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In-vitro Phytotoxic effect of Chromium and EDTA on seed germination of *Sesbania grandiflora* L.

S Revathi and V Subhashree*

School of Biosciences and Technology, VIT University, Vellore- 632014, Tamil Nadu, India.

ABSTRACT

The phytotoxic effect of five different concentrations of chromium (Cr) from 20-100mg with and without the presence of Ethylenediaminetetraacetic acid (EDTA) was tested on the seed germination of *Sesbania grandiflora* a plant belonging to the family Leguminoseae. Three replicates were maintained for each concentration. The control seeds were treated separately with distilled water and were free from any treatment or the metal. A comparison was made between the varying concentrations of Cr without EDTA and varying concentrations of EDTA from 0.1-0.5mM with differing concentrations of Cr and 0.35mM of EDTA with different concentration of Cr. The parameters like germination percentage, Metal Tolerance Index, Germination Index and Percentage phytotoxicity was calculated for every 24 hrs for 4 days. The results obtained from this treatment shows that with increase in concentration of chromium there was decrease in growth and in the presence of 0.35mM of EDTA, thus showing a positive effect on the root elongation and growth of the plant. Thus EDTA even at very low concentrations is effective in phytoextraction of Chromium.

Keywords: Chromium, Phytotoxicity, EDTA, Germination, Phytoremediation.

Corresponding author

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INTRODUCTION

Phytoremediation is a technique that uses plants for removal of hazardous metal contaminants through the process of physical, chemical and biological process from the polluted soil [1]. It is an attractive, environmental friendly and cost-effective method, which is a promising alternative to conventional methods [2, 3]. This method of phytoextraction reduce the heavy metal level below regulatory limits within a reasonable time frame which is achieved by genetic and physiological capacity [4] of the plant to accumulate, translocate and resist high levels of heavy metals and produce high amount of biomass [5-7].

Plants are called hyperaccumulators, when they can accumulate more than 0.1% Pb,Co or Cr and more than 1% Mn,Ni or Zi in plant shoots when grown in their natural habits [8-10].The genus *Sesbania* contains about 70 widespread tropical and subtropical species, including annuals and perennials, herbaceous shrubs and trees. The genus has gained importance due to its fast growth, high yield, flooding tolerance, root and stem nodulation [11] and high Nitrogen fixation [12].

Chromium (Cr) is considered as a serious environmental pollutant due to its wide industrial applications, through the process of inadequate storage and poor disposal procedures of steel, alloys, cast iron, chrome plating, dye and pigments, textiles, leather tanning and wood preserving. Cr exists in two stable forms as trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) species. The trivalent is less toxic and is an essential element of a balanced human and animal diet but high concentrations of Cr^{6+} is hazardous and are toxic to living being, that causes carcinogenic and mutagenic properties [13,14].

The uptake and translocation of such heavy metal can be made bioavailable to the plant by the addition of natural and/or synthetic chelator [15-17]. Among various synthetic chelators, Ethylenediaminetetraacetic acid (EDTA) has been used and tested more intensively [18,19] since they increase the metal translocation from root to shoots [20], due to its strong complex forming ability. Its high efficiency relies on the solubilization of poorly available metals in soils (e.g. lead, chromium, copper), followed by a large passive accumulation of metal complexes in plant shoots through the transpiration stream [21, 22].

The seeds are intended to observe the environmental conditions closely. Seed germination is considered as the first physiological process to be affected by Cr and the germination inhibition appears to be the first defense mechanism that a seed exhibits when environmental conditions are adverse [23]. Hence the ability of the seed to germinate in a medium containing Cr is considered [24] to be the main factor limiting plant growth in heavy metal phytotoxicity [25, 26].

The objective of this phytotoxicity study is to test the effect of heavy metal chromium (Cr) with and without a chelator EDTA on the seed germination of *Sesbania grandiflora* .

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MATERIALS AND METHODS

Seeds of *Sesbania grandiflora* were obtained from an agricultural company at Coimbatore. The chemicals were obtained from HiMedia Limited, Mumbai. The 1 Molar stock solution of Chromium (prepared from Potassium dichromate $K_2Cr_2O_7$), and EDTA was prepared. Three treatments of five different working concentrations of Cr containing 20, 40, 60, 80 and 100 mg of Cr without EDTA (Treatment I). Cr with EDTA concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5mM of EDTA respective to Cr concentration (Treatment II) and 0.35mM EDTA (Treatment III) was kept constant for all the five different concentrations of Cr. Seeds were surface sterilized in 3% (v/v) formaldehyde for five minutes to avoid fungal contamination and then washed in running distilled water (DW) and placed on Petri dishes (10cm diameter) lined with double layer of filter paper (Whatman No.1) wetted with 5ml of different concentrations of Cr. The concentration of chelator EDTA ranged for various treatments. Triplicates were maintained for each concentration and Control was free of the metal and the chelator used. Each Petri plate received a number of 20 seeds and the plates were kept at room temperature for 4 days (ie 96hrs) for determining the germination percentage each day (ie for every 24hrs). The seeds were considered germinated once the radicle emerged about 2 mm out from the seeds. The radicle length of germinated seeds, however were measured after 48hrs of germination.

Using the formula given by Turner and Marshal [27] the Metal Tolerance Index of the seed was calculated for every 24hrs.

$$\text{Metal Tolerance Index(MTI)} = \frac{\text{Radicle Length of Seed in Test}}{\text{Radicle Length of Seed in Control}} \times 100$$

Germination Index was calculated using the formula of IRSA [28] in which the seed germination and root elongation of the test plant was compared with that of the control.
To facilitate the comparison between different tests, the GI is expressed here as a percentage in comparison to control (100%).

$$\text{Germination Index(GI)} = \frac{\text{Germination Percentage of Sample x Radicle Length of Sample}}{\text{Germination Percentage of Control x Radicle Length of Control}}$$

The Percent Phytotoxicity for the treatments was calculated by using the formula of Chou and Lin [29]; Ray and Banerjee [30].

$$\text{Percentage Phytotoxicity} = \frac{\text{Radicle Length of Control - Radicle Length of Test}}{\text{Radicle Length of Control}} \times 100$$

The data observed in the experiment were statistically analyzed for the calculation of mean, standard deviation and standard error (S.E.) and least significant difference (LSD) test were performed to determine the statistical significance of the differences between means of treatments.

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RESULTS AND DISCUSSION

The germination of *Sesbania grandiflora* seeds started after 24hrs. The results of the Germination percentage, Radicle length, Metal Tolerance Index, Germination Index and Percentage phytotoxicity at varying concentrations of Cr and EDTA are presented in the tables 1-3. When compared with the control, the treatments with different concentration of chromium with and without the presence of EDTA showed a toxic effect on seed germination and radicle length over time. Fig 1-4 summarize the results of the effect of metal Cr and Cr in combination with different concentration of the chelator on seed Germination, Radicle length, Metal Tolerance Index , Germination Index and Percentage phytotoxicity of *Sesbania grandiflora*. The results presented here are the average of triplicates and the SD were calculated with ±SE value. Statistically, the results showed that the heavy metal chromium with increasing concentrations significantly affected the radicle length with increase in time. Similarly the reports of Zayed and Terry [31], states that high levels of Cr^{2+} supply can inhibit seed germination and subsequent seedling growth.

Effect of metal and chelator on germination percentage:

The germination percentage was found to be reduced at higher concentrations of chromium with and without EDTA Fig1 and the intensity of inhibition of seed germination increased as the concentration of Cr increased. The Control which is free of metal or any treatment showed 100% germination. Similarly there was 100% germination for lower concentrations of Cr at 20 and 40 mg of Cr and for 20 and 40mg of Cr with 0.1 and 0.2mM EDTA concentrations respectively. The higher concentrations of 60, 80 and 100mg of Cr had the percentage value of 97.5%, 92.5% and 92.5% respectively. Similar results were obtained for Cr in combination with (0.3, 0.4 and 0.5mM) EDTA. The treatment having varying concentration of Cr (20-100mg) with constant EDTA concentration (0.35mM) showed a germination percentage value from 97.5- 85% (Table1-3).

Table 1: Effect of chromium on germination and growth of *Sesbania grandiflora*

Concentration of Cr (in mg)	Germination Percentage	Metal Tolerance Index	Germination Index	Radicle length	Percentage phytotoxicity
Control	100±2.27	-	-	2.98±0.12	-
20	100±2.27	98.32±1.68	0.98±0.03	2.93±0.13	1.67±1.52
40	100±2.27	97.48±2.02	0.97±0.02	2.90±0.13	2.51±1.48
60	97.5±2.5	95.73±0.47	0.93±0.04	2.85±0.17	4.26±1.5
80	92.5±1.4	94.76±1.24	0.87±0.03	2.82±0.16	5.22±1.6
100	92.5±1.4	89.76±1.8	0.83±0.02	2.67±0.16*	10.2±1.58

Statistical significance: **= significant at both the levels ie.P≤0.05 and P≤0.01, *=significant at one level ie.P≤0.05

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Table 2: Effect of different concentration of chromium and different concentration of EDTA on germination and growth of *Sesbania grandiflora*

Concentration of Cr (in mg) + EDTA	Germination Percentage	Metal Tolerance Index	Germination Index	Radicle length	Percentage phytotoxicity
Control	100±2.27	-	-	2.98±0.12	-
20+0.1	100±2.27	90.16±1.84	0.90±0.04	2.68±0.14*	9.83±2.46
40+0.2	100±2.27	88.99±2.9	0.88±0.03	2.65±0.13*	11.0±2.5
60+0.3	97.5±2.5	85.00±3.0	0.82±0.03	2.53±0.15**	15.0±2.5
80+0.4	92.5±1.4	81.07±2.8	0.74±0.02	2.41±0.15**	18.9±2.3
100+0.5	92.5±1.4	77.24±2.65	0.71±0.04	2.30±0.16**	22.7±2.35

Statistical significance: **= significant at both the levels ie.P≤0.05 and P≤0.01 *=significant at one level ie.P≤0.05

Table 3: Effect of different concentration of chromium with constant concentration of 0.35mM EDTA on germination and growth of *Sesbania grandiflora*

Concentration of Cr (in mg) + EDTA	Germination Percentage	Metal Tolerance Index	Germination Index	Radicle length	Percentage phytotoxicity
Control	100±2.27	-	-	2.98±0.12	-
20+0.35	97.5±2.5	86.04±0.96	0.83±0.02	2.56±0.13**	13.9±0.95
40+0.35	92.5±1.4	85.23±0.27	0.78±0.02	2.54±0.15**	14.7±1.06
60+0.35	92.5±1.4	82.51±1.49	0.76±0.04	2.45±0.16**	17.4±1.02
80+0.35	85±0.1	81.61±1.3	0.69±0.03	2.43±0.18**	18.3±1.02
100+0.35	85±0.1	81.40±1.5	0.69±0.03	2.42±0.19**	18.5±1.01

Statistical significance: **= significant at both the levels ie.P≤0.05 and P≤0.01

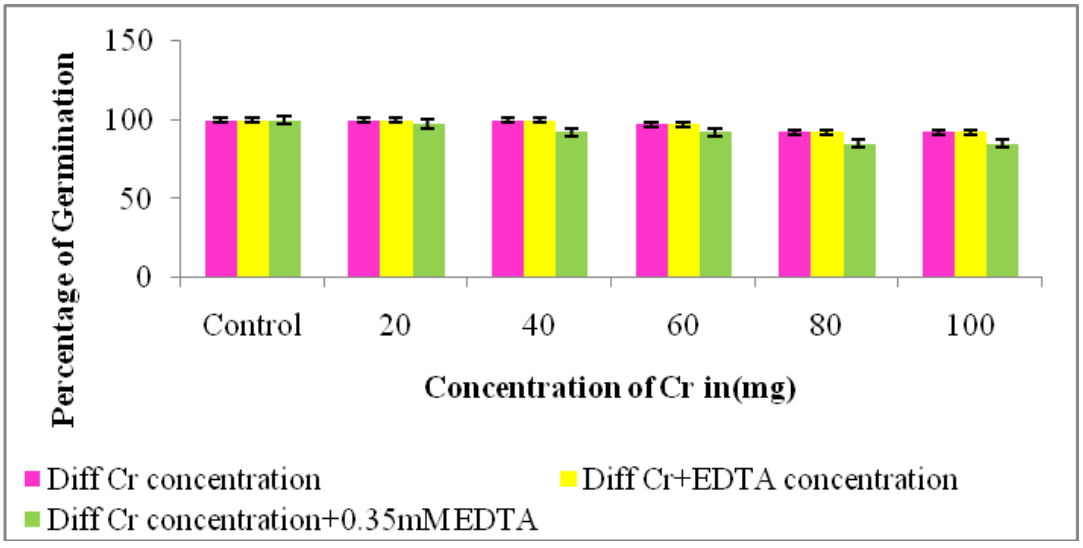

Fig 1: Effect of different concentration of Chromium with varying concentration of Chelator EDTA on Germination percentage of *Sesbania grandifora*

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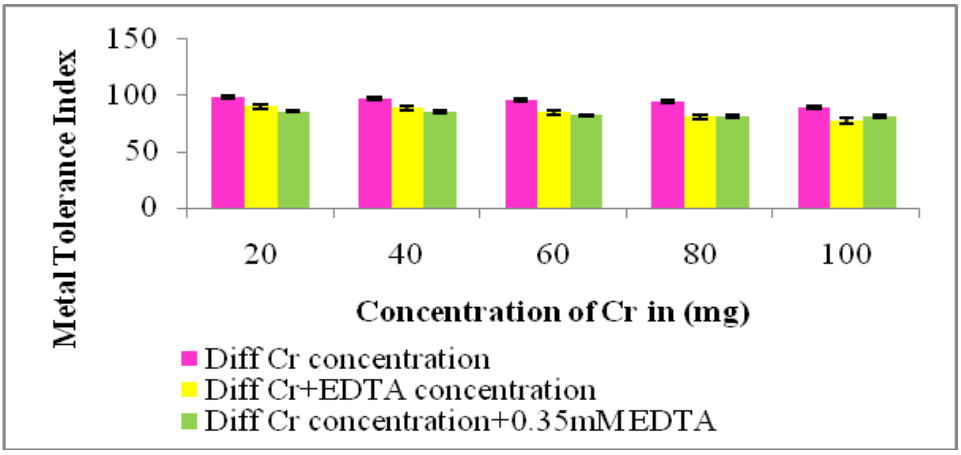

Fig 2: Effect of different concentration of Chromium with varying concentration of Chelator EDTA on Metal Tolerance Index of *Sesbania grandifora*

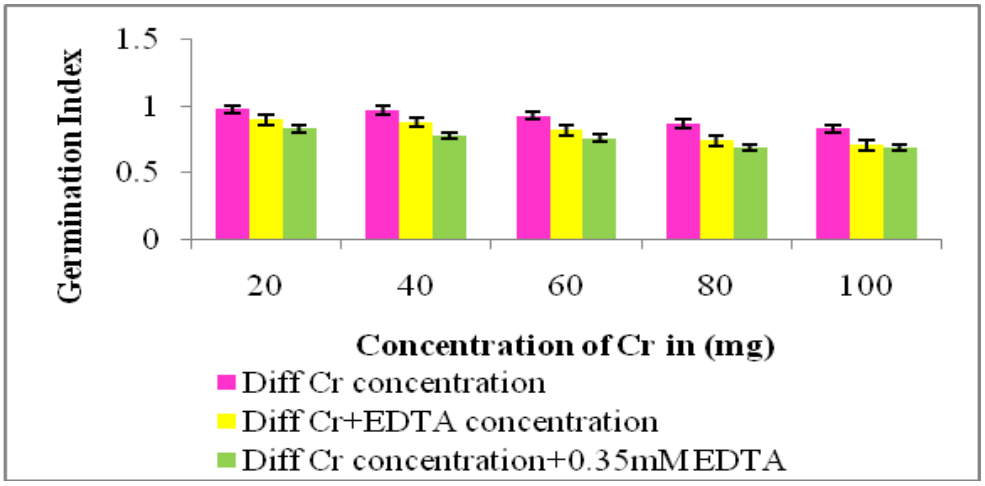

Fig 3: Effect of different concentration of Chromium with varying concentration of Chelator EDTA on Germination Index of *Sesbania grandifora*

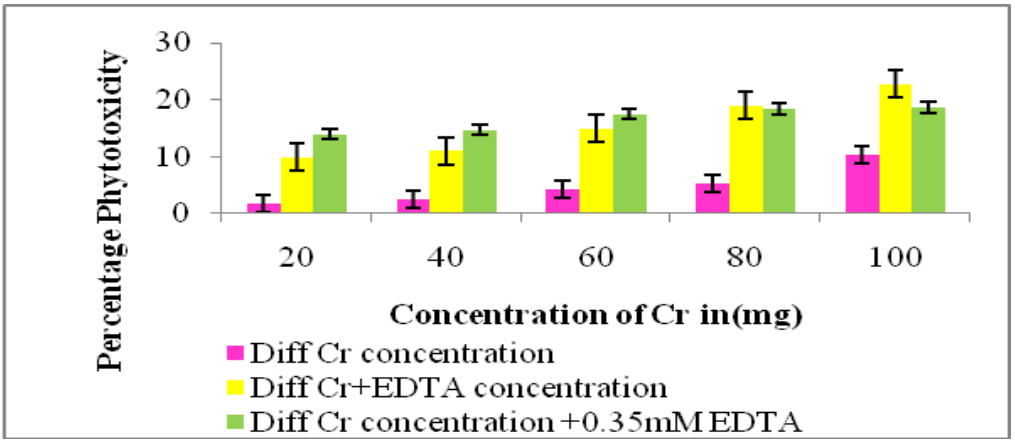

Fig 4: Effect of different concentration of Chromium with varying concentration of Chelator EDTA on Phytotoxicity percentage of *Sesbania grandifora*

Effect of metal and chelator on radicle length

The radicle length for increasing concentrations of Cr of 20-100mg was found to be 2.95-2.57cm, which clearly shows that with increasing metal concentrations there is a decrease in the radicle length Fig 2. The treatment with varying Cr and varying EDTA concentration showed a radicle length of 2.68- 2.30 cm and for varying Cr and constant EDTA of 0.35mM concentration the radical length was found to be 2.56-2.42cm. The radical length for the treatment with increasing concentration of Cr without EDTA did not show any significant difference ($P \leq 0.01$ and $P \leq 0.05$) for 20-80mg of Cr, were as 100mg of Cr showed a significant difference at ($P \leq 0.05$) but no such significance at $P \leq 0.01$. The treatment with varying concentrations of Cr and varying concentrations of EDTA showed a significant difference for concentrations 20mg+0.1mM and 40mg+0.2mM (Cr+EDTA) at $P \leq 0.05$, but did not show significance for $P \leq 0.01$, were as the other concentrations were significantly different at both $P \leq 0.01$ and $P \leq 0.05$. Similarly the treatment with varying concentration of Cr with constant 0.35mM EDTA concentration showed a significant difference for both $P \leq 0.01$ and $P \leq 0.05$ for all the varying concentrations of Cr with 0.35mM EDTA. Reports of Shanker *et al.* [32] hypothesized that; the root growth inhibition due to Cr toxicity could be due to inhibition of root cell division or root elongation or to the extension of cell cycle. Similarly as reported by Monalisa and Patra., [33] in mung bean seedling, 10µM concentration of EDTA in Cr^{+6}-EDTA complex showed possible root germination and high proline and total chrolophyll content.

The Metal Tolerance Index (MTI) and Germination Index (GI) values are denoted in Fig 3 and 4, from the figures it can be seen that the both the Index values for Cr treated plants in the absence of EDTA were higher. But while comparing the values between the second treatments (varying Cr with varying EDTA) and the third treatments (varying Cr with constant EDTA concentration) it was found that the MTI and GI values were higher for varying concentrations of EDTA corresponding to varying Cr concentrations, instead of constant EDTA concentration. The Percentage phytotoxicity values also varied differently with different treatments. From the fig 4 it was clear that the percentage phytotoxicity value at the concentrations 80mg of Cr with 0.4mM EDTA and 100mg of Cr with 0.5mM EDTA was higher when compared with other treatments.

According to some studies Cr toxicity reduces seed germination and radicle growth in plants, where a similar study with *Phaseolus mungo* by Kamlesh, *et al*., [34], reported the inhibition in the germination percentage with increase in Cr concentrations. According to Jamal, *et al*., [35] in *Triticum aestivum*, Cr had more toxic effect on root growth, shoot growth and seedling length individually and also in combination with other metals. In a similar study with *Triticum aestivum*, by Isak, *et al*., [36] found that the increasing concentration of heavy metals Cr,Cd,Mn,Zn cause a decrease in the percentage of germination and radicle growth. In a study by [37] in Cr tolerance to rice, it was reported that at higher concentrations of Cr, the germination percentage decreased considerably. The concentration of 400ppm was toxic to seed germination and concentration above 800ppm of Cr was toxic to the plant. Similar findings by Bonet et al. [38] reported the inhibitory effect of higher chromium concentration on bush bean (Phaseolus vulgaris L.). A report by Ibrahim *et al.,* [39] in alfalfa seeds, showed the

decrease in the inhibition of high concentrations of Co and Cr in the presence of a 10mM concentration of EDTA.

Hence the outcome of this present study with *S.grandiflora* show that the processes of germination and root elongation are affected differently with different concentrations of Cr and EDTA, and Cr being more toxic.The germination of the seeds was more severely affected, and the level of inhibition of germination percentage and root elongation was related to the concentration of Cr. The metal tolerance index and percentage of phytotoxicity was found to be higher for varying concentration of EDTA corresponding to Cr concentrations than with EDTA having a constant concentration. Under laboratory conditions, it can be suggested that, the germination processes will be severely affected when higher concentrations of the heavy metal are used against the seeds, in the absence of chelator, since EDTA treatments alleviated the inhibitory effect of the high concentrations of Cr and reduced the toxicity to some extent.

CONCLUSION

The increasing concentration of the heavy metal increased the phytotoxicity. In the present study it has been observed that, chelating agents reduces the toxicity effect of Cr to some extent. Hence it is concluded that the presence of EDTA is effective in the uptake of the metal, only when the metal is present at lower concentrations, were as with increasing concentrations of Cr with corresponding increase in chelator the plant did not show any positive effect on the increased radicle length or percentage of germination. Thus *S.grandiflora* could be suitably used along with EDTA at constant concentration for remediating land contaminated with chromium.

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