Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 2813-2815
Received: 11-09-2018
Accepted: 14-10-2018

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Chelating agent and chemical fertilizer effect on copper uptake by *Helianthus annuus*

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Abstract
Phytoremediation has shown great potential as an alternative treatment for the remediation of heavy metal contaminated soils. The objective of this research was to investigate the ability of chelating agent and chemical fertilizer for enhancing the phytoremeditaion of copper. Ethylenediamine tetraacetic acid (EDTA) and ammonium chloride (NH_4Cl) were applied to the soil at various dosages to elevate metal mobility. It was found that maximum copper extraction was observed by high dosage of chelating agent and chemical fertilizer alone with respect to control. The best treatment for maximum uptake of copper was noted in pots applied with combination of 9 mM EDTA/kg and 300 mg/kg of NH_4Cl with respect to chelating agent and chemical fertilizer alone and control. Chelating agent was much better for phytoextraction of copper than ammonium chloride but ammonium chloride in combination with chelating agent gave the better results.

Keywords: Copper, chelating agent (EDTA), chemical fertilizer (NH_4Cl), phytoextraction, sunflower

Introduction
Land application of biosolids, either as liquid sludge or composted material, often adds heavy metals into the soil. Soil pollution is one of the most vital environmental problem over the last decades (Raskin *et al.*, 1997) [15]. Heavy metal toxicity and its bioaccumulation in the food chain represent one of the major environmental and health problems of our society. Major sources of metals pollution are burning of fuels, mining and smelting of metallic ferrous ores, municipal sewage, fertilizers and pesticides. The most common pollutants of the environment are Copper, Chromium, Copper, Mercury, Lead, Nickel and Zinc. Cu in excess amounts caused chlorosis, reducing of root and shoot growth (Ait Ali *et al.*, 2002) [1]. Copper is usually much more bioavailable in soils, and thus particularly toxic to many plant species (Pahlsson, 1989) [12]. However, few studies have been performed with biodegradable chelates as the ligand to enhance the phytoextraction of Cu from contaminated soils (Kulli *et al*., 1999; Kayser *et al*., 2000) [9, 8]. The removal of heavy metals from contaminated soil is a major problem and requires new techniques to enhance the process. Biological processes are being used to decontaminate soils. Some plants are hyper accumulators that actively take up heavy metals and accumulate at in aerial parts with high concentration from soil (Brooks, 1998) [3], such plants can absorb and accumulate about 10,000 µg/g of Zn and 1,000 µg/g of Cu (Turgut *et al.*, 2005) [12]. Due to the advancement in technology, Plants are used as green technology or phytoremediation for soil amendments and agronomic practices to remove pollutants from the environment or to minimize its toxicity (Raskin and Ensley, 2000; Jing *et al.*, 2007) [14, 7].
Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for phytoremediation because it has a strong chelating ability for different metals and it also increases the bioavailability and plant uptake of the metals in the soil (Salt *et al*., 1998; Huang *et al*., 1997; Norvell, 1991) [16, 6, 11]. About 80% of the total soil metal is solubilized and becomes available for phytoextraction when EDTA is applied (Haag- Kerwer *et al*., 1999) [5]. Synthetic chelating agents such as EDTA allow plants which are not considered as hyper-accumulators to be usable for phytoremediation purposes, because chelates induce plants to take up more heavy metals than they normally accumulate (Liphadzi *et al*., 2003) [10]. Nitrogen fertilizers play a vital role in phytoextraction along with plant growth and enhance heavy metal stress tolerance and absorption (Boroujerdnia and Ansari, 2007) [2]. The aim of this research work is to study the effects of chelating agent and Chemical fertilizer either alone or in combination for phytoextraction of copper through *Helianthus annuus* plant.

Materials and methods
Soil preparation and Experimental procedure
The soil was collected from the agricultural field of College of Forestry, SHUATS, Allahabad, thoroughly mixed, dried and then sieved through 4 mm mesh sieve to remove the fiber and other non-soil particulates from the soil.

Plastic pots (cm height and cm diameter) were filled with 5 kg soil that had been previously sieved using 4 mm mesh sieve. The soil was artificially spiked with heavy metal like copper in the form of $CuSo_4.5H_2o$ at the rate of 150 mg/kg five days before sowing. After 5 days of heavy metal application, the seeds of sunflower were sown in each pot. After growing for 3-4 weeks, a dose of 0, 5, 7 and 9 mM / kg of chelating agent (EDTA) and 0, 100, 200 and 300 mg / kg of chemical fertilizer (NH_4Cl) respectively were applied to soil. Data on heavy metal concentration was collected after harvesting of crop. Different agronomic procedures were done during the entire experiment.

Plant analysis

The plants after harvesting were first washed with tap water, distilled water and finally by double distilled water to remove attached soil, separated into roots, stem and leaves, shredded and dried. The vegetation was dried first at room air temperature and then in an oven at 65^0C. Dried plant parts were then crushed and powdered separately. Powdered plant samples were then put separately in well washed dried and suitably labeled flasks. A di-acid mixture comprised of concentrated HNO_3 and $HClO_4$ was used. To one gram of plant material, 5 ml of concentrated HNO_3 was added and kept overnight. Then next day, 12 ml of di-acid mixture (conc. HNO_3 + $HClO_4$ in the ratio of 3:1) was added and digested on hot plate till white reddish brown fumes of perchloric acid comes out. Plant samples slowly begin to dissolve and digest in di-acid mixture. After few hours, plant samples dissolved completely in the digestion mixture and the solution was then evaporated until only about 2 ml was left in the flask. After cooling double distilled water was added and the samples were filtered through Whatman No. 42 filter paper. Digested samples were then transferred for heavy metal analysis using Atomic Absorption spectrophotometer.

Results and Discussion

Phyto-accumulation of Copper in different parts

The effect of different treatments on the accumulation of Cu in the roots and its translocation into the stem and leaf tissues were evaluated. The level of Cu content in the roots stems, and leaf tissues are presented in Table 1. Our results indicated that Chelating agent and Chemical fertilizer alone and in combination played different roles in the accumulation and translocation of Cu into different parts of the plant. The accumulation of Cu in roots, stem and leaves significantly increased with all treatments as compared to control.

The data revealed that maximum copper uptake 2.99, 2.51 and 1.25 ppm in case of chemical fertilizer alone was found in roots, stem and leaves respectively in F_3 (300 mg / kg NH_4Cl) and minimum 2.33, 1.87 and 0.96 ppm respectively were recorded in F_0 (0 mg / kg NH_4Cl). The data also suggested that in case of chelating agent alone, the maximum copper uptake 4.03, 3.43 and 1.92 ppm was recorded in C_3 (9 mM EDTA/kg) and minimum 1.25, 0.91, 0.34 ppm was recorded in C_0 (0 mM/kg of EDTA), but in case of interaction effect of both EDTA and NH_4Cl, it was observed that the maximum uptake of copper 4.31, 3.61 and 2.23 ppm was found in roots, stem and leaves respectively in T_{15} (9 mM / kg EDTA and 300 mg / kg NH_4Cl) and minimum 0.56, 0.30 and 0.19 ppm respectively were recorded in T_0 (no chelant and chemical fertilizer). The data clearly revealed that both increasing concentration of ammonium chloride and EDTA increased copper uptake and chelating agent was much better than ammonium chloride. From the study it is clear that the EDTA in combination with ammonium chloride boosted the copper uptake more than the EDTA and ammonium chloride did alone. The results indicated that the sunflower can tolerate heavy metal concentration and EDTA and NH_4Cl had potential to promote the uptake of copper. Combined effect of 9 mM EDTA / kg and 300 mg / kg NH_4Cl was found best but low dose of EDTA should be used because of their environmental risks. Our results were also in accordance with (Ebrahimi, 2013) [4] who found that Cu content in the roots of the plant increased when the dose of EDTA was 10 mmolkg^{-1}. Soil amended with N fertilizer resulted in the highest accumulation of Pb and Cu (Rahman *et al.*, 2013) [13]. Nitrogen fertilizer boosted growth parameters increasing root and shoot dry matter significantly, allowing a higher uptake of toxic metals by plants (Ebrahimi 2013) [4].

Table 1: Effect of Chelating agent and Chemical fertilizer on uptake of Copper by root, stem and leaves of Sunflower.

Treatment combination	Root	Stem	Leaves
T_0 (C_0F_0)	0.56	0.30	0.19
T_1 (C_0F_1)	1.29	0.92	0.31
T_2 (C_0F_2)	1.47	1.13	0.39
T_3 (C_0F_3)	1.69	1.29	0.48
T_4 (C_1F_0)	2.07	1.52	0.70
T_5 (C_1F_1)	2.23	1.75	0.73
T_6 (C_1F_2)	2.36	1.95	0.80
T_7 (C_1F_3)	2.52	2.14	0.83
T_8 (C_2F_0)	2.92	2.44	1.22
T_9 (C_2F_1)	3.11	2.63	1.28
T_{10} (C_2F_2)	3.25	2.81	1.34
T_{11} (C_2F_3)	3.44	3.00	1.46
T_{12} (C_3F_0)	3.76	3.21	1.72
T_{13} (C_3F_1)	3.98	3.39	1.81
T_{14} (C_3F_2)	4.08	3.49	1.92
T_{15} (C_3F_3)	4.31	3.61	2.23
CD (P=0.05)	0.030	0.034	0.036

C= chelating agent, F= chemical fertilizer

T_0 = 0 mM EDTA/kg + 0 mg/kg NH_4Cl, T_1 = 100 mg/kg NH_4Cl, T_2 = 200 mg/kg NH_4Cl, T_3 = 300 mg/kg NH_4Cl, T_4 = 5 mM EDTA/kg, T_5 = 5 mM EDTA/kg + 100 mg/kg NH_4Cl, T_6 = 5 mM EDTA/kg + 200 mg/kg NH_4Cl, T_7 = 5 mM EDTA/kg + 300 mg/kg NH_4Cl, T_8 = 7 mM EDTA/kg, T_9 = 7 mM EDTA/kg + 100 mg/kg NH_4Cl, T_{10} = 7 mM EDTA/kg + 200 mg/kg NH_4Cl, T_{11} = 7 mM EDTA/kg + 300 mg/kg NH_4Cl, T_{12} = 9 mM EDTA/kg, T_{13} = 9 mM EDTA/kg + 100 mg/kg NH_4Cl, T_{14} = 9 mM EDTA/kg + 200 mg/kg NH_4Cl, T_{15} = 9 mM EDTA/kg + 300 mg/kg NH_4Cl.

Conclusion

The study demonstrated that chelating agent and chemical fertilizer are efficient in increasing the concentrations of Cu in different parts of Sunflower grown in metal contaminated soil. Chelating agent was much more efficient than chemical fertilizer in enhancing concentration of Copper. Application of 9 mM / kg EDTA and 300 mg / kg NH_4Cl appeared to be the best treatment for phytoextraction of Cu from contaminated soil. From the study it is clear that chemical fertilizer should be used with chelating agent in order to have a good biomass and maximum uptake of heavy metal. Sunflower was the best plant to carry out the phytoextraction of Copper.

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