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Morphological and ultra structural changes induced by chromium and chelate assisted phytoextraction in *Sesbania grandiflora* (L.) Pers

Revathi S. and Subhashree V.*

ABSTRACT: *Field experiments using pot trials were conducted to study the effects of the presence and absence of EDTA (ethylene diamine tetra acetic acid) on morphological changes and ultra structural alterations in* Sesbania grandiflora *(L.) Pers. Two sets of treatments were maintained, one with different concentration of chromium Cr (VI) without EDTA ranging from 20-100mg Cr (VI) Kg^{-1} and the other with different concentration of Cr (VI) in the presence of 0.35mM EDTA range from (20-100mg Cr with 0.35mM EDTA Kg^{-1}) and a control set without chromium or EDTA was maintained. The results showed that with increase in the concentration of chromium in the presence of EDTA the plant* Sesbania grandiflora *is altered both in terms of morphological parameters and ultra structural variations, which was studied for a period of 30,60 and 90 days. There is a decline seen in the fresh biomass and dry matter of both root and shoots of the plant at higher concentrations of chromium in the presence of EDTA, and the structural changes were also evident. Thus the presence of EDTA enhances the extraction of heavy metal.*

Keywords: *Chromium, EDTA,* Sesbania grandiflora, *biomass, SEM-EDAX.*

INTRODUCTION

Heavy metal pollution is responsible for many harmful consequences both for human health and the environment**.** Currently followed technologies for cleaning such contaminated sites using mechanical or chemical treatment are usually expensive, labor intensive, and soil disturbing. Phytoextraction is a green technology that extracts inorganic contaminants, primarily heavy metals, from soil and water. It is a strategic technology that utilizes metal accumulating plants for hyper accumulation, which is a natural phytoextraction technique. It is also a cost-effective and efficient solution to clean up heavy metal contaminants. [1-3]. This approach includes overall biological, chemical and physical processes that enable uptake, sequestration, degradation and metabolization of contaminants by plants [4,5].

The presence of heavy metal can be attributed by two ways (a) natural and (b) anthropogenic. A variety of anthropogenic sources, such as mining processing, electroplating, wood preservation, iron and steel production, pigment manufacture, smelters, power station industry, production and application of metal-containing pesticides, can lead to soils acquiring heavy metal contents substantially in excess of natural levels. Some of these metals, at relatively low concentrations, may stimulate the biological life [6, 7]. Chromium a heavy metal released by such anthropogenic activities, is a toxic non-essential element for microorganism and plants [8]. The absorption and translocation of chromium can be modified by soil pH, organic matter content and chelating agents [9].

For many years, chelating agents and organic acids have been employed for analytical purposes to displace metals from soils and sediments. A number of process schemes exist for extracting metals from contaminated soils. But the leaching process by acid or a chelating agent is the most appealing for heavy metals removal from the polluted soil, since it generally creates less surface damage, requires a minimal amount of facilities and reduces the potential for human exposure [10, 11]. EDTA (ethylene diamine tetra acetic acid) which is a synthetic chelator is largely used for such phytoextraction studies. This chelating agent was often used to enhance metal uptake by

* Biomolecules and Genetics Division, School of Biosciences and Technology, VIT University, Vellore, India
*Corresponding author E-mail: v_subhashree@hotmail.com

plants in field and pot experiments [12, 13]. The presence of EDTA alters the metal speciation and metal phytotoxicity [14, 15].

The aim of the present study was thus to investigate the changes undergone by the plant *Sesbania grandiflora*, with and without EDTA in the garden soil spiked with different concentrations of chromium and to study the disparity on morphological characteristics and ultra structural amendments.

MATERIALS AND METHODS

Seed and Treatments

The seeds of *Sesbania grandiflora* was procured from Agri clinic, Ministry of Agri, Govt. of India approved centre, Coimbatore, India. Pot experiments were conducted in VIT garden, VIT University.

Two set of Treatments were followed in this study to assess the efficiency of chromium accumulation in *Sesbania grandiflora*. The first set of treatment (C1-C5) assessed the phytotoxic effects of increasing concentrations of chromium from 20-100mg Kg^{-1} in the absence of chelator. The second set of treatment (CE1-CE5) assessed the role of EDTA in extracting chromium by *Sesbania grandiflora*. A comparison between both the treatments was supported with the morphological and biochemical variations.

Chromium Solution Preparation

Chromium stock solution was prepared by dissolving 0.5916g of chromium from Potassium dichromate ($K_2Cr_2O_7$) in 100ml of distilled water. From this stock solution different concentration of (20, 40, 60, 80 and 100mg/L) chromium Cr (VI) solutions were prepared and used for first set of treatments. Similarly to the five different concentrations of Cr (VI) solutions, 0.35mM EDTA was dissolved giving a second set of treatment.

Experimental Setup and Soil Spiking

The soil used in this study was taken from VIT garden VIT University, the soil was air-dried and sieved. For first set of treatment 2.7kg of soil+0.3kg of manure was mixed manually with five different concentrations of chromium and filled in five different pots respectively. In the second treatment to the 3kg of soil (soil +manure), five different concentrations ranging from 20-100mg Kg^{-1} of Cr (VI) containing 0.35mM EDTA was mixed manually to the soil and filled in five different pots respectively. Control set was maintained without chromium or chelator. Triplicates were maintained. Totally 33pots were used for the study. The control and the spiked soil pots were left to equilibrate for 3 weeks. The pots were occasionally turned and mixed during the incubation period to ensure thorough mixing.

The seeds of *Sesbania grandiflora* were sterilized in 3% formaldehyde and sown in plastic trays for one week to attain the seedling stage. About 5 to 6 seedlings were transferred to the spiked soil pots after three weeks of incubation.

Physico-chemical properties of spiked soil

The experimentally spiked soil samples were collected manually at a depth of 5-10cm in each pot, from both the treatment pots involving the presence and absence of EDTA, so as to compare the metal accumulation efficiency of EDTA on plants. The physico-chemical properties such as pH, electrical conductivity and concentration of chromium were analyzed for the control soil and the spiked soil after 90 days by the process of soil digestion.

Plant Harvesting and measurement of parameters

The plant samples were removed from both the treatment pots at various time period of growth as 30, 60 and 90 days after sowing. Various morphological parameters like shoot and root length (cm), nodule number, shoot and root biomass and shoot and root dry weight (gms) were weighed separately after drying and recorded for each of the individual treatments and concentrations.

Scanning Electron Micrograph (SEM) Analysis coupled with EDAX

Root samples from the highest concentration of chromium without EDTA (C5), with EDTA (CE5), and control plants were prepared for SEM-EDAX instrument (model quanta 250, FEI Czech Republic) equipped with Everhart Thornley detector operated at high voltage with a vacuum of $3.99e^{-4}$Pa. The roots were approximately cut with 5mm length. The specimens were dried through carbon dioxide and mounted on stubs, sputter coated with palladium and the materials were observed under SEM-EDAX to determine the morphology and elemental composition of the specimen.

Statistical analysis

All the experiments were done in triplicates and the data presented in the figures are the mean of the experiments. The data were analyzed statistically and the mean±SE were calculated. Significance between control and treatments were compared at 0.05 and 0.01 probability levels.

RESULTS AND DISCUSSION

Effect of Chromium on Root length and Shoot length

The root length of *Sesbania grandiflora* plants at different stages of growth under chromium stress is represented in figure 1. Root length of *S.grandiflora* increased with the increase in time period, whereas decreased with increase in concentration of chromium with EDTA, than chromium without EDTA. At 30DAS the highest root length was found to be at 20 mg $kg^{-1}$of Cr (VI) without EDTA (7.95cm) and the lowest was at 100 mg kg^{-1} Cr (VI) without EDTA (6.25cm). At 60DAS all the values differed significantly. At 90DAS the highest root length was found to be at 20 mg $kg^{-1}$of Cr (VI) without EDTA (19.5cm) and the lowest was at 100 mg kg^{-1} Cr (VI) without EDTA (10.75cm). The highest root length for Cr^{6+} and EDTA treated plants was found to be at 20 mg $kg^{-1}$of Cr (VI) (8cm) and the lowest was at 100 mg kg^{-1} Cr (VI) (4cm). The highest root length for Cr (VI) and EDTA treatment was recorded for 90DAS was found to be at 20 mg $kg^{-1}$of chromium (17cm) and the lowest was at 100 mg kg^{-1} chromium (7cm). Similarly all the values differed significantly at the LSD of PdŠ0.05 and PdŠ0.01 for 90 DAS. The control showed relatively increasing root length with increase in time period.

Shoot length of *Sesbania grandiflora* at different stages of growth under chromium stress is represented in figure 1. Maximum shoot length was recorded at 90 days for control plants with (79cm), and minimum shoot length was observed at 100mg kg^{-1} chromium with EDTA (C5=14.5cm). When compared with the above observations with that of chromium and EDTA treated plants, it was observed that there was an increase in shoot length with increase in the time period, but with increasing concentrations of chromium and EDTA the shoot length decreased, in this the maximum shoot length was noted at 20 mg kg^{-1} chromium and EDTA (CE1=65.5cms) and the minimum shoot length at 100 mg kg^{-1} chromium and EDTA (CE5=49cm). At 30DAS statistically there was difference at the levels PdŠ0.05 and PdŠ0.01 for (CE2-CE5), but the treatments C4 and C5 showed significant difference only at PdŠ0.05. There was statistical difference seen for all the treatments at both the levels for 60 and 90 DAS. When compared with other treatments control showed an increasing shoot length. Shankar *et al.*, [16] reported that, chromium have a direct impact on the shoot metabolism causing reduction in plant height due to its transport to the upper part of the stem and this reduction of plant height are mainly due to the reduced root growth due to lack of nutrients and transport of water across the plant, which correlates with the present study.

Effect of Chromium on Nodule number

Nodule number of *Sesbania grandiflora* plants at various levels of chromium with and without EDTA is given in figure 2. From the figure it can be concluded that though the number of nodules increased with the time period, but it decreased with the increase in the concentration of chromium. Maximum number of nodules of grandiflora was observed for control at 90^{th} day of treatment (30 nodules $plant^{-1}$). The minimum nodule number was recorded at 100 mg kg^{-1} chromium with EDTA level on 30th day (1nodule $plant^{-1}$) for *Sesbania* plant. When compared with the control and chromium treated plants without EDTA which showed decrease in nodule number, there is less number of nodules or absence of nodule formation at 60^{th} and 90^{th} day for plants treated with EDTA at concentrations above 60mg of chromium with EDTA. Statistically significant difference at LSD PdŠ0.05 was noted for the treatment CE 4. Similarly Unnikannan *et al.,* [17] reported the lowest number of root nodules in *Cassia auriculate* with 11.8 nodules $plant^{-1}$ at 50mg kg^{-1} chromium treated plants.

Effect of Chromium on Fresh Biomass of root and shoot

The root fresh weight of *Sesbania grandiflora* plants at different stages of growth under chromium stress is represented in figure 3. The fresh weight of *S.grandiflora* increased with the increase in time period from 30^{th} day to 90^{th} day, but decreased with increase in concentration of chromium with EDTA, than chromium without EDTA. At 30 days the highest root fresh weight was found to be at 20 mg $kg^{-1}$of Cr (VI) without EDTA (0.57 gms) and the lowest was at 100 mg kg^{-1} Cr (VI) without EDTA (0.32gms). Significant differences were noticed for the treatments C4 and C5 in the absence of EDTA and CE 4 and CE 5 treatments in the presence of EDTA at 60 days. At 90 days the highest root fresh weight was found to be at 20 mg $kg^{-1}$of Cr (VI) without EDTA (10.83gms) and the lowest was at 100 mg kg^{-1} Cr (VI) without EDTA (4.16gms). The highest root fresh weight for Cr (VI) and EDTA treated plants was found to be at 20 mg $kg^{-1}$of Cr (VI) (0.37gms) and the lowest was at 100 mg

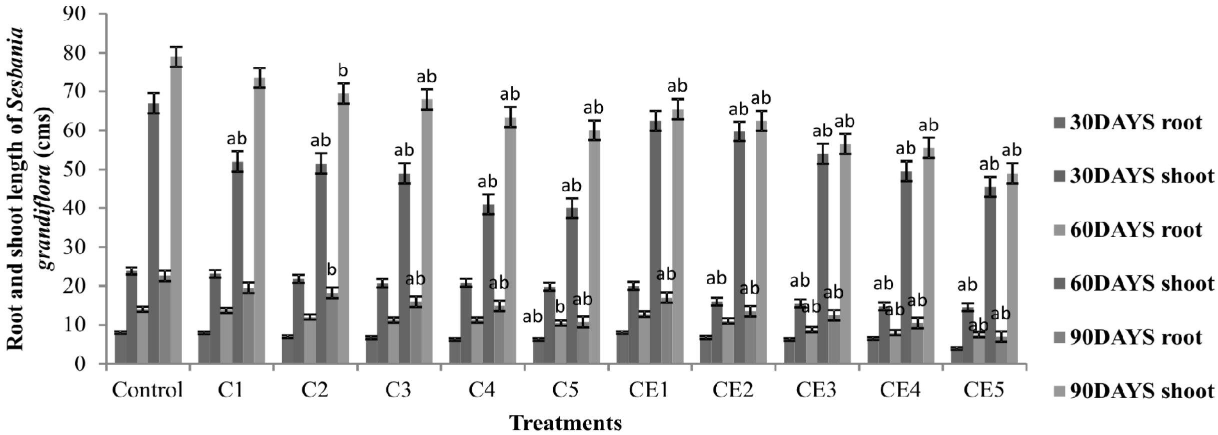

Figure 1: Root and shoot length of *Sesbania grandiflora* in different chromium concentrations with and without EDTA

Significance at P≤0.05=b, significance at P≤0.01andP≤0.05=ab

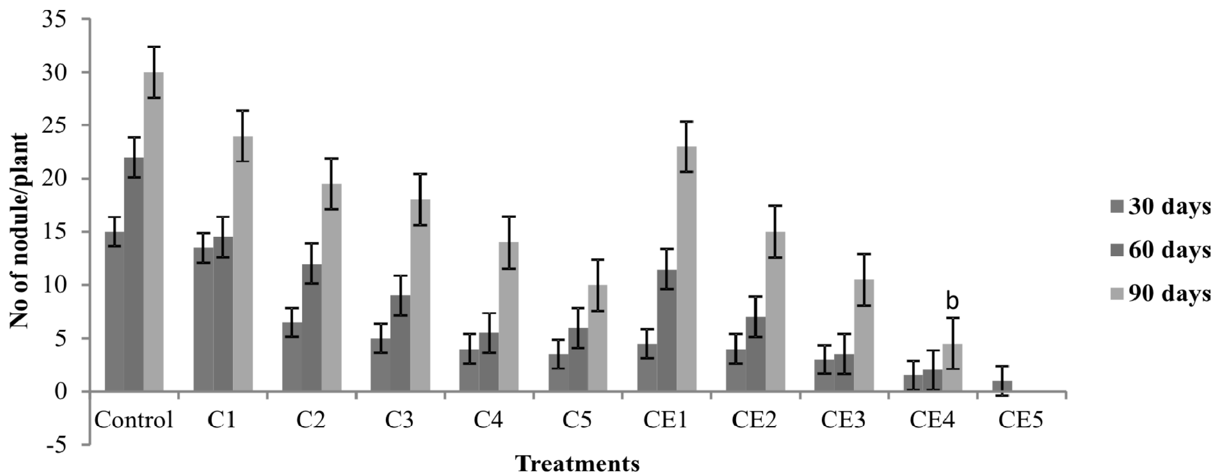

Figure 2: Nodule number of *Sesbania grandiflora* in different chromium concentrations with and without EDTA

Significance at P≤0.05=b, significance at P≤0.01 and P≤0.05=ab

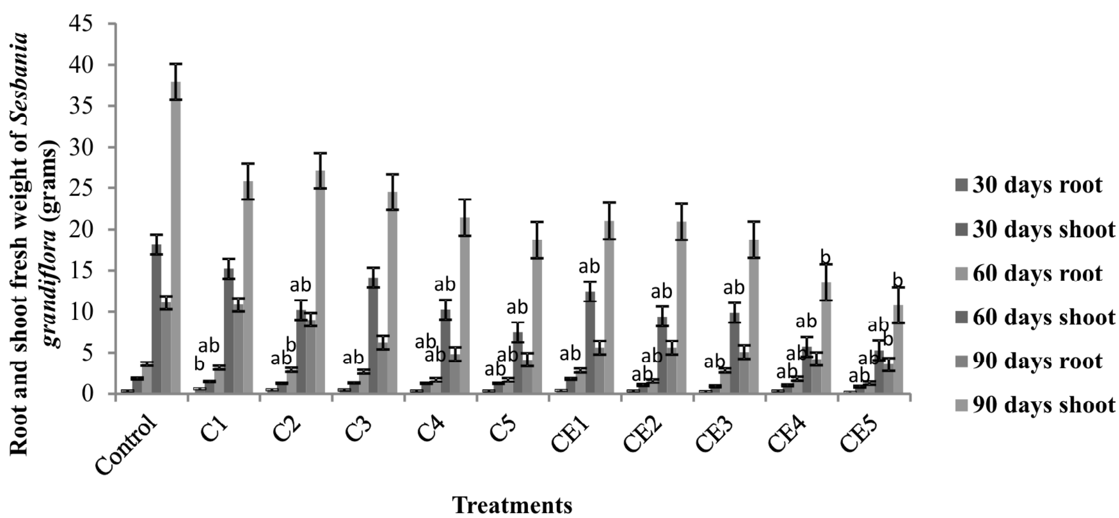

Figure 3: Root and shoot fresh weight of *Sesbania grandiflora* in different chromium concentrations with and without EDTA

Significance at P≤0.05=b, significance at P≤0.01 and P≤ 0.05=ab kg^{-1} Cr (VI) (0.17gms). The highest root fresh weight for Cr (VI) and EDTA treatment was recorded for 90 days and was found to be at 20 mg kg^{-1}of Cr (VI) (5.6gms) and the lowest was at 100 mg kg^{-1} Cr (VI) (3.5gms).

Shoot fresh weight of the test plant grown under chromium stress with and without EDTA is represented in figure 3. Maximum shoot fresh weight was recorded at 90DAS for 20 mg kg^{-1} Cr (VI) without EDTA (C1=25.76gms), and minimum shoot fresh weight was observed at 100mg kg^{-1} chromium without EDTA (C5=18.72gms). A comparison between the above observations with that of Cr (VI) and EDTA treated plants, showed the shoot fresh weight increases only with the increase in the time period from 30th to 90th day, but not with the increasing levels of chromium with and without EDTA. Statistically there was significant difference noted for LSD of PdŠ0.05 and PdŠ0.01 for both 30 and 60 DAS. With increasing concentrations of chromium and EDTA the shoot fresh weight decreased, the maximum fresh weight in shoot was noted at 20 mg kg^{-1} Cr (VI) and EDTA (CE 1=21gms) and the minimum fresh weight in shoots at 100 mg kg^{-1} Cr (VI) and EDTA (CE 5=10.75gms). The control plants without any treatment showed a substantial increase in shoot fresh weight.

Effect of Chromium on Shoot and Root Dry matter production

The root dry weight of *Sesbania grandiflora* plants raised in various treatments of chromium concentration at different stages of growth is furnished in figure 4. When compared to the control the root dry weight at concentration of chromium 20-100mg of Cr (VI) without EDTA and in the presence of EDTA showed a steady increase with an increase in the time period (30th-90th days), but the value decreased considerably with increase in concentration of Cr (VI) in the presence of EDTA. The difference in LSD at both ($P<0.05$ and $P<0.001$) was observed for values at 30 days, but for 90 days treatment it showed significant difference at only one level (PdŠ0.05).

The results showed by figure 4 indicated that the maximum shoot dry weight value occurred at 20 mg kg^{-1}of Cr (VI) without EDTA of species *grandiflora* (3.66gms) on 90th day. Minimum dry weight of shoot was observed on 30th day at 100 mg kg^{-1}of Cr (VI) with EDTA (0.025gms). Similar views were reported by various authors [18-20], on the toxicity of Cr (VI) and the physiological changes induced by increasing concentrations of metal.

Chromium accumulation and physiochemical analysis of soil

The physio-chemical characters of soil before and after spiking and control soil such as pH, EC and Cr (VI) were analyzed and the results are given in table 1

Table 1
Physicochemical composition and chromium concentration of Spiked soil after 90days

Treatments	*Parameters*		
	pH	*EC (dSm^{-1})*	*Cr(mg/kg)*
Control	9.24	0.01	-
C1	8.96	0.01	0.15
C2	8.96	0.01	0.18
C3	8.94	0.02	0.20
C4	8.90	0.03	0.25
C5	8.82	0.03	0.25
C1+EDTA	8.90	0.02	0.20
C2+EDTA	8.89	0.02	0.25
C3+EDTA	8.64	0.02	0.28
C4+EDTA	8.56	0.03	0.30
C5+EDTA	8.54	0.05	0.35

SEM observations

Scanning Electron Microscopy equipped with Energy Dispersive X-ray Spectrometer (EDX) analysis was performed to determine the location and transport of chromium metal in roots of *Sesbania grandiflora* 100 mg/Kg Cr concentration with EDTA and without ETDA. It was observed that most of the Cr metal ion had accumulated in the roots. A distinct signal and high atomic values for Cr were also noticed in Energy dispersive X-ray (EDX) analysis. The peaks for the corresponding concentration of Cr samples with and without EDTA are given in the figures (5, 6, and 7). The figures denotes the spectrum of the peak with the highest concentration of the metal and from the peaks it is evident that there is higher metal accumulation in the plant with 100mg Cr/Kg with EDTA, than when compared to the control and the other treatment 100mg Cr/Kg without EDTA. From the figures in can also be noted that the treated plants with highest concentration of chromium in the presence of EDTA (CE-5) showed complete destruction of cortex tissues and endoderm cells when compared with the control and for the treatments in the absence of EDTA. Thus when compared with the control plants and chromium treated plants without EDTA, it is clear that in the presence of a chelator EDTA the metal accumulation is high and it has therefore brought changes in the roots internally.

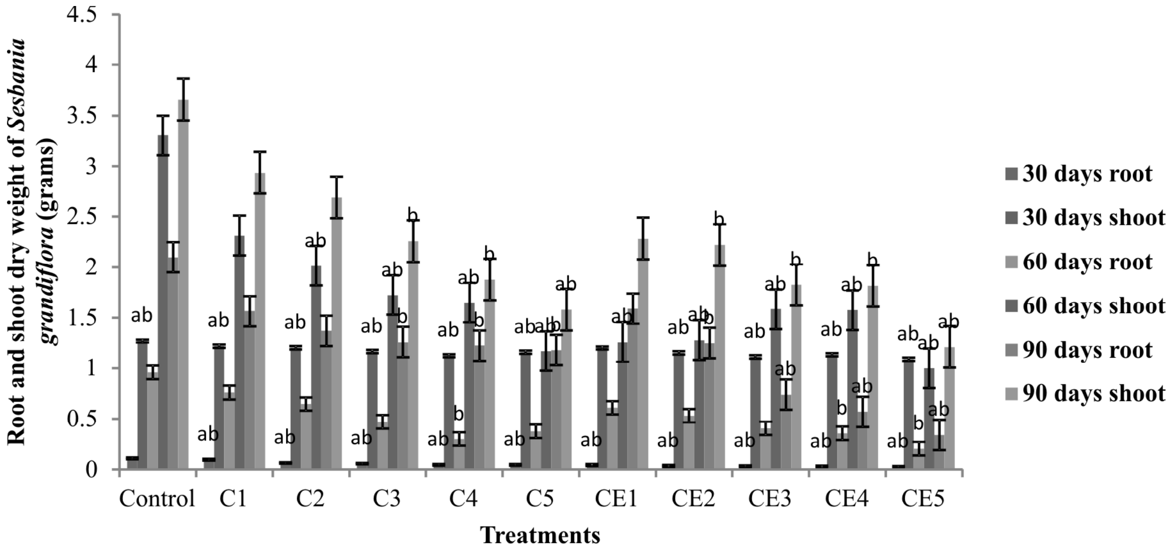

Figure 4: Root and shoot dry weight of *Sesbania grandiflora* in different chromium concentrations with and without EDTA

Significance at P≤0.05=b, significance at P≤0.01andP≤0.05=ab

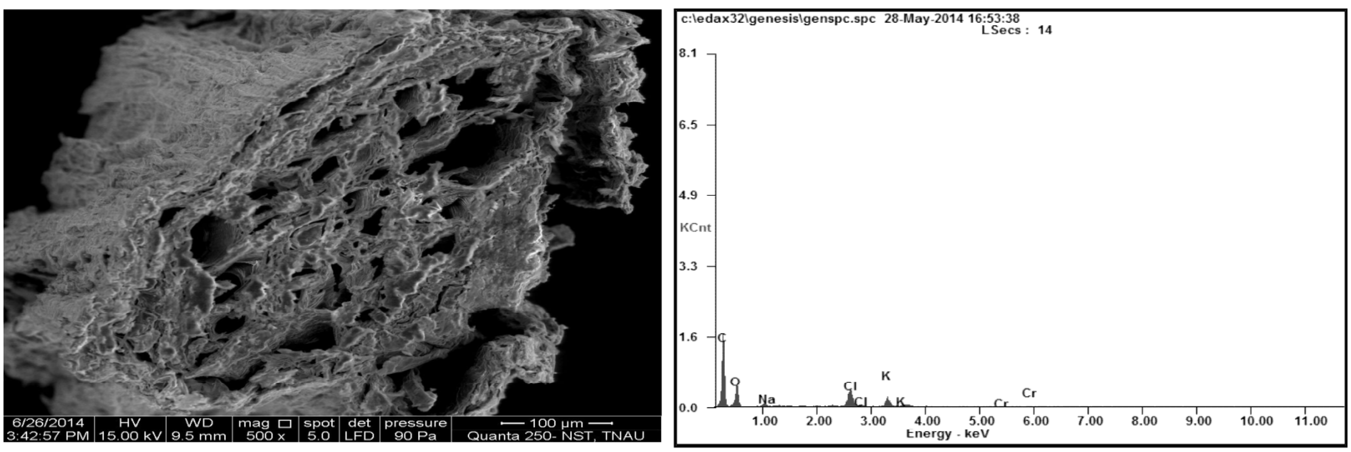

Figure 5: SEM micrograph and EDAX spectra for control plant

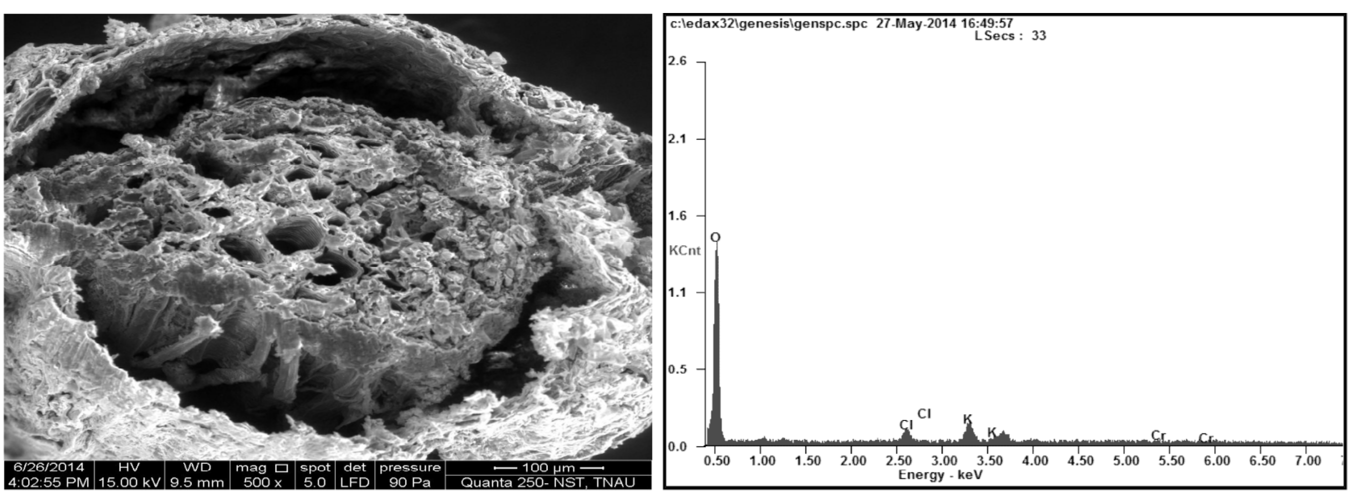

Figure 6: SEM micrograph and EDAX spectra of root tissue of *Sesbania grandiflora* grown in 100mg of chromium concentration in the absence of EDTA

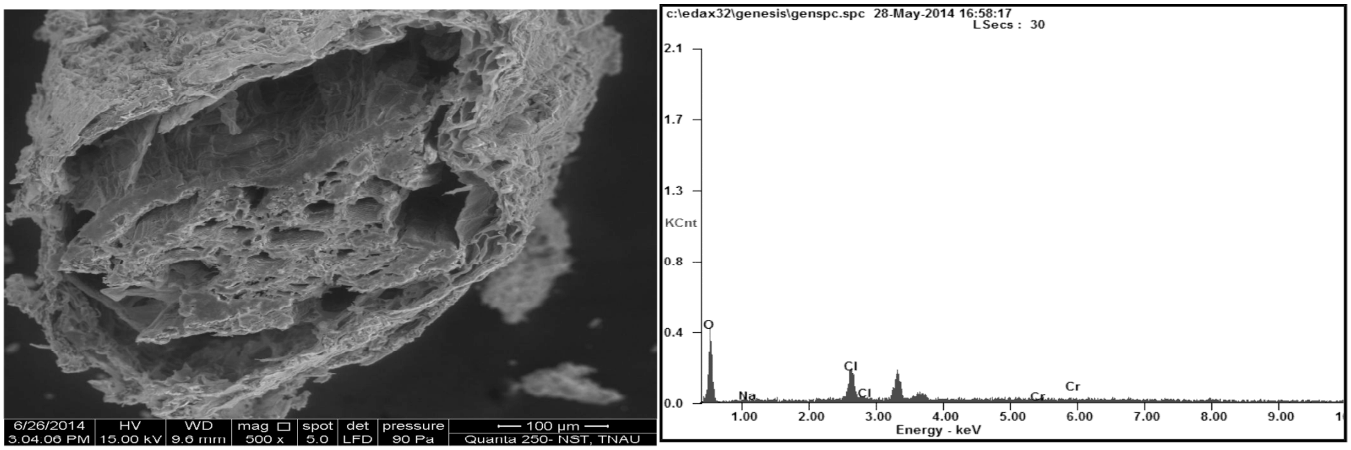

Figure 7: SEM micrograph and EDAX spectra of root tissue of *Sesbania grandiflora* grown in 100mg of chromium concentration in the presence of 0.35mM EDTA

CONCLUSIONS

From the present study it is concluded that, the presence of EDTA has a potential output in enhanced uptake of chromium by *Sesbania grandiflora* plant and this is clearly reflected from their morphological and ultra structural studies. Yet lower concentrations of EDTA (0.35mM/L) are effective in extracting the metal with the help of the plant and this shows the efficiency of the plant in uptake of metal facilitated with a chelator. Thus *Sesbania grandiflora* could be widely used in phytoremediation technique.

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