



Potential for Phytoextraction of Cu by *Sesamum indicum* L. and *Cyamopsis tetragonoloba* L.: A Green Solution to Decontaminate Soil

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Abstract

Phytoextraction is a plant based-technique for removing toxic heavy metals from polluted soil. The experiment reported in this paper was undertaken to study the basic Cu phytoextraction potential of *Sesamum indicum* in comparison with *Cyamopsis tetragonoloba* for remediation of Cu contaminated soil in the framework of a pot-experiment. Plants were subjected to seven Cu concentrations (0, 25, 50, 100, 150, 200, and 300 mg kg⁻¹ soil) for 12 weeks. The morphological (i.e. growth) and biochemical (i.e. chlorophyll) parameters of both the plant species were observed throughout the experimental period; the phytoextraction efficiency of *S. indicum* and *C. tetragonoloba* were also determined. Most growth parameters were reduced under high Cu stress. Our results shows that at low concentration (25 mg Cu kg⁻¹) all the growth and biochemical parameters were increased but at elevated Cu concentrations, root length, shoot length, and biomass (fresh and dry) were all significantly decreased ($p < 0.05$). Chlorophyll contents also declined with increasing concentrations of Cu, when compared with control. A consistent increase of Cu accumulation in root and shoot of both *S. indicum* and *C. tetragonoloba* with rising concentrations of Cu in soil was noted for all tested treatments. In this study, both plant species showed quite high Cu tolerance and accumulation efficiency, even though *C. tetragonoloba* have higher Cu accumulation and tolerance indices than that of *S. indicum*. At 300 mg Cu kg⁻¹, the highest Cu concentration was found in the root (282.08 mg Cu kg⁻¹) followed by leaf (105.78 mg Cu kg⁻¹), stem (65.30 mg Cu kg⁻¹), and pod (8.13 mg Cu kg⁻¹) of *S. indicum*. In contrast, *C. tetragonoloba* had highest Cu concentration primarily in the root (158.45 mg Cu kg⁻¹) followed by the stem (154.73 mg Cu kg⁻¹), leaf (152.32 mg Cu kg⁻¹), and pod (8.13 mg Cu kg⁻¹). Considering rapid growth, high biomass, tolerance, accumulation efficiency, bioconcentration factor (BCF) > 1, bioaccumulation coefficient (BAC) > 1 and translocation factor (TF) > 1 established *C. tetragonoloba* as a potential candidate plant for the decontamination of slightly Cu-polluted soil where the growth of plants would not be impaired and the extraction of Cu could be maintained at satisfying levels. Therefore, the present study suggested that *C. tetragonoloba* could possibly be used as a viable tool for phytoextraction.

Keywords Soil pollution · Copper · Accumulation · Translocation · Phytoextraction

1 Introduction

Agricultural soil contamination due to hazardous heavy metals is a significant environmental problem. The accumulation of substantial toxic metals in soil profile adversely affects agricultural production. As a consequence of heavy metal contaminated soil, the harmful metals turn into a part of a natural way of life and furthermore cause serious risk to plants, animals, humans, and entire soil condition including soil organisms (microbes) (Nagajyoti et al. 2010; Singh and Prasad 2014).

Rapid industrialization and urbanization, improper use and transfer of unsafe metal containing squanders,

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inappropriate utilization of manure, commercial fertilizers and pesticides are the primary sources for heavy metal entry into the agricultural soil (Dmitry et al. 2015; Amel et al. 2016). Toxic heavy metals, including lead, copper, zinc, cadmium, chromium, nickel, cobalt, and iron are major environmental contaminant components, especially in regions with high anthropogenic activities (Nagajyoti et al. 2010; Subhashini et al. 2013).

Among heavy metals, copper (Cu) is the most abundant transition heavy metal in earth's crust, known as a coinage metal, that exists in the monovalent and divalent oxidation states. Cu has assumed an essential part of human social, cultural, modern industrial, and technical advancement since early times. Like other transition metals (e.g. Ag and Au) Cu has broadly utilized as a part of an extensive variety of activities, for example, fungicides and pesticides, wood additives, pigments used in glasses, paints and furthermore as a superconductor (Rebecca 2006). Cu is considered as basic essential micronutrient (trace element) in low concentration for animal (Wintz et al. 2002) and plant biological functions (Mahmood and Islam 2006; Muhammad et al. 2015). In an enzyme chemical reaction, Cu acts as cofactor and activator enlightening enzymes/substrate metal complex (Mildvan 1970) and in addition, serves as catalysts for homogeneous and heterogeneous chemical reactions. Cu phytotoxicity in plants changed the take-up of other essential nutrients. In plants, excess Cu is more toxic relatively with other essential trace elements, such as Zn and Mn which are possibly toxic in excess (Dresler et al. 2014). High take-up of Cu well beyond plant prerequisites brings about toxic impacts on plant normal functions (Monni et al. 2000). Excess Cu has become deleterious to plants and is responsible for reduced seed germination, stunted root and shoot development, reduced yield, oxidative stress generation and formation of reactive oxygen species (ROS) (Stadtman and Oliver 1991; Azooz et al. 2012) that causes damaging influence to metabolic pathways and harm to macromolecules (Hegedus et al. 2001).

Soil contamination by Cu is reliant on both natural and also anthropogenic sources. Agricultural soil receives a substantial amount of Cu from environmental contamination that came about because of anthropogenic activities which frequently utilize Cu for agricultural farming, industrial and mechanical purposes (Jiang et al. 2004). The inappropriate, inequitable and untreated utilization of Cu-containing pesticides to manage plant pests, diseases and infections have brought about the surplus accumulation of Cu in agricultural soil profile (Mackie et al. 2012; Muhammad et al. 2015). Some of the distinguished anthropogenic activities including mining and smelting operations, industrial and urban activities, inorganic–organic fertilizers, liming, sewage sludge and wastewater for irrigation are the most common sources of Cu to the agricultural soil (Herawati et al. 2000).

In this way, the sustainable clean-up of Cu-contaminated soil to confine its impact on the environment is required. Remediation of substantial metal contaminated soil by conventional physical and chemical techniques are well-reported in literature but these are insufficient and unacceptable for agricultural lands as they require huge venture and innovative resources (Oh et al. 2013). Along these lines, a plant-based technology has been developed as a cure for the manageable control of elevated levels of different toxic heavy metals in soil.

Phytoextraction (or else known as phytoaccumulation/phytoabsorption/phytosequestration) a promising, cheaper, eco-efficient technique for the remediation of heavy metal polluted soil has attracted immense consideration in recent times. Phytoextraction is the take-up of heavy metals from soil to root by metal-accumulating plants and their transfer to easily harvestable parts (i.e. aboveground shoot/biomass) (Seema et al. 2015; Amanullah et al. 2016).

The plant species appropriate for phytoextraction purpose should have great tolerance to the harmful impacts of the substantial metals in soil, high accumulation and transfer of metals from below-ground to above-ground parts, fast growth and development rate, high production of above-ground biomass (shoot) and must be simple to cultivate and harvest (Shabani and Sayadi 2012; Hazrat et al. 2013). Several workers have reported the phytoremediation potentials of plant species belonging to botanicals families, specifically the Brassicaceae, Asteraceae, Fabaceae, Poaceae and Chenopodiaceae. Even phytoremediation potentials by Chlorophyceae are well documented in the literature (Gawronski and Gawronska 2007; Anjum et al. 2014; Balaji et al. 2014a, b, 2016). The heavy metal take-up limit, accumulation, exclusion, compartmentation and mechanisms of metal tolerance differ among various plant species and furthermore between different parts of plants (Sharma et al. 2014).

Sesame (*Sesamum indicum* L.) is an important oilseed crop of family Pedaliaceae (Elleuch et al. 2007). Sesame seeds are considered to be the oldest oilseed crop well-known to man, highly resistant to drought conditions and have the ability to grow where most of the crops are unsuccessful to cultivate (Dawodu et al. 2014). Recent studies illustrate that sesame seeds have been widely used as alternative feedstock for biodiesel production. The methyl ester extracted from sesame seeds can effectively be utilized as petrodiesel (Ahmad et al. 2011). The viscosity and density of methyl esters of sesame seed oil were observed to be near that of diesel, while the heating (7.5%) and calorific (5.4%) values were lower than that of diesel to some extent (Saydut et al. 2008).

Moreover, guar (*Cyamopsis tetragonoloba* L.) is a remarkable legume crop plant of family Fabaceae. The endosperm of guar seeds contains gum, which has to pick up significance as a non-food product (Ashraf et al. 2002). The

utilization of fluid (gum) extracted from guar in fracking for oil has extended the significance of guar around the world. The oil business industry has begun utilizing extracted fluid from guar in pressure-driven cracking of rocks or coal beds (process of hydraulic fracturing) attributable to its high consistency and proficiency for petroleum and natural gas extraction (Deepak et al. 2014; Abidi et al. 2015). A key factor of using legumes for phytoremediation is their function in giving additional N-compounds to the soil, therefore enhancing soil richness and fertility and sustaining the development of plants and soil organisms (Xiuli et al. 2013).

This study was set up to investigate a plant species that could tolerate high toxicity of Cu treatment levels in soil. Both the plant species, i.e. *S. indicum* and *C. tetragonoloba* have fast growth and development rate also high above-ground biomass. It was estimated that plant species with high phytotolerance could then be exploit for their phytoextraction potential. In this way, considering above mentioned points, the objectives of the present study are to (1) observe the phytotoxicity of Cu on morphological and biochemical parameters of *S. indicum* and *C. tetragonoloba*, (2) examine the Cu accumulation efficiency in below and above ground biomass growing on a Cu polluted soil and (3) to investigate the possibility of using *S. indicum* and *C. tetragonoloba* for Cu phytoextraction.

2 Materials and Methods

2.1 Seed Procurement and Surface Disinfection Process

Seeds of two plant species, i.e. *Sesamum indicum* (sesame) variety TH-6, from the Institute of Oilseeds Research Program and *Cyamopsis tetragonoloba* (guar) variety BR-99 were procured from the Institute of Fodder Research Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan. Seeds were surface cleaned (disinfected) with 0.1% HgCl_2 for 10 min and rinsed seven times with deionized (DI) water, to keep away from any microbial infection (Pourakbar et al. 2007).

2.2 Test Soil Sample Collection

Soil samples (with sandy-loam texture) were collected from uncontaminated farming fields situated in Jamshoro, Sindh, Pakistan, at depth of 0–15 cm using hand shovel.

Soil samples were collected from equidistant (2 m) interval and mixed well to make single uniform bulk soil. The soil samples were air dried for about 15 days and ground with pestle and mortar to pass through a size of 2 mm mesh and use for further study. In order to increase soil porosity, sandy-loam soil samples were mixed together with sand in 3:1 proportion.

2.3 Soil Sample Measurements

For characterization, Soil pH was measured with a pH-meter (InoLab-WTB GmbH; Weilheim, Germany) using glass electrode at 1:2 (w/v) ratio of soil to water suspension (Rachit et al. 2016). The electrical conductivity (EC) was measured with an electrical conductivity meter (WTW—330i) at the 1:2 (w/v) ratio of soil to water suspension (Rachit et al. 2016). Organic matter (OM) and organic carbon (OC) (%) were measured according to Walkley and Black (chromic acid titration) method (Fanrong et al. 2011).

2.4 Preliminary Screening for Cu Treatment Levels

For the selection of Cu treatment levels, different concentrations of CuCl_2 (0, 50, 100, 200, 500, 700, 1000, 1500 and 2000 mg kg^{-1}) were undertaken in the preliminary screening of the *S. indicum* and *C. tetragonoloba* for 20 days. In light of the Cu phytotoxicity, morphological growth and development of the plant seedlings, the subsequent concentration levels (0, 25, 50, 100, 150, 200 300 mg kg^{-1}) were finally selected (Table 1).

2.5 Pot Experiment

Plastic pots were filled with 5 kg sieved soil, after which soil was artificially spiked with Cu (aqueous solution) using CuCl_2 salt with increasing concentration levels (25, 50, 100, 150, 200 300 mg Cu kg^{-1}) to each pot with three replicates and set aside for 15 days to get stability. The uncontaminated soil without Cu spiking was utilized as control (T_0). Experimental pots were arranged in a complete randomized design (CRD). Following 15 days of stabilization, soil in pots was mixed well, and 20 surface disinfected (i.e. sterilized) seeds were sown in each replicate pot. One week (7 days) after seed germination, seedlings were thinned down to five for each pot. A plastic plate was placed below the pot for the collection of liquid (leachate) that drains out, which was returned back to the pot at subsequent watering. Overall investigation was performed in

Table 1 Cu treatment levels selected for pot experiment

Heavy metal	Salt used	Treatments (mg kg^{-1} soil)						
		T1	T2	T3	T4	T5	T6	T7
Copper (Cu)	CuCl_2	0	25	50	100	150	200	300

a greenhouse for a period of 12 weeks. The phytotoxic effects of metal displayed by plants were apparently noted throughout the test time frame. Plant species were harvested after 90 days of germination. Likewise, plant morphological (growth) and biochemical (chlorophyll) parameters were measured. Soil test samples (in triplicate) were also collected for investigation of Cu by an Atomic Absorption Spectrophotometer (Perkin-Elmer, AAnalyst 800).

2.6 Germination Percentage (%)

Germination percentage is an estimation of the viability of seeds, calculated as total number of germinated seeds to the total number of seeds sown expressed in percentage (Talebi et al. 2014):

$$\text{Germination percentage (\%)} = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of seeds sown}} \times 100.$$

2.7 Morphological Parameters

Plant samples were carefully uprooted from each treatment pot to quantify morphological (growth) parameters. Plant root and shoot lengths were measured using metric scale. Fresh weights of root and shoot were measured as well with the assistance of analytical weight balance. Plant samples were air dried for 1 week. After that plants were oven-dried at 80 °C to attain a constant weight and at that point their dry weights were recorded.

2.8 Chlorophyll Contents

Extraction of chlorophyll content, in completely extended foliage (leaf) from each replicate pot, was carried out by taking 0.5 g of fresh leaf material, ground with 10 ml of 80% acetone. Following filtration, 1 ml of the suspension was diluted with an extra 2 ml of acetone, and optical density was determined with a UV–Visible spectrophotometer (Biochrom Libra S22), using two wavelengths (663 and 645 nm) against blank. Chlorophyll content was evaluated by Arnon (1949).

2.9 Determination of Tolerance Index (TI)

Tolerance index (TI) was expressed as the ratio between growth parameters (root/shoot length, root/shoot fresh and dry weight) of the plant species in contaminated soil in relation to the growth parameters of plants from non-polluted soil calculated using the following equation (Wilkins 1978):

$$\text{TI (\%)} = \frac{[\text{Growth parameter}]_{\text{Cu contaminated soil}}}{[\text{Growth parameter}]_{\text{Control soil}}} \times 100.$$

2.10 Quality Control and Quality Assurance

All the glassware utilized throughout the present investigation was comprised of high-quality Pyrex glass material which has great resistance to acid. The analytical grade reagents with a certified purity of 99% and metal standard stock solution (1000 ppm) for AAS analysis were secured from E. Merck (Germany). Working standards were prepared by proper dilutions of standard stock solutions with double-distilled water.

2.11 Plant Sample Preparation, Digestion and Cu Determination

To determine Cu accumulation in different plant tissues (i.e. root, stem, leaf and pod), harvested plant parts were rinsed thoroughly by means of tap water, then with deionized (DI) water to clean adhered components of soil and then oven-dried at 80 °C till steady weight. The oven-dried plant tissues were ground carefully using an electric grinder and passed through a 1.0-mm mesh strainer. The ground plant tissue samples were digested by HNO₃ along with HClO₄ mixed at a ratio of 3:1 (v/v) according to the protocols devised by Altaf et al. (2017). 0.5 g of plant sample was digested with 12 ml of 3:1 HNO₃/HClO₄ di-acid mixture on the hot plate. After cooling, the digested solution was filtered through Whatman's filter paper and finally the volume made up to mark 50 ml by adding deionized (DI) water.

The quantification of copper (Cu) in respective tissues was carried out by atomic absorption spectrophotometer (Perkin-Elmer, AAnalyst 800) provided with a copper cathode lamp, under optimum analytical conditions for the estimation of copper. The optimum conditions for AAS used throughout these studies are given in Table 2. The standard calibration method was adopted for the quantification of results and triplicate samples were run to insure the precision of quantitative results. The concentration of Cu as well as accumulation in plant root and shoot was calculated according to Monni et al. (2000):

$$\text{Cu Conc. (mg/kg)} = \frac{\text{AAS interpretation (reading)} \times \text{dilution factor}}{\text{dry wt. of plant tissues (root, stem, leaf, pod)}}$$

Table 2 Measurement conditions of F-AAS for copper (Cu) determination

Parameters	Values
Wave length (nm)	327.4
Hollow cathode lamp current (mA)	5.0
Flame type	Air–C ₂ H ₂
Background correction	On
Slit-width (nm)	1.0
Flame condition	Oxidizing
Expansion factor	1

Cu Acc. ($\mu\text{g}/\text{plant}$) = Cu conc. $\times$ dry wt. of plant tissues.

2.12 Soil Sample Preparation, Digestion and Cu Determination

Soil samples were air dried at room temperature, ground, mixed well, and kept in plastic (polyethylene) sealed lock bags used for subsequent metal analysis. Digestions of soil samples were done using aqua regia method. To quantify the Cu content in soil, sample of 1 g soil was digested by means of wet acid digestion method through HNO_3 along with HCl in proportion of 3:1 (v/v) and heated on a hot plate for 2 h at a temperature of 110 °C until the solution became clear. After cooling, the volume was completed to 50 mL by adding distilled water. The solution was filtered through Whatman's filter paper and consequently, examined for Cu contents with Atomic Absorption Spectrophotometer.

2.13 Evaluation of Phytoextraction Efficiency

To evaluate the phytoextraction potential of *S. indicum* and *C. tetragonoloba*, the following factors were calculated according to Rohan et al. 2013.

2.13.1 Bioconcentration Factor (BCF)

The bioconcentration factor was expressed as the ratio of Cu concentration in plant roots in relation to Cu concentration in soil medium, calculated as follows:

$$\text{Bioconcentration factor [BCF]} = \frac{[\text{Cu}]_{\text{root}}}{[\text{Cu}]_{\text{soil}}}.$$

2.13.2 Bioaccumulation Coefficient (BAC)

The bioaccumulation coefficient was determined as the ratio of Cu concentration in plant shoots to that of Cu concentration in soil medium, calculated as follows:

$$\text{Bioaccumulation coefficient [BAC]} = \frac{[\text{Cu}]_{\text{shoot}}}{[\text{Cu}]_{\text{soil}}}.$$

2.13.3 Translocation Factor (TF)

The translocation factor was measured as the ratio of Cu concentration in plant shoots in relation to Cu concentration in plant roots, calculated as follows:

$$\text{Translocation factor [TF]} = \frac{[\text{Cu}]_{\text{shoot}}}{[\text{Cu}]_{\text{root}}}.$$

2.14 Statistical Data Analysis

All conducted tests were carried out with three replicates and the data were statistically analyzed with PASW® Statistics 18 (SPSS Inc., Chicago, IL, USA). For the comparison of treatment means, analysis of variance (ANOVA) was performed and to observe the significance difference among treatment means Duncan's multiple range Post Hoc tests were applied at a significance level of $p < 0.05$.

3 Results and Discussion

3.1 Characterization of Tested Soil

The tested soil was sandy loam in texture with an average pH value of 6.89 ± 0.04 and electrical conductivity (EC) of $1662 \pm 11 \mu\text{S cm}^{-1}$. Organic carbon (OC) content of the soil was 2.20%, while organic matter (OM) of the tested soil was found to be 3.79%. Among soil properties, mobility and bioavailability of Cu strongly depend on pH of soil and organic matter (OM) contents present in soil (Bravin et al. 2012). Soil pH has direct affect on the solubility of heavy metals together in soil as well as soil solution. Adriano (2001) reported that, both the mobility and Cu bioavailability increased with decreased soil pH, while organic matter (OM) makes available a variety of organic chemical substances to the soil solution that carried out function as chelating agents (chelates) and enhances mobility and availability of metal to plants (McCauley et al. 2009; Fanrong et al. 2011). Therefore, in accordance with soil properties, Cu is more mobile and more bioavailable.

3.2 Cu-Induced Phytotoxic Effects

Copper is an essential micronutrient at low concentration; the maximum values for all tested growth and biochemical parameter (chlorophyll content) in two plant species were observed at 25 mg kg^{-1} , but gradual increase in Cu concentration significantly ($p < 0.05$) reduced these parameters. In the current investigation, the germination percentage of *S. indicum* and *C. tetragonoloba* seed was affected significantly ($p < 0.05$) at $300 \text{ mg Cu kg}^{-1}$ as compared to control (Table 3). The reduced germination percentages (20 and 75%) were recorded at $300 \text{ mg Cu kg}^{-1}$ in *S. indicum* and *C. tetragonoloba*, respectively. It has been well documented in the literature that germination is a fundamental process in the life a plant species to decide the impacts of Cu toxicity. According to Li et al. (2005) seed is the only stage in the whole plant life well protected against the heavy metal toxicity. The outermost layer of seed acts like a barrier that prevents the entrance of substantial heavy metal such as Cu inside the embryo from toxic soil environment

Tables 3 Phytotoxic effects of Cu on growth parameters of *Sesamum indicum* L. and *Cyamopsis tetragonoloba* L.

Plant species	Cu applied (mg kg ⁻¹)	Germination (%)	Root length (cm)	Shoot length (cm)	Root fresh weight (g plant ⁻¹)	Shoot fresh weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)
<i>S. indicum</i>	0	90.00 ^a ± 13.23	12.26 ^b ± 0.43	113.80 ^b ± 5.67	11.50 ^b ± 0.61	35.93 ^{ab} ± 4.32	7.32 ^b ± 0.67	19.67 ^b ± 2.11
	25	90.00 ^a ± 0.00	14.17 ^a ± 0.25	125.77 ^a ± 3.86	15.83 ^a ± 0.72	40.78 ^a ± 3.19	9.63 ^a ± 0.48	23.10 ^a ± 1.15
	50	90.00 ^a ± 5.00	11.26 ^c ± 0.93	93.96 ^c ± 1.30	10.10 ^c ± 0.18	31.13 ^{bc} ± 1.21	6.86 ^b ± 1.61	15.44 ^c ± 1.10
	100	90.00 ^a ± 5.00	9.30 ^d ± 0.38	87.67 ^d ± 1.39	8.25 ^d ± 0.45	27.27 ^{cd} ± 2.03	5.11 ^c ± 0.84	11.55 ^d ± 0.58
	150	70.00 ^b ± 18.03	8.94 ^d ± 0.26	78.40 ^e ± 2.33	7.33 ^d ± 0.66	25.59 ^{cd} ± 5.06	4.37 ^c ± 0.47	10.59 ^d ± 1.21
	200	25.00 ^c ± 10.00	8.68 ^d ± 0.13	77.69 ^e ± 1.36	6.83 ^{ef} ± 0.50	21.79 ^d ± 3.72	3.63 ^{cd} ± 0.57	7.99 ^e ± 1.53
	300	20.00 ^c ± 5.00	7.43 ^e ± 0.21	73.11 ^e ± 1.17	5.81 ^f ± 0.88	17.89 ^e ± 1.73	2.81 ^d ± 0.33	6.13 ^e ± 1.12
<i>C. tetragonoloba</i>	0	95.00 ^a ± 5.00	14.07 ^b ± 0.78	117.04 ^b ± 1.20	14.26 ^b ± 0.73	39.11 ^b ± 4.50	8.28 ^b ± 1.05	21.32 ^b ± 1.19
	25	95.00 ^a ± 0.00	20.17 ^a ± 1.16	135.13 ^a ± 4.49	16.17 ^a ± 0.67	44.26 ^a ± 2.56	11.03 ^a ± 0.91	25.17 ^a ± 2.10
	50	90.00 ^{ab} ± 5.00	14.05 ^b ± 1.08	111.86 ^b ± 0.36	10.45 ^c ± 0.67	34.18 ^c ± 1.03	10.55 ^a ± 0.77	16.66 ^c ± 1.14
	100	90.00 ^{ab} ± 0.00	13.25 ^b ± 1.00	102.27 ^c ± 6.90	9.07 ^d ± 0.85	29.26 ^d ± 3.73	7.16 ^{bc} ± 0.71	15.51 ^{cd} ± 1.14
	150	85.00 ^{bc} ± 5.00	11.03 ^c ± 1.42	96.29 ^{cd} ± 1.18	8.10 ^{de} ± 0.91	28.25 ^d ± 1.01	6.04 ^c ± 1.03	13.14 ^{de} ± 2.76
	200	80.00 ^{cd} ± 5.00	10.02 ^{cd} ± 0.96	91.78 ^d ± 2.89	7.05 ^{ef} ± 0.50	27.18 ^d ± 1.00	4.63 ^d ± 0.55	11.40 ^{ef} ± 1.16
	300	75.00 ^d ± 5.00	9.04 ^d ± 0.80	81.08 ^e ± 1.77	6.64 ^f ± 0.15	26.11 ^d ± 0.95	4.10 ^d ± 0.10	9.03 ^f ± 0.83

Similar letters in same column are statistically non-significant according to Duncan's Multiple Range Test ($p < 0.05$); data are means ($n = 3 \pm \text{SD}$)

^aSignificantly highest followed by later alphabets for lower means

and ensures the protection of embryo from Cu phytotoxicity. Sesame (*S. indicum*) and guar (*C. tetragonoloba*) seeds are able to germinate in the presence of low to moderate level of Cu concentrations in soil. The consideration of past studies reported by Ansari et al. (2013) on phytotoxicity of Cu in seed germination of various plants species signifies the variability of heavy metal tolerance and resistance within the same and among different plant species.

Root and shoot lengths (Seedling's height) are among the most important determinants of plant morphological (growth) parameters. In this study, the root and shoot lengths in terms of growth parameter were significantly ($p < 0.05$) affected under Cu stress (Table 3). The elevated Cu concentration has direct influence on plant morphology. In *S. indicum* and *C. tetragonoloba* the longest roots (14.17 and 20.17 cm) and shoots (125.77 and 135.13 cm) were found in lower treatment at 25 mg Cu kg⁻¹, respectively. At 300 mg Cu kg⁻¹ treatment, root length decreased by 7.43 and 9.04 cm while shoot length reduced by 73.11 and 81.08 cm in both *S. indicum* and *C. tetragonoloba*, respectively. Heavy metal stress is related to a common process of plant growth inhibition or with retarded growth. Elongation of plant root and shoot has demonstrated a notable sensitivity to over excess Cu present in soil. Barbosa et al. (2013) have reported that height of maize plant directly (linearly) reduced with higher Cu treatments. Increased level of Cu in the soil decreases root length which directly influences root growth and specific superficial area, decreasing the absorption capacity of water and nutrients. The shoot length was decreased consequently because of Cu hindrance with metabolic system of the plants which reduced mineral elements uptake and increased substantial amount of Cu inside the

growing shoot that results yellowing of leaf, i.e., chlorotic symptoms due to mineral nutrients deficiency and eventually leading to stunted plant growth (Muhammad et al. 2015).

Cu contamination showed significant ($p < 0.05$) affects on both fresh and dry weights (biomass) of *S. indicum* and *C. tetragonoloba* at higher concentration (Table 3). Cu toxicity at 300 mg kg⁻¹ decreased root fresh weight (5.81 and 6.64 g plant⁻¹) and shoot fresh weight (17.89 and 26.11 g plant⁻¹) in *S. indicum* and *C. tetragonoloba*, respectively. The dry biomass follows the same trend as fresh biomass. At higher concentration (300 mg kg⁻¹) Cu stress reduced root dry weight (2.81 and 4.10 g plant⁻¹) and shoot dry weight (6.13 and 9.03 g plant⁻¹) in *S. indicum* and *C. tetragonoloba*, respectively. Plant biomass is an excellent indicator for describing the growth and developmental changes of plants within the prospect of heavy metal toxicity. The reduction in plant biomass might be related with disturbed metabolic activities because of decreased take-up of fundamental mineral nutrients when developed under Cu toxicity (Muhammad et al. 2015). Moreover, plants species which can generate high shoot (above-ground) biomass and have the capacity to accumulate heavy metals could be utilized for phytoextraction purposes including exclusion of heavy metals from contaminated soil. Various examinations demonstrate the phytotoxic effects of increased levels of Cu on plant biomass (fresh and dry) cultivated in Cu contaminated soil. Our outcomes for Cu phytotoxicity were obvious from hindered growth and development and also reduced fresh and dry weights that are in consonance with a similar studies on maize seedlings under Cu stress (Dresler et al. 2014).

Chlorophyll contents decreased significantly ($p < 0.05$) with steady raise of Cu concentrations from 25 to 300 mg

Cu kg^{-1} (Fig. 1). In *S. indicum* and *C. tetragonoloba*, the maximum amount of chlorophyll contents were measured at 25 mg Cu kg^{-1} , while the lowest concentration of chlorophyll *a* (0.07 and 0.09 mg g^{-1} f.w.), chlorophyll *b* (0.08 and 0.06 mg g^{-1} f.w.) and total chlorophyll (0.15 mg g^{-1} f.w.) was at 300 mg Cu kg^{-1} , respectively. Cu concentration in excess amount showed distinctive phytotoxic symptoms in foliage (leaves) of various plants species. The reduction in photosynthetic pigments is likely because of chloroplast damage during development phase upon exposure of Cu in soil system (Ali et al. 2015). Kabata-Pendias and Pendias (2001) have presented a strong evidence regarding the chlorophyll biosynthesis reduction which might be linked with the destruction of photosynthetic organization at thylakoid level and also the hindrance of Cu with organized system of chlorophyll (Wodala et al. 2012).

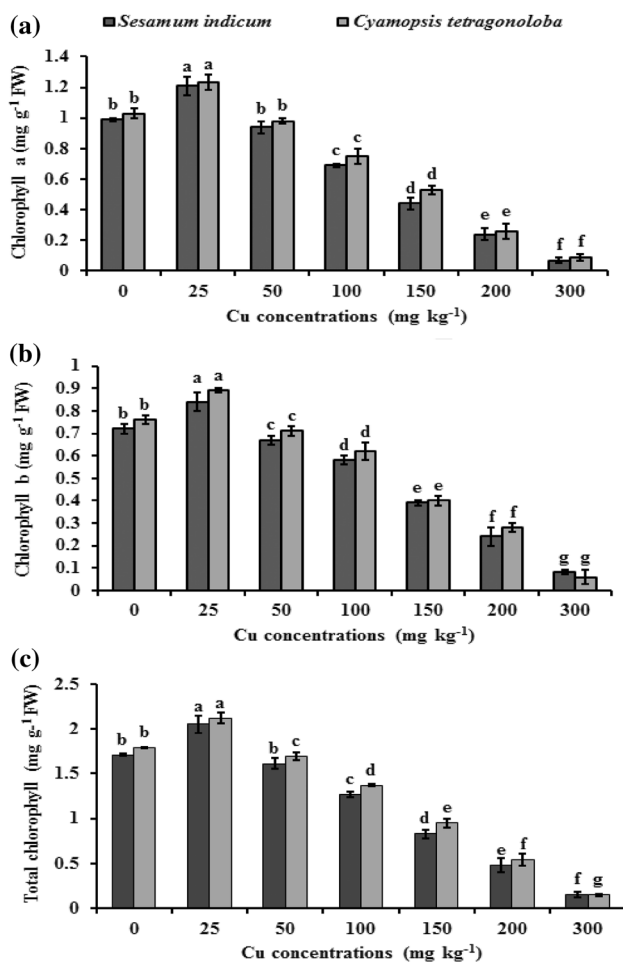


Fig. 1 Effect of Cu stress on photosynthetic pigments chlorophyll-*a* (a), chlorophyll-*b* (b) and total chlorophyll (*a* + *b*) (c), on *S. indicum* and *C. tetragonoloba* after 12-week growth in soil medium with varying concentrations of Cu. Similar letters are statistically non-significant according to Duncan's Multiple Range Test ($p < 0.05$); data are means ($n = 3 \pm \text{SD}$). Superscript a represents significantly highest followed by later alphabets for lower means

Tolerance indices (TIs) were also affected by Cu toxicity. Both plant species had different tolerance indices (TIs) under Cu stress (Table 4). In this study, *C. tetragonoloba* was more tolerant to Cu stress than *S. indicum*. At 300 mg kg^{-1} Cu treatment, *S. indicum* and *C. tetragonoloba* had the TIs for root lengths (60.66 and 64.49%) and shoot lengths (64.36 and 69.28%), root fresh weights (50.53 and 46.67%) and shoot fresh weights (50.17 and 67.22%), root dry weights (38.63 and 49.97%) and shoot dry weights (31.43 and 42.29%), respectively. Metal tolerance of plant is a fundamental requirement to find out the plant-metal interactions prior to exploit for phytoextraction purpose. As reported by Salt et al. (1998), a plant species utilized for phytoremediation must have high tolerance and metal accumulation capacity in their harvested biomass. Therefore, plant tolerance to substantial metal toxicity is evaluated in accordance with their root or potential shoot development limitations by the metal present in a medium (Ali et al. 2002). Growth and development hindrance might be a typical response of plant to heavy metal stress and is likewise a prominent factor amongst the most imperative agricultural indices for substantial heavy metal stress tolerance (Monni et al. 2000). According to Audet and Charest (2007), if TI values < 1 , this indicates that the plant experienced a stress owing to metal contamination with a net reduced in plant biomass. By contrast, TI > 1 suggested that plant species have developed tolerance with a net increase in biomass (hyper-accumulator). If TI values equal to 1, the plant is unaffected by metal pollution, indicating no difference relative to control treatments.

3.3 Cu Concentration in Plant Tissues

The Cu concentrations among the different plant tissues (root, stem leaf, and pod) of both plant species are presented in Table 5. In *S. indicum*, the maximum concentration of Cu accumulated in the root: 282.08 mg Cu kg^{-1} followed by leaf: 105.78 mg Cu kg^{-1} , stem: 65.30 mg Cu kg^{-1} , and pod: 8.13 mg Cu kg^{-1} at 300 mg Cu kg^{-1} treatment. However, in *C. tetragonoloba* Cu accumulated primarily in the root: 158.45 mg Cu kg^{-1} followed by stem: 154.73 mg Cu kg^{-1} , leaf: 152.32 mg Cu kg^{-1} , and pod: 8.13 mg Cu kg^{-1} at 300 mg Cu kg^{-1} treatments. The elevated Cu contents in the plant tissues (e.g. root, stem, leaf and pod) are noticeably associated with the increasing metal concentration in the soil environment. Studies have demonstrated the take-up of metals; their distribution and translocation to various plant parts and also the extent of tolerance is reliant on the metal, its bioavailability, the plants species and their metabolic systems (Rohan et al. 2013). The Cu accumulation capacity exceptionally differs among various plants species, based on their availabilities in the soil and also influenced by different soil conditions, as reviewed by Muhammad et al. (2015).

Table 4 Effect of Cu stress on the tolerance indices (TIs) of *Sesamum indicum* L. and *Cyamopsis tetragonoloba* L.

Plant species	Cu applied (mg kg ⁻¹)	Tolerance indices					
		Root length (%)	Shoot length (%)	Root fresh weight (%)	Shoot fresh weight (%)	Root dry weight (%)	Shoot dry weight (%)
<i>S. indicum</i>	25	115.60 ^a ± 2.00	110.73 ^a ± 7.15	137.76 ^a ± 5.92	114.73 ^a ± 18.19	131.99 ^a ± 7.95	118.65 ^a ± 16.99
	50	91.95 ^b ± 9.22	82.69 ^b ± 4.03	87.94 ^b ± 4.27	87.26 ^b ± 7.76	95.15 ^b ± 29.85	79.00 ^b ± 8.81
	100	75.99 ^c ± 5.74	77.17 ^{bc} ± 4.15	71.93 ^c ± 6.78	77.07 ^{bc} ± 14.85	70.67 ^{bc} ± 17.04	59.25 ^c ± 7.93
	150	73.05 ^c ± 4.67	69.01 ^{cd} ± 4.15	63.64 ^{de} ± 2.40	72.43 ^{bc} ± 18.89	59.91 ^{cd} ± 7.16	54.39 ^c ± 9.84
	200	70.91 ^c ± 3.53	68.41 ^{cd} ± 4.35	59.48 ^{ef} ± 4.89	61.53 ^{bc} ± 15.00	49.47 ^{cd} ± 3.78	41.28 ^{cd} ± 11.08
	300	60.66 ^d ± 3.09	64.36 ^d ± 3.50	50.53 ^f ± 7.86	50.17 ^c ± 6.39	38.63 ^d ± 6.03	31.43 ^d ± 7.05
<i>C. tetragonoloba</i>	25	143.83 ^a ± 15.27	115.45 ^a ± 3.03	113.44 ^a ± 1.46	113.98 ^a ± 12.70	133.97 ^a ± 9.91	118.64 ^a ± 16.51
	50	99.83 ^b ± 4.95	95.59 ^b ± 1.29	73.42 ^b ± 5.37	87.95 ^b ± 7.28	128.19 ^a ± 9.10	78.51 ^b ± 9.93
	100	94.05 ^{bc} ± 2.44	87.38 ^c ± 5.86	63.61 ^c ± 5.15	75.01 ^{bc} ± 8.27	88.11 ^b ± 19.89	72.98 ^{bc} ± 8.06
	150	78.15 ^{cd} ± 6.06	82.28 ^{cd} ± 1.86	56.84 ^c ± 5.71	72.98 ^{bc} ± 9.87	74.64 ^{bc} ± 22.03	61.79 ^{bcd} ± 13.59
	200	71.55 ^d ± 10.87	78.44 ^d ± 3.24	49.42 ^d ± 2.32	70.16 ^c ± 9.36	56.05 ^c ± 3.68	53.69 ^{cd} ± 7.57
	300	64.49 ^d ± 8.69	69.28 ^e ± 1.56	46.67 ^d ± 2.96	67.22 ^c ± 6.67	49.97 ^c ± 5.19	42.29 ^d ± 1.71

Similar letters in same column are statistically non-significant according to Duncan's Multiple Range Test ($p < 0.05$); data are means ($n = 3 \pm \text{SD}$)

^aSignificantly highest followed by later alphabets for lower means

Tables 5 Cu concentration, bioconcentration factor (BCF), bioaccumulation factor (BAC) and translocation factor (TF) of *Sesamum indicum* L. and *Cyamopsis tetragonoloba* L.

Plant species	Cu applied (mg kg ⁻¹)	Cu concentration				BCF	BAC	TF
		Root (mg kg ⁻¹)	Stem (mg kg ⁻¹)	Leaf (mg kg ⁻¹)	Pod (mg kg ⁻¹)			
<i>S. indicum</i>	25	33.17 ^f ± 2.80	11.09 ^f ± 1.24	9.40 ^f ± 0.94	2.20 ^f ± 0.10	1.33 ^a ± 0.11	0.91 ^a ± 0.09	0.69 ^a ± 0.12
	50	60.33 ^e ± 4.04	18.32 ^e ± 0.64	17.37 ^e ± 0.97	3.32 ^e ± 0.30	1.21 ^b ± 0.08	0.78 ^b ± 0.02	0.65 ^a ± 0.03
	100	114.40 ^d ± 5.71	31.30 ^d ± 1.47	36.22 ^d ± 6.99	4.35 ^d ± 0.87	1.14 ^{bc} ± 0.06	0.72 ^{bc} ± 0.06	0.63 ^a ± 0.07
	150	162.33 ^c ± 6.43	41.32 ^c ± 1.51	54.94 ^c ± 2.44	5.53 ^c ± 0.38	1.08 ^{bc} ± 0.04	0.68 ^c ± 0.01	0.63 ^a ± 0.02
	200	210.45 ^b ± 10.32	50.30 ^b ± 1.13	70.56 ^b ± 4.46	7.10 ^b ± 0.36	1.05 ^{cd} ± 0.05	0.64 ^{cd} ± 0.02	0.61 ^a ± 0.03
	300	282.08 ^a ± 3.88	65.30 ^a ± 0.01	105.78 ^a ± 4.87	8.13 ^a ± 0.06	0.94 ^d ± 0.01	0.60 ^d ± 0.01	0.64 ^a ± 0.00
<i>C. tetragonoloba</i>	25	21.43 ^f ± 1.89	18.23 ^e ± 0.95	12.69 ^e ± 1.28	0.91 ^f ± 0.20	0.86 ^a ± 0.08	1.27 ^a ± 0.08	1.49 ^c ± 0.09
	50	40.45 ^e ± 1.31	33.33 ^d ± 3.20	18.70 ^d ± 0.46	2.00 ^e ± 0.10	0.81 ^{ab} ± 0.03	1.08 ^b ± 0.07	1.34 ^c ± 0.12
	100	74.40 ^d ± 2.71	74.88 ^c ± 4.51	22.29 ^d ± 1.67	3.20 ^d ± 0.95	0.74 ^b ± 0.03	1.00 ^b ± 0.05	1.35 ^c ± 0.11
	150	91.32 ^c ± 6.14	74.98 ^c ± 4.27	74.21 ^c ± 3.51	4.17 ^c ± 0.06	0.61 ^c ± 0.04	1.02 ^b ± 0.02	1.69 ^b ± 0.14
	200	128.73 ^b ± 3.29	107.33 ^b ± 12.19	104.60 ^b ± 6.66	7.24 ^b ± 0.16	0.64 ^c ± 0.02	1.10 ^b ± 0.05	1.70 ^b ± 0.03
	300	158.45 ^a ± 2.68	154.73 ^a ± 10.77	152.32 ^a ± 2.45	8.13 ^a ± 0.06	0.53 ^d ± 0.01	1.05 ^b ± 0.03	1.99 ^a ± 0.02

Similar letters in same column are statistically non-significant according to Duncan's Multiple Range Test ($p < 0.05$); data are means ($n = 3 \pm \text{SD}$)

^aSignificantly highest followed by later alphabets for lower means

3.4 Cu Accumulation in Root and Shoot

Along with concentrations, the overall amount of metals accumulated in the above-ground biomass (i.e. shoot) is considered as the fundamental parameter to assess the plant's capability for phytoextraction (Hanen et al. 2010). Hence, for the evaluation of accumulation potential, it is important to consider plant biomass. Consequently, metal accumulation in plant biomass most probably relies upon both factors,

i.e. metal concentration and biomass, for accurate quantity measurements (Vymazal 2016). A considerable increase of Cu accumulation in root and shoot per plant varied with respect to Cu concentrations in soil for both plant species (Fig. 2). In this study, both *S. indicum* and *C. tetragonoloba* accumulated more Cu contents in shoot than root. Root accumulation of Cu in *S. indicum* and *C. tetragonoloba* was increased from 25 to 300 mg Cu kg⁻¹. The highest value for Cu accumulation in *S. indicum* and *C. tetragonoloba* root

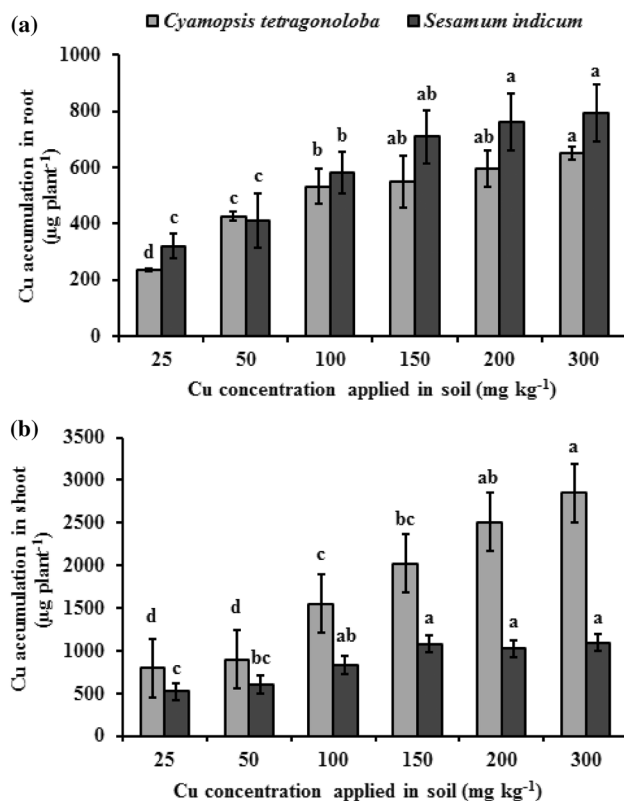


Fig. 2 Accumulation of Cu in root (a) and shoot (b) of *S. indicum* and *C. tetragonoloba* after 12-week growth in soil medium with varying concentrations of Cu. Similar letters are statistically non-significant according to Duncan's Multiple Range Test ($p < 0.05$). Data are means ($n = 3 \pm \text{SD}$). Superscript a represents significantly highest followed by later alphabets for lower means

(793.48 and 649.75 $\mu\text{g plant}^{-1}$) was found at 300 mg Cu kg⁻¹, respectively. Likewise, shoot accumulation of Cu in *S. indicum* and *C. tetragonoloba* was also increased from 25 to 300 mg Cu kg⁻¹. The highest value for Cu accumulation in *S. indicum* and *C. tetragonoloba* shoot (1096.98 and 2849.73 $\mu\text{g plant}^{-1}$) was also found at 300 mg Cu kg⁻¹, respectively.

3.5 Phytoextraction Potential

The plant species appropriate for phytoextraction or phytostabilization can be identified by elucidating the accumulation potential and translocation behaviors of heavy metals within plant and soil system. The Phytoextraction efficiency for *S. indicum* and *C. tetragonoloba* was quantified by evaluating the bioconcentration factor (BCF), bioaccumulation coefficient (BAC) and translocation factor (TF) values (Table 5). According to Fitz and Wenzel (2002), the suitable criteria for plants species used in phytoextraction of metal contaminated soil should have the bioconcentration factor, bioaccumulation coefficient and translocation factor values

higher than 1, under heavy metal stress, are considered as good phytoextractor whereas, those with bioconcentration factor and translocation factor values lower than 1 are not suitable candidate for phytoextraction. Following the criteria, plants species with bioconcentration factor (BCF) values > 1 and translocation factor (TF) values < 1 would likely be suitable for phytostabilization (Mendez and Maier 2008).

Between tested plant species, *S. indicum* had BCF values > 1 from 25 to 200 mg Cu kg⁻¹ and BAC and TF values < 1 at all treatments, indicating that *S. indicum* can be identified as phytostabilizer and utilized for phytostabilization of Cu-polluted soil. In contrast, *C. tetragonoloba* had the BCF values < 1 from 25 to 300 mg Cu kg⁻¹, while both bioaccumulation coefficient (BAC) and translocation factor (TF) values > 1 were found at all treatments, indicating that *C. tetragonoloba* could be a high-efficiency plant for Cu translocation from root to the shoot and used as a valuable tool for phytoextraction of Cu from soil.

4 Conclusions

The investigation concluded that no plant species were identified as metal hyperaccumulator. However, *C. tetragonoloba* had considerably higher Cu accumulation than *S. indicum* in light of its better growth and development, efficient tolerance and accumulation efficiency. Moreover, the noteworthy estimation of BCFs, BACs and TFs recommend that *C. tetragonoloba* is a prospective candidate for remediating Cu-polluted soil in quick and successive flushes than *S. indicum*. In addition, the two plant species have economic and ecological values. With the use of these plant species in the remediation of metal polluted soil, the immense positive attributes are that the cost is low in contrast with other physiochemical techniques, and can expel contaminations from soil and diminish their development towards groundwater, manage the soil properties and may enhance soil quality and profitability. Furthermore, after harvesting the metal accumulated biomass could be burned (incinerated) and reduced for metal recovery and would also be utilized as biofuel. Further study is required to understand the mechanisms of Cu absorption in plants.

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