soil is mixed with natural soil by 1:4)

suggested that addition of Cu-contaminated soil to natural soil increased the activities of antioxidants in the roots and leaves of *L. usitatissimum.* The activity of SOD increased by 443, 353, 303 and 231% in the roots while increased by 604, 536, 434 and 286% in the leaves when Cu-contaminated soil was mixed with natural soil at 1:0, 1:1, 1:2 and 1:4, respectively,

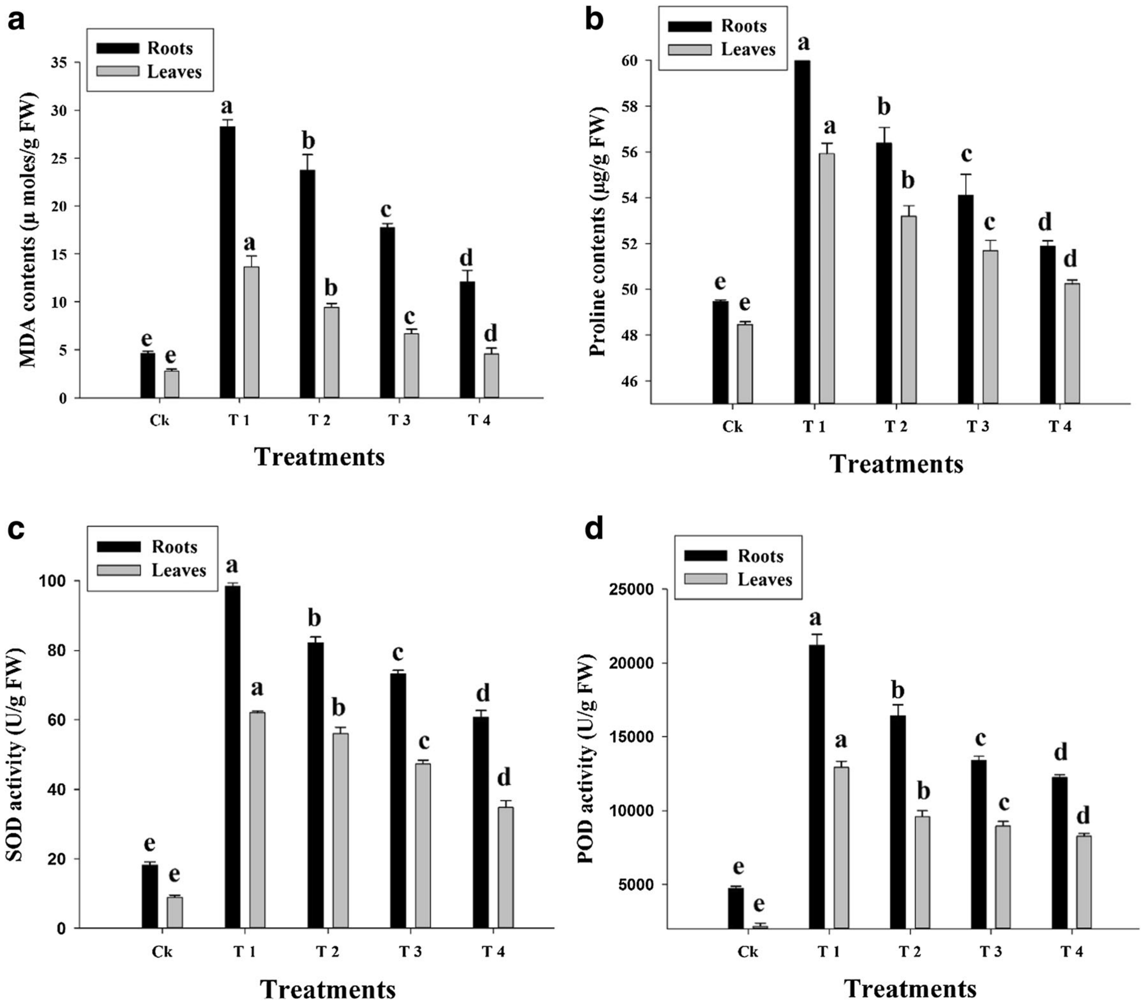

Fig. 2 Effect of different levels of Cu-contaminated soil mixed with natural soil on MDA (**a**), proline (**b**), SOD (**c**) and POD (**d**) in the roots and leaves of *L. usitatissimum*. Values in the table are just one harvest. Mean ± SD ($n = 3$). Different letters within a column indicate significant difference between the treatments ($P < 0.05$). Relative radiance of plastic filter used: Ck control (soil without Cu concentration), T1 (Cu-contaminated soil is mixed with natural soil by 1:0), T2 (Cu-contaminated soil is mixed with natural soil by 1:1), T3 (Cu-contaminated soil is mixed with natural soil by 1:2) and T4 (Cu-contaminated soil is mixed with natural soil by 1:4)

Table 2 Effect of different levels of Cu-contaminated soil mixed with natural soil on soil pHs, electrical conductivity (EC) (μscm^{-1}) and cation exchange capacity (CEC) (cmol/kg) at post-harvest stage

Treatments	pH	Electrical conductivity	Cation exchange capacity
Ck	6.35 ± 0.03 e	205 ± 2 e	11 ± 0.15 e
T1	6.90 ± 0.01 a	273 ± 2 a	15.4 ± 0.1 a
T2	6.76 ± 0.04 b	250 ± 5 b	14.7 ± 0.1 b
T3	6.56 ± 0.02 c	232 ± 4 c	12.5 ± 0.1 c
T4	6.45 ± 0.04 d	217 ± 2 d	11.9 ± 0.1 d

Values in the table are just one sampling. Mean ± SD ($n = 3$). Different letters within a column indicate significant difference between the treatments ($P < 0.05$). Relative radiance of plastic filter used: Ck control (soil without Cu concentration), T1 (Cu-contaminated soil is mixed with natural soil by 1:0), T2 (Cu-contaminated soil is mixed with natural soil by 1:1), T3 (Cu-contaminated soil is mixed with natural soil by 1:2) and T4 (Cu-contaminated soil is mixed with natural soil by 1:4)

compared with control, i.e., 0:1. Compared with the control, the activity of POD increased by 350, 248, 183 and 159% in the roots while increased by 499, 344, 314 and 283% in the leaves by mixing of Cu-contaminated soil to natural soil at 1:0, 1:1, 1:2 and 1:4, respectively.

Soil characteristics and extractable Cu concentration in soil

In the present study, different agrochemical properties of soil such as pH, electrical conductivity (EC) and cation exchange capacity (CEC) at post-harvesting stage of the plants were also determined (Table 2). These results suggesting that addition of different ratios of Cu-contaminated soil to the natural soil significantly ($P \leq 0.05$) increased soil properties compared with control. Our results revealed that soil pH increased by 9, 6, 3 and 2% when Cu-contaminated soil mixed with natural soil at 1:0, 1:1, 1:2 and 1:4, respectively, compared with control, i.e., 0:1. Compared with control, soil EC increased by 33, 22, 13 and 6% while CEC increased by 40, 34, 14 and 8% by mixing of Cu-contaminated soil to natural soil at 1:0, 1:1, 1:2 and 1:4, respectively.

In the present study, Cu concentration at post-harvest stage of *L. usitatissimum* from the soil were also measured (Table 3). Increasing Cu concentration in the soil causes more uptake of Cu by the plants. After harvesting *L. usitatissimum*, the final concentration of Cu was less than the initial concentration of Cu. The results suggested that maximum Cu concentration was up taken by the plant grown under 1:0 (Cu concentration mixed with natural soil), i.e., 36 mg kg^{-1} Cu while minimum Cu concentration was up taken by the plants grown under 1:4 (Cu concentration mixed with natural soil), i.e., 11 mg kg^{-1} Cu compared with the plants grown without any concentration of Cu. These results also suggested that *L. usitatissimum* has potential to revoke Cu in ranging from 22 to 27% from Cu-contaminated soil.

Correlation between growth parameters, chlorophyll contents and Cu up taken by *L. usitatissimum* from the soil

The Pearson correlation analysis was carried out to quantify the relationship between various studied parameters (Fig. 3). Cu up taken from the soil is negatively correlated with plant height, plant diameter, plant fresh weight, plant dry weight and total chlorophyll contents in the leaves of *L. usitatissimum*. However, all other growth attributes are positively correlated with each other but negatively correlated

Table 3 The efficiency of *L. usitatissimum* in extracting of Cu concentration (mg kg^{-1}) from the soil at post-harvesting stage

Treatments	Cu concentration	Available Cu	Final Concentration of Cu	Uptake of Cu	Removal of Cu (%)
Ck	37.5 ± 2 e	-	29 ± 1 d	8.5	22
T1	2153 ± 13 a	143 ± 3 a	107 ± 5 a	36	25
T2	1315 ± 23 b	95 ± 3 b	74 ± 4 b	21	22
T3	575 ± 16 c	66 ± 4 c	48 ± 2 c	18	27
T4	325 ± 32 d	43 ± 5 d	32 ± 2 d	11	26

Values in the table are just one sampling. Mean ± SD ($n = 3$). Different letters within a column indicate significant difference between the treatments ($P < 0.05$). Relative radiance of plastic filter used: Ck control (soil without Cu concentration), T1 (Cu-contaminated soil is mixed with natural soil by 1:0), T2 (Cu-contaminated soil is mixed with natural soil by 1:1), T3 (Cu-contaminated soil is mixed with natural soil by 1:2) and T4 (Cu-contaminated soil is mixed with natural soil by 1:4)

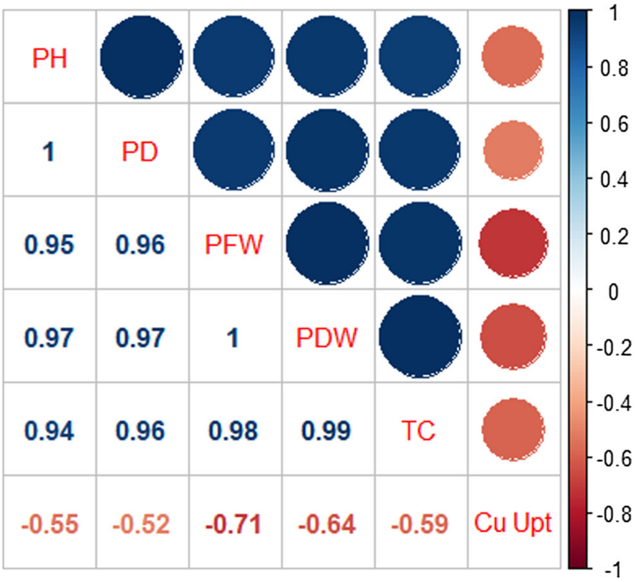

Fig. 3 Correlation of Cu uptake with growth attributes and chlorophyll contents in *L. usitatissimum*. PH (plant height), PD (plant diameter), PFW (plant fresh weight), PDW (plant dry weight), TC (total chlorophyll contents) and Cu Upt (Cu up taken by plants from the soil)

with Cu up taken from the soil. This correlation reflected the close connection between Cu uptake and growth in *L. usitatissimum*.

Discussion

For the past few decades, industrialization and urbanization are the main causes of increasing heavy metal concentration in agricultural land, which also have effects on ecosystem (Manousaki et al. 2008; Murakami and Ae 2009; Nagajyoti et al. 2010; Rehman et al. 2019b). Heavy metal contamination is toxic for the plants, but heavy metal such as Cu is also required by the plants for normal growth and development (Xu et al. 2006; Li et al. 2018; Saleem et al. 2019). Although Cu is a micronutrient for the plants, excess Cu may cause toxicity, which ultimately affects crop yield and plant productivity (Bouazizi et al. 2010). Normal concentration of Cu in the plants is 20–30 mg kg^{-1} of dry mass. The Cu level accumulated by the plants must be transported and distributed to various parts of the plants for healthy growth and development. Contrastingly, high concentration of Cu in the plant tissues/cell causes chlorosis, inhibited root growth, bronzing and necrosis (Stojek 2013; Waters and Armbrust 2013). In the present study, addition of Cu-contaminated soil to the natural soil causes significant reduction in plant growth and biomass (Table 1). However, the reduction in the growth of *L. usitatissimum* under high levels of Cu suggested the Cu-induced toxicity at its elevated concentrations. Phytotoxicity of Cu-affected plant growth and composition has been showed by many studies (Habiba et al. 2015; Zaheer et al. 2015; Race et al. 2016). Reduction in plant biomass is a common response in plants exposed to an excess of Cu (Pietrzak and Uren 2011). These findings are in agreement with Li et al. (2018) who reported significant reductions in the biomass of Cu-treated plants of *Brassica napus* L. The observed reduction in plant dry mass is also in conformity with similar investigations on *Festuca arudinacea* L. and *Lolium perenne* L. seedlings exposed to Cu stress (Zhao et al. 2010). Increasing Cu concentration in the soil has also been reported to reduce both shoot and root growth in *Arabidopsis thaliana* L. (Kolbert et al. 2012). Liu et al. (2015) adds additional supporting evidence to our present findings.

Determination of chlorophyll contents from the leaves are important biological parameter for the evaluation of plant stress. It was observed that increasing concentration of Cu in the soil adversely affects leaf chlorophyll (Rehman et al. 2019b). In addition to plant growth and biomass, leaf chlorophyll contents were decreased by Cu toxicity (Table 1). Decrease in chlorophyll contents might be result of displacement of Mg required for chlorophyll biosynthesis or ultra-structural alteration of chloroplast under metal toxicity. This reduction in chlorophyll contents might be due to the inhibited activities of various enzymes associated with chlorophyll biosynthesis (Martins and Mourato 2006; Zvezdanović et al. 2007). Similar results were showed by Sánchez-Pardo et al. (2014) when they studied *Lupinus albus* L. and *Glycine max* L. under Cu stress.

When a plant undergoes stress condition, the dynamic equilibrium of reactive oxygen species (ROS) is disturbed and causes generation of these species, which causes ROS accumulation in the cell/tissues and causes lipid peroxidation (Sgherri et al. 2007; Rizwan et al. 2016). This accumulation of ROS is toxic for the plants and may cause cellular damage. Although, the accumulation of ROS is a common feature of the plants under stress environment, which induced oxidative damage in the plants (Andrade et al. 2010; Thounaojam et al. 2012). Heavy metal accumulation in the cell/tissues is involved in the direct or indirect generation of ROS in the following ways: (1) direct transfer of electrons, (2) disturbance of metabolic pathways and (3) reduced activities of antioxidants (Sun et al. 2010; Halliwell and Gutteridge 2015). Antioxidants such as superoxidase dismutase (SOD) and peroxidase (POD) are involved in the scavenging of ROS (Meng et al. 2007; Liu et al. 2018). The SOD catalyzes the dismutation of superoxide to H_2O_2, and molecular oxygen and POD decomposes H_2O_2 by oxidation of cosubstrates (Chandrasekhar and Ray 2017). In the present study, high Cu concentration in the soil causes oxidative stress by increasing MDA contents, and, due to generation of ROS, antioxidants (SOD and POD) come into play for the scavenging of ROS (Fig. 2). Upregulation of activity of these enzymes shows the capacity of plants to scavenge excessive ROS in the cells. Increasing activities of antioxidants under high concentration of Cu in the soil indicated that *L. usitatissimum* could tolerate Cu stress by enhancing antioxidative defense system. When plants undergo heavy metal stress, they believed to accumulate high contents of proline in their tissue to reduce metal toxicity as showed by Ku et al. (2012) in *Nicotiana benthamiana* L. In the present study, significant increase in antioxidant enzyme activities can be considered as an indicator of increased ROS production and mitigation (Upadhyay and Panda 2010; Shin et al. 2012). Goswami and Das (2016) studied *Calandula officinalis* L. under Cu stress and noticed that phytotoxicity of Cu induced oxidative damage, which was overcome by increasing activities of antioxidants. Production of antioxidant enzymes (SOD and POD) in *L. usitatissimum*, consequently, serves as an approach to strengthen cell antioxidant system and overcome the risk of ROS production due to metal stress (Rout and Sahoo 2013).

In the present study, a significant ($P \leq 0.05$) change in the chemical properties of soil at post-harvest stage was observed. Results from the present study revealed that addition of Cu-contaminated soil to the natural soil increased soil pH, EC and CEC (Table 2). The increased in soil pH, EC and CEC at post-harvest stage might be due to the high physiochemical properties of soil in Cu-contaminated soil. The similar soil (Cu-contaminated soil) was used by Rehman et al. (2019a), but they added rice straw biochar (RSB) in it to reduce phytotoxicity of Cu and noticed that addition of RSW significantly increased plant growth and biomass while reduced the metal accumulation by the plants. But our objective is accumulation of Cu by the plant to remove heavy metals from the soil. Although very few literature are available of mixing of two different soils taken from two different sites, we have observed that addition of Cu-contaminated to natural soil affected plant growth and composition (Table 1). Furthermore, *L. usitatissimum* is a potential candidate to remove heavy metals from the metal-contaminated soil studied by many researchers (Szynkowska et al. 2009; Hradilová et al. 2010; Smykalova et al. 2010; Vrbová et al. 2013). Our results also suggested that *L. usitatissimum* seedlings have ability to remove Cu from the Cu-contaminated soil ranging from 22 to 27%. Hosman et al. (2017) studied *L. usitatissimum* plants under different concentrations of lead (Pb), cadmium (Cd) and zinc (Zn) and showed that *L. usitatissimum* has ability to revoke high concentration of heavy metals from the soil. Similar findings were obtained by Amna et al. (2015) and Uddin Nizam et al. (2016) when they studied *L. usitatissimum* under the concentration of different heavy metals and noticed that *L. usitatissimum* has potential to revoke huge amount of heavy metals from the soil and can be used as phytoremediation for different heavy metals under metal-contaminated soil.

Conclusions

Study concludes that *L. usitatissimum* could be considered as a phytoremediator plant and classified as an accumulator for the tested Cu with different mechanisms and considered a good accumulator. Furthermore, *L. usitatissimum* has a considerable potential to cope with high concentration of Cu in the soil due to active antioxidative defense mechanism. However, addition of Cu-contaminated soil to natural soil significantly reduced plant height, plant diameter and fresh and dry biomass of the plant and chlorophyll contents in the leaves. Moreover, excess Cu in the soil also induces oxidative damage in *L. usitatissimum*, which overcomes by the activities of antioxidants (SOD and POD). Chemical properties (pH, EC and CEC) of soil are also affected by addition of Cu as post-harvesting stage of the plant. Contrastingly, *L. usitatissimum* has potential to cope huge amount of Cu from the soil and can be used as a heavy metal accumulator for metal-contaminated soil. However, future research is needed on the effects of Cu stress on quality of both fiber and flaxseed from *L. usitatissimum*. Moreover, potential for *L. usitatissimum* in remediation of soils polluted with heavy metals should be tested under field conditions.

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Compliance with ethical standards

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

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