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PHYTOEXTRACTION OF COPPER BY *CICER ARIENTUM*

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ABSTRACT

Environmental pollution is a global problem that affects both the developing and developed countries. To an equal extent both human and natural processes contribute to environmental pollution. The contaminants are can be either organic or inorganic. The aim of this study was to find the remediation potential of *Cicer arientum* against different concentration of Copper in hydroponics culture at germination and postgermination stage and It was seen that the percentage of remediation after third week is around 78.63% which is approximately twice of 38.4 % that was observed in first week. Impact of toxicity was also observed in plant height and chlorophyll content.

KEYWORDS: ***Cicer arientum*, Phytoremediation, metal-accumulation, heavy metals, pollutants**.

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INTRODUCTION

Phytoremediation can be defined as the use of plants to remove pollutants present in soil such as metals, pesticides, explosives, antibiotics and oil or to reduce its toxicity.[1] Phytoremediation technique are developed due to need of innovative, economical and environmentally compatible approaches to remove heavy metals from the environment.[2] Apart from having the metal hyper accumulating properties, plants also cover the ground to shield people from direct contact with the soil and also prevents the blowing of contaminated dust away from the site to surrounding areas or deep underground.[3] However, they work best where contaminant levels are low because high concentrations may limit plant growth and take too long to clean up.[4] Phytoremediation also takes advantage of natural plant processes and requires less equipment and labor than other remediation methods since plants do most of the work. It is a cost effective and non-destructive in situ technology.[5] Metals can be recycled after plants have grown, by harvesting or incineration.[6] several crop growth cycles may be needed to decrease contaminant levels to allowable limits. If the plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of the ash is much smaller than the volume of contaminated soil if dug out and removed for treatment.[7] Phytoextraction is done with hyper accumulator plants which absorb unusually large amounts of metals in comparison to other plants.[8] Hyper-accumulating plants can contain more than 1,000 milligrams per kilogram of cobalt, copper, chromium, lead, or nickel; or 10,000 milligrams per kilogram (1 %) of manganese or zinc in dry matter.[9] One or more of these plant types are planted at a particular site based on the kinds of metals present and site conditions. *Copper* is essential substance to human life, but long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomach aches, dizziness, vomiting and diarrhea.[10] Moreover, uptake of more than 1.3 milligrams per liter of copper may cause liver and kidney damage and even death. Thus, this was conducted to determine phytoextraction potential of *Cicer arientum* seeds against of copper (in media) during germination.

MATERIALS AND METHODS

Plant selection

This is a very important step while planning a phytoremediation experiment as correct choice of plant is very important to achieve significant remediation. While choosing a plant things to keep in mind are: It should be able to tolerate the pollution level in soil; easy to grow in laboratory; easy to cultivate; fast growing. Keeping these points in mind, we have selected *Cicer arientum***.**

Preparation of Standard Curve of copper

1 mg Cu2+ /ml standard solution was prepared by dissolving 1 gram of CuSO4.5H2O to 1 liter of deionized water. OD was taken at the wavelength range of 500 - 700 nm using the spectrophotometer and peak was found to be at 608 nm. A standard curve was plotted using the calculated concentrations and their corresponding OD.

Chlorophyll estimation

The Chlorophyll content was estimated. 1gm of fresh leaf was homogenized with 80% (v/v) acetone. The extract was centrifuged at 3000rpm for 15 min and made upto 25ml with 80% (v/v) acetone. The supernatant was transferred to a cuvette and the optical density was measured at 645 and 663 nm in UV spectrophotometer. The chlorophyll (µg/ml) was estimated = (20.2XOD at 645nm) + (8.02XOD at 663nm).

Growth medium (Hydroponic solution)

*Cicer arientum***,** *s*eeds were grown *in-vitro* in Hoagland solution (pH 6.0) with different copper concentrations (50-500 ppm) till they germinate. Impact of toxicity was observed in germinated and post germinated seeds (7 and 21days respectively), and percentage remediation after 7days is 38.4% whereas after 21 days is around 78.63% using a UV spectrophotometer at 608 nm for copper respectively.[11]

Total Biomass

The plants were weighed for fresh weight immediately after taking out from media. Plants were dried in oven by setting at low heat (100°C) over night, and then cooled in a dry environment after that weighed for dry weight.

RESULTS

The effect of different Copper concentrations on *Cicer arientum* seeds was observed.

Remediation of Cu^{2+} using Cicer arientum seeds during post germination period of 7 days

Table1
Remediation of Cu^{2+} using C.arientum seeds during germination period of 7 days.

Cu Conc. (ppm) on 0 day	Conc. Observed (ppm) after first week	Amount remediated(ppm)	% Remediation
50	32.166	17.834	35.66
100	64.2	35.8	35.8
250	158	92	36.8
500	308	192	38.4

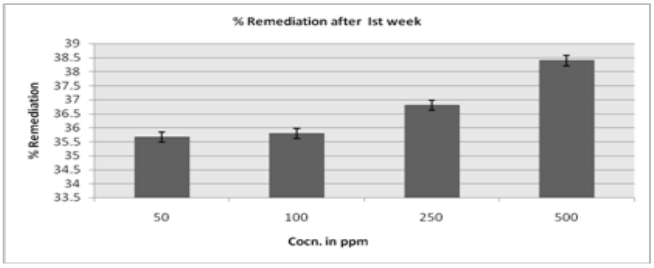

Figure 1
Remediation of Copper using Cicer arientum seeds during germination period

When Cicer arientum seeds were grown with lower concentrations of copper (50ppm to 500ppm), significant remediation was observed, But when the concentration was increased to 50, 100, 250 and 500 ppm. Cicer arientum seeds turned black and neither any remediation nor any growth was observed. The reason behind this observation is that such high concentrations of copper were toxic to Cicer arientum. Remediation rate was found to increase with an increase in copper concentration upto 38.4%.

Effect of Copper on Plant Length of Cicer arientum with various concentrations during germination period.

Table 2
Root and shoot length of Cicer arientum seeds grown in different copper concentrations during germination period of 7 days.

	SHOOT			ROOT		
Copper Conc. (ppm)	**Length (cm)**	**Dec. In length (cm)**	**%Dec.**	**Length (cm)**	**Dec. In length(cm)**	**%Dec.**
BLANK	9.42			7.07		
50 ppm	5.35	4.07	43.20	2.135	4.935	69
100 ppm	2.95	6.47	68.68	1.85	5.22	73.8
250 ppm	1.96	7.46	79.19	1.4	5.66	80.05
500 ppm	1.225	8.195	87	1.25	5.75	81.9

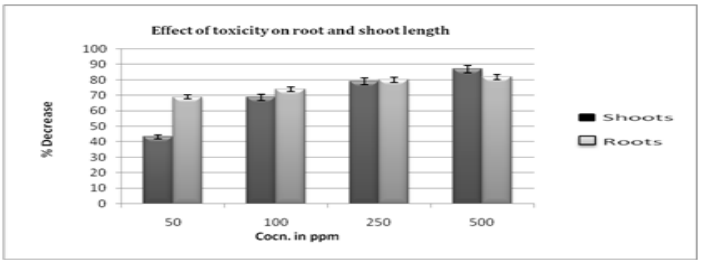

Figure 2
Root and shoot length of Cicer arientum grown in different copper concentrations during germination period of 7 days

According to shoot and root length data presented above, there is a considerable decrease in shoot length and root length after the concentration of 100 ppm which is around 68.68% and 73.8% respectively. This decrease was due to toxicity of copper. Although significant amount of toxicity was observed, in terms of decrease in root and shoot length, the percentage remediation was found to increase with the increase in concentration. Maximum toxicity was observed in root and shoot length at 500 ppm. High level treatments of Copper caused detrimental effect on shoot length and root length implying that higher concentration of copper was not conducive to vegetative growth. The reduction in the shoot length may be due to direct inhibition of cell division or cell elongation at higher Cu levels as well as retarded root growth and lesser nutrients and water transport to the shoot parts of the plant.[12] A decrease in shoot and root length of 87% and 81.9 % at the concentration of 500 ppm was observed.

Remediation of Cu^{2+} using Cicer arientum seeds during post germination period of 21 days.

Table 3
Remediation of Cu using C.arientum seeds during post germination period

Cu Conc. (ppm) on 0 day	Cu Conc. (ppm) on 21 day	Amount remediated (ppm)	% Remediation
50	12.185	37.81	75.63
100	37.88	62.12	62.12
250	93.13	156.87	62.74
500	267.85	232.15	46.43

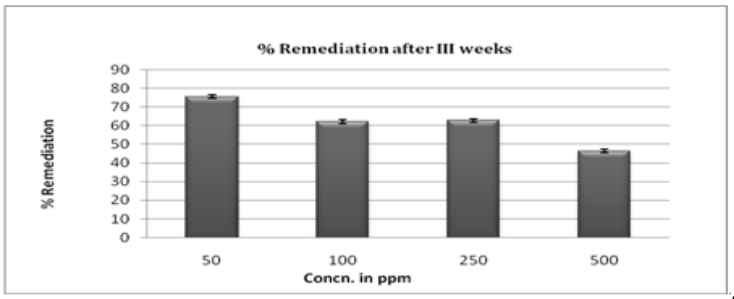

Figure 3
Remediation of Cu using Cicer arientum seeds during post germination

When Cicer arientum was treated with copper concentrations (i.e., 50,100,250 and 500), it was observed that there was a decrease in percentage remediation with an increase in copper concentration increased toxicity also increased. Percentage of remediation after 21 days is around 78.63% which is approximately twice of 38.4 %, observed in 7days.

Effect of Copper on Plant Length of Cicer arientum at various concentrations during post germination period of 21 days.

Table 4
Root and shoot length of Cicer arientum grown in different copper concentrations during post germination period

	SHOOT			ROOT		
Copper Conc. in ppm	**Length (cm)**	**Dec. In length (cm)**	**% Dec.**	**Length (cm)**	**Dec. In length (cm)**	**% Dec.**
BLANK	1.4			4.5		
50	1	0.4	28.57	2.3	2.2	48.88
100	1	0.4	28.57	2	2.5	55.55
250	0.7	0.7	50	1.1	3.4	75.55
500	0.4	1	71.42	0.8	3.7	82.22

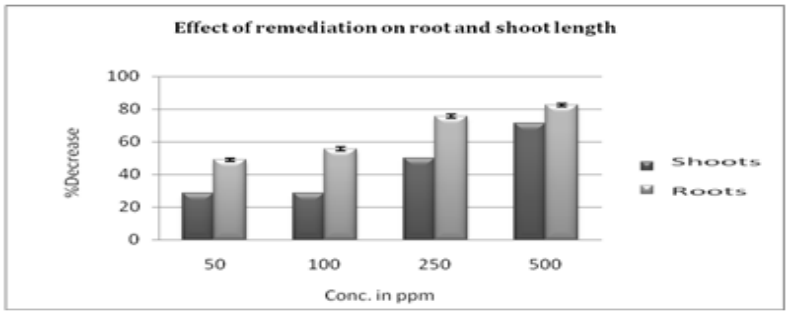

Figure 4
Root and shoot length of Cicer arientum grown in different copper concentrations during post germination period.

The results clearly indicate that, all the growth parameters were suppressed gradually with the increase in concentration of Copper. A marked inhibition in plant length became evident at 500 ppm concentration of Cu. Application of Copper at 50, 100, 250 and 500 ppm suppressed the plant length as compared to control plants. There was a significant (P<0.01) reduction in plant length compared to control plants. Decrease in percentage remediation is observed with an increase in concentration, this is caused due to toxicity of copper. Maximum remediation is observed at 50 ppm and minimum remediation at 500 ppm. But the amount of remediation in post germinated seeds is much higher as compared to 7 days old seeds. High level of toxicity is evident in the readings presented above as there is percentage decrease in shoot and root length of 71.42 % and 82.22% at the concentration of 500 ppm.

Effect of Copper on Total Chlorophyll content of Cicer arientum at various concentrations:

Table 5
Total Chlorophyll content of Cicer arientum at various concentrations

Copper Conc. (ppm)	Chlorophyll (mg/ml) on Ist week	Chlorophyll (mg/ml) on IIIrd week
Control	12.334	15.784
50	10.624	3.816
100	10.518	3.712
250	10.423	3.527
500	10.324	2.165

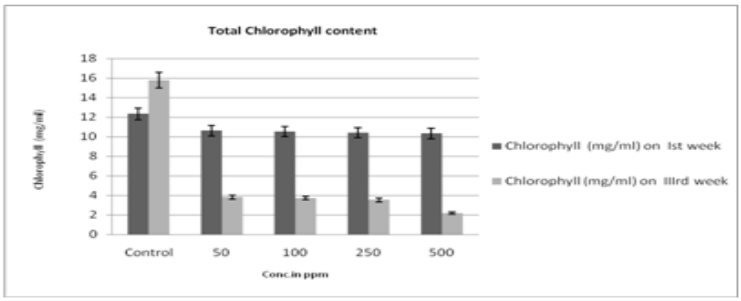

Figure 5

Total Chlorophyll content of Cicer arientum at various concentrations

As copper exposure increased from I week to III week the total chlorophyll content in leaves of of Cicer arientum showed decreasing trend at various concentrations compared to controls plants. At 50 ppm the percentage of total chlorophyll content varied from 10.624 to 3.816, at 100 ppm from 10.518 to 3.712 at 250 ppm from 10.423 to 3..527 and the total chlorophyll content at 500 ppm was varied from 10.324 to 2.165 within time interval of three weeks. In the present study, the significant decrease in chlorophyll content may be due to reduced chlorophyll biosynthesis by inhibiting electron transport during Calvin cycle which may cause oxidative stress in plants.[13]

Effect of Copper on Biomass of Cicer arientum at various concentrations.

Table 6

Effect of Copper on Biomass of Cicer arientum at various concentrations

Cu Conc. (ppm)	Fresh weight	Dry weight
Control	1.567	2.456
50	0.9231	0.6031
100	1.6195	1.4395
250	0.4064	0.3164
500	0.2085	0.1808

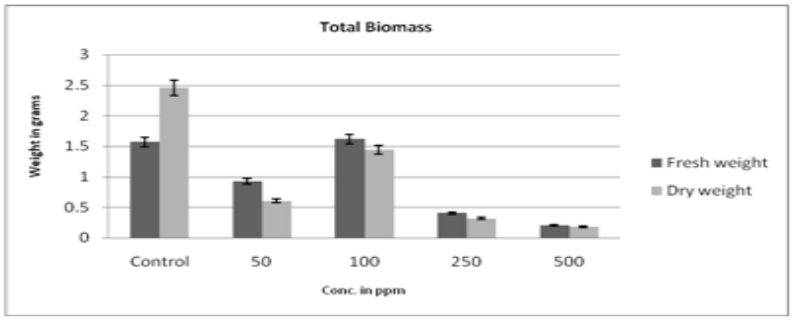

Figure 6

Effect of Copper on Biomass of Cicer arientum at various concentrations

Copper had inhibitory effects on plant growth and reduced the dry biomass, indicating phytotoxic effect[14]. Even the plants exposed to the 500 ppm, copper survived and no visual Cu toxicity symptoms such as necrosis and chlorosis were observed in these plants. For the biomass, the dry weight declined with the increase of Cu2+ dosage. From the above data we can get inference that the total biomass decreases as concentration of copper increased with respect to control plant in all the selected plant. In Cicer arientum total biomass decreased from 2.456gms (control) to 0.1808 gms (at 500 ppm Cu) The variation of the dry weight of tissue biomass for plants exposed to Cu treatments shows that Cu depressed biomass production in all four concentration of Cu (50,100,250 and 500ppm), compared with the control that shows the enhanced biomasses in all the plants.

DISCUSSION

There is a considerable decrease in shoot and root length after the concentration of 100 ppm which is around 68.68% and 73.8% respectively, which indicates the impact of copper toxicity on *Cicer arientum.* A decrease in the lengths of root and shoot of the germinating seeds was observed which indicates the remediating properties of the germinated plant**.** Percentage of remediation after three weeks is around 78.63% which is approximately twice of 38.4 % observed in 7days. The decrease in biomass(dry weight in grams) of *Cicer arientum* are in according to those recorded in the root biomass of *Brassica Juncea* which significantly decreased with increasing Zn, Cd, Cu and Pb contamination[15]. Reduction of biomass of plants was also reported in many plants maize wheat [16] when exposed to Zn and Cu stress. Reduction in dry weight could be due to their interference with metabolic process that is associated with normal development[17.] Copper

lowered chlorophyll content in plant which could be due to inhibition of chlorophyll synthesis and stimulation of chlorophyll degrading chlorophyllase activity by copper toxicity[18]. Copper accumulation in plants can reduce growth, induce chlorosis in young leaves, reduce pigment content[19], alter enzymatic functions, damage root cells and cause ultrastructure modifications of chloroplasts and cell studied impact of copper in the environment and its phytotoxicity effect [20].

CONCLUSION

The present study on phytoremediation potential of *Cicer arientum* indicated that it is a potential candidate. *Cicer arientum* is a fast growing plant and has the ability to tolerate high Cr (500 ppm) concentrations and can remediate high amounts of copper (78.3%).The knowledge on the phytoremediation potential of Cicer arientum may help us to adopt different strategies of purification and improvement of the environment through use of plants which tolerate and accumulate high levels of heavy metal.

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CONFLICT OF INTEREST

Conflict of interest declared None.

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