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Copper Effects on Growth Parameters of Hollyhock (*Althaea rosea* L.)

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Abstract

Copper is an essential micronutrient for plant growth which is involved in many metabolic processes. However excessive amounts of copper may cause environmental pollution. With an increase in the contamination of urban areas with heavy metals, more attention should be paid to the role of ornamental plants in removing pollutants from the soils. The effects of heavy metals on the growth parameters of plants also should be determined. In this research the effects of four levels of Cu ($CuSO_4.5\ H_2O$) including 0, 20, 40 and 80mg Cu kg soil on growth parameters of hollyhock plants were investigated. Results showed that in treated plants root and shoot elongation, root dry weight and shoot fresh weight were not significantly differentiate from control. However, a significant decrease in Chl.a, Chl.b and total chlorophyll content was observed by increasing the cu level in the soil. Proline content in the leaf tissues reached to the highest values when plants were treated with 80 mg Cu kg soil. Electrolyte leakage of treated plant with 20 mg Cu kg soil was not significant differentiate from control. The concentration of Cu in the shoots and roots significantly increased with increasing the Cu level in the soil. Translocation factor at all Cu concentrations significantly decreased in compared to the control. Generally, results showed that A.rosea is a suitable alternative for phytoremediation of copper contaminated area.

Keywords: *Althaea rosea*, Copper, Growth parameter.

INTRODUCTION

Soil and irrigation water contamination with heavy metals is one of the most serious environmental problems to limit plant production and threat human health. The major sources of contamination in urban areas are industrial wastes, home applications which are disposed in correctly and municipal sewage sludge (Prabha *et al*., 2007;Qian *et al.*, 2005).

For plants, copper (Cu) is an essential micronutrient. It plays a structural and catalytic role as it is a component of several proteins and enzymes involved in electron transfer chain, oxidation and reduction reactions, charge accumulations and for phytosystem II activity(Ducic and Polle., 2005). Cu is normally present in plant tissues at 10 µg g^{-1} plant dry weight (Ducic and Polle., 2005). However, excessive amount of Cu affects nitrogen and protein metabolism, causes chlorosis of leaves, inhibits photosynthesis and disturbs mineral uptake (Wei *et al*., 2008). Cu interferes with the biosynthesis of photosynthetic machinery and decreases net photosynthetic rate (Qian *et al*., 2005). In the nature, Cu contamination usually results from human activities, such as mining, smelting, industrial waste disposal, sewage sludge application to agricultural soils, and the use of some types of fertilizers and pesticides (Ducic and Polle., 2005; Wei *et al*., 2008).

Up to now, many plants have been found as remediation plants, but there was little report about ornamental plants that can remedy contaminated soils. In fact, ornamental resources are very abundant, and they can indicate and monitor atmospheric pollutants. Especially for urban areas, ornamentals can beautify the environment and also resolve heavy metal pollution at the same time. Phytoremediation has received more attentions in recent years since it has been shown to be cost effective and more easy to apply than other conventional technologies for removing contaminates from the soils (Moteshare zadeh *et al*., 2008). Generally, phytoremediation is the use of green plants to remove contaminants from soils (Moteshare zadeh *et al*., 2008). There are many plants species currently used in phytoremediation, such as *Ammania baccifere* which can accumulate up to 1000 mg cu kg root dry weight(Mukhopadhyay and Maiti., 2010).

To our knowledge, there are few reports about ornamental plants that can absorb Cu from contaminated soils. The aim of this study was to identify the capability of *A. rosea* to remove Cu from contaminated soils by application of a concentration gradient of Cu, and also to evaluate the effects of different concentration levels of copper on growth parameters of *A. rosea*.

MATERIAL AND METHODS

An experiment was conducted in Shahid Bahonar University of Kerman Agricultural research station in 2010. Seeds of *A.rosaea* were sterilized in 0.5% (w/v) $NaClO_3$ for 5 min and rinsed four times in deionized water. In April 2010, soil samples were collected from the 0-30 cm depth of university landscape and were analyzed to determine the level of Cu. The samples without contamination were selected and sieved through a 4 mm sieve and filled into 1:2000 wagner pots. Copperic sulfate ($CuSO_4.5H_2O$) solution was added to the soil in each experimental pot at 20, 40 and 80 mg Cu kg soil. Soils were then completely mixed and rested to be completely equilibrated for 40 days. Seedlings of A.rosea all at similar growth stage were transplanted into the pots. Pots were then arranged in a completely randomized design with four replications. Number of seedlings per pots was reduced to 3 as one experimental plot and pots were replicated 3 times to minimize the experimental errors. Growing conditions was adjusted as follows: light intensity 150 µmol photon m^{-2} s^{-1}; maximum temperature 35℃, minimum temperature 15℃, photoperiod 16h/8h light/night and 60% relative humidity. Water lost by each pot was measured regularly by weighing out the pots every other days and sufficient amount of water applied to maintain soil water content at 85% of soil water holding capacity. Plants were harvested after 120 days.

Estimation of chlorophyll content

Chlorophyll content in the leaf tissues was determined according to the method described by Arnone (1949). Briefly 0.1 g of fresh leaf samples were extracted with 10 ml of ethanol and then absorbance of the extract was measured by spectrophotometer (SPUV-26) at 663 and 645 nm (Arnone, 1949).

Estimation of proline content

Proline content was determined by the method of Bates *et al*. (1973). Leaf samples were homogenized in 3% aqueous sulfosalicylic acid, and the homogenate was centrifuged (Universal 320R) at 10,000 rpm. The supernatant was used for estimation of the proline content. The absorbance was read by a Spectrophotometer (SPUV-26) at 520 nm (Bates *et al*., 1973).

Estimation of electrolyte leakage

One gram of tissue was cut to in 2cm segments, rinsed in deionized water for 24 h at 24℃. The electrical conductivity of the solution was determined using a conductivity meter (Winlab). Tubs were then air thighted and placed in boiling water bath for 20 min to disrupt the tissues and cells, and then cooled to 24℃. The electrical conductivity was again measured. Membrane ion leakage was calculated as the ratio of the conductivity after 12 h to the conductivity after boiling (Pang *et al*., 2003).

Estimation of copper content

Plants were rinsed, cut and separated into the shoots and roots. Each part was dried in an oven at 65℃ for 72 h. Samples dry weight were recorded. All dried parts were grounded using a morter and pistel , mixed thoroughly and digested with HCl (0.1 N). After filtration extracts were analyzed for copper content by an ICP set (AAS varian BV model) (Rothery, 1988).

Estimation of number and length of stomat

Leaf samples imprints prepared by nail polish and used to estimate of number and length stomata. Epidermis were viewed with a light microscope (40X Objective, 10X ocular,). Observations were made on an average of 10 fields. Measurements were made with a calibrated eyepiece micrometer. All data were then converted to µm.

Estimation of translocation factor

The ratio of metal concentrations in shoot to root is defined as translocation factor (TF) which refers to the ability of plant to translocate metals from the root to the shoot. (Roongtanakiat, 2009).

All data were subjected to the analysis of variance using one-way ANOVA model (SAS program version 9 for Windows) and tested at 1% level of significance.

RESULTS AND DISCUSSION

Morphological parameters

Values of stem length, stem fresh weight and root dry weight of plants treated with different levels of Cu were not significantly different from what were found in untreated ones. Even though the highest values of root fresh weight and shoot dry weight were found in plants treated with 20 mg Cu kg soil, these values were significantly different from those of untreated plants. Increasing levels of Cu in the soil significantly increased the stomatal number and length. However, root length significantly decreased in treated Cu compared to the control plants (Table 1).

It has been shown that root growth of Elsholtzia haichowensis had more sensitive to high Cu levels than shoot growth (Qian *et al*., 2005). Root elongation and biomass reduction were reported to be the most sensitive parameter in plants exposed to heavy metals (Wei *et al*., 2008). The inhibitory effect of Cu on root growth supposed to be due to reduction in cell division and retardation of normal root cell growth (Wei *et al*., 2008).

Chlorophyll content

Increasing the Cu levels in the soil significantly decreased chl.a, chl.b and total chlorophyll content of plant leaves. Lowest amount of photosynthetic pigments was found in plants grown in the soil containing 80 mg Cu kg soil (Table 2).

It has been shown that at lower Cu concentrations, the central Mg^{2+} ion of the chlorophyll is replaced by Cu^{2+}(Singh *et al*., 2010). The loss of photosynthetic pigment content is generally due to the direct peroxidative breakdown of pigments and chloroplast membrane lipids by the reactive oxygen species (Singh *et al*., 2010). Chlorophyll content in Chrysanthemum coronarium L. has shown to decrease with increasing the level of Cu, significantly (Wei *et al*., 2008).

Proline content

Highest level of proline content was found in plants treated with 80 mg Cu kg soil. The amount of Cu absorbed by plants treated with different levels of Cu was significantly higher than that of untreated ones (Table 2). Proline can play an important protective role against heavy metal stress. Proline has shown to improve Cu tolerance of chickpea genotypes effectively by controlling oxidative stress, an important cause of copper toxicity(Singh *et al*., 2010).

Electrolyte leakage

Ion leakage in plants treated with 20 mg Cu kg soil was not significantly different from untreated plants. However, increasing the level of Cu in the soil to 40 and 80 mg Cu kg soil, significantly increased ion leakage of the leaf tissues (Table 2).

The accumulation of Cu^{2+} ions may induce the formation of reactive oxygen species (ROS), H_2O_2 and HO and a subsequent decrease of antioxidants to avoid cell damages due to ROS accumulation. Additionally, Cu^{2+} ions can interact with S and N groups in cell proteins and cause an alteration of the ionic channels of the membrane, which promotes a higher flow of ions in the leaf cells (Bakor *et al*., 2007). Cu-induced stress has been shown to causes membrane damage in Azolla accession during the first hours after exposure (Sanchez- viveros *et al*., 2010).

Cu content in *A.rosea*

Increasing the concentration of the Cu in the soil, increasing the Cu absorbed by roots and shoot parts. The maximum concentration of Cu in the roots and shoot parts of plants were 152 and 85 ppm in experimental pots with 80 mg Cu kg^{-1} soil, respectively. Generally the amount of Cu was higher in the roots compared to the shoot parts (Fig. 1 and 2).

The level of Cu in the cell wall fractions in roots and shoots of *Chrysanthemum coronarium* L. also showed an increasing trend with the increase of Cu concentration in nutrient solution (Wei *et al*., 2008). It was suggested that Cu transportation over the tonolplast and final storing in the vacuole plays a roll in the detoxification process (Wei *et al*., 2008).

It is obvious that copper accumulation may also be affected by soil properties such soil pH and soil moisture and the changing environmental individual genotypic variability and varying degrees of soil contamination affected Cu accumulation in plants (Ariyakanon and Winaipanich., 2006).

Translocation factor

Translocation factor was found to be highest in untreated plants. Increasing the Cu level to 20, 40 and 80 mg^{-1}Cu kg soil significantly decreased the Cu translocation factor compared to the control plants. However, the difference among all treated plants were not significant (Table 2).

The heavy metal translocation ability of vetiver grass grown in industrial wastewaters varied depending on the characteristic of growth media and metal types (Roongtanakiat, 2009). This factor in amaranthus was much better than that in sunflower and amaranthus had more successful phytoremediation (Ducic and Polle., 2005).

CONCLUSION

The results from the present investigation show that this plant could not be classified as a Cu hyperaccumulator because the Cu concentration in the roots was greater than that in the shoots, it was tolerant to Cu because it grew well in soils with 80 mg kg^{-1} Cu. Thus *A. rosea* has great potential to be used for phytostabilization remediation of contaminated soils by Cu. What was more significant was *A. rosea* could remedy contaminated soils while beautifying the environment at the same time, especially in urban areas this has an important and practical significance.

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Tables

Table 1. Mean values of growth characteristics of hollyhock plants treated with different levels of copper concentrations.

Cu level (mg/kg)	Stomata length (µm)	stomata number	Root dry weight(g)	Shoot dry weight (g)	Root fresh weight (g)	Shoot fresh weight (g)	Root length (cm)	Shoot length(cm)
0	15.75d	11.75c	5.68a	6.1b	14.16b	13.12a	20.59a	20.29a
20	18.93c	14.5b	5.87a	7.24a	16.74a	13.56a	18.63ab	20.71a
40	22.12b	17.33a	5.91a	6.61ab	15.28ab	13.63a	15.34bc	21.83a
80	24.31a	18.6a	5.67a	6.31ab	14.58ab	13.09a	13.06c	19.25a

Differences between means which are followed by the same letter are not significantly different at 1% level of significance.

Table 2. Mean values of physiological characteristics of hollyhock plants treated with different levels of copper concentrations.

Cu level(mg/kg)	Translocation factor(Tf)	Proline (µm/l)	Electrolyt leakage (ds)	Total chlorophyll	Chlorophyll b (mg/g fresh weight)	Chlorophyll a (mg/g fresh weight)
0	0.85a	65.71c	26.63b	42.26a	19.04a	23.22a
20	0.59b	76.68c	26.85b	36.77b	15.4b	21.07ab
40	0.56b	119.97b	36.85a	35.13cb	14.37cb	20.76ab
80	0.55b	172.95a	37.16a	31.66c	11.92c	19.90b

Differences between means which are followed by the same letter are not significantly different at 1% level of significance.

Figures

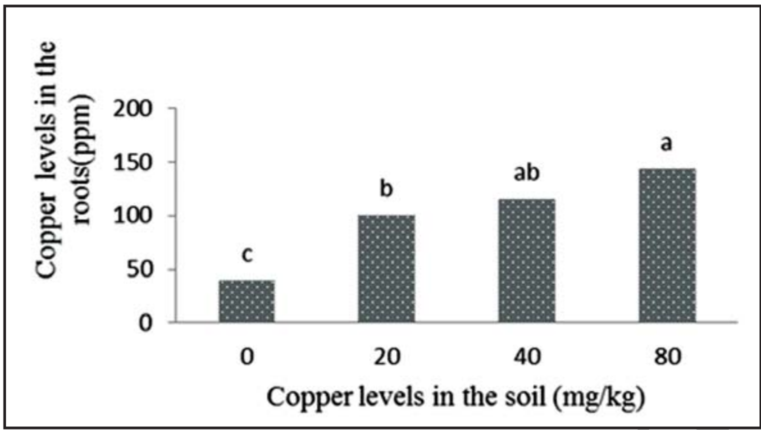

Fig.1. Effect of Cu concentration on the accumulation of Cu in the roots of *A. rosea*

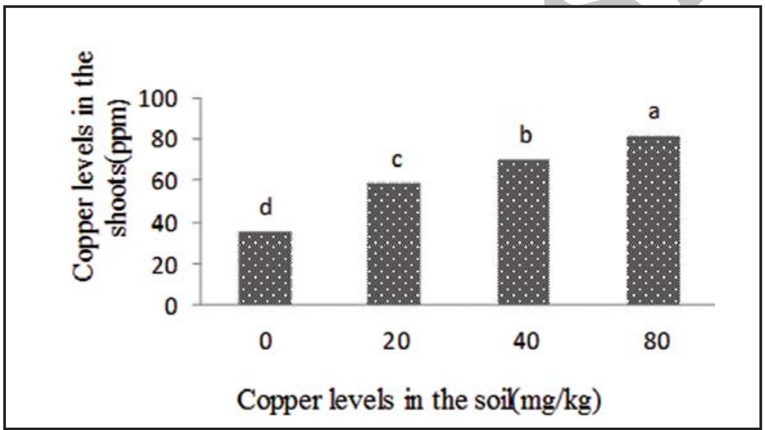

Fig.2. Effect of Cu concentration on the accumulation of Cu in the shoots of *A. rosea*