

Chromium toxicity in *Sesbania sesban* (L.) Merr

R. Bakiyaraj, T. Mahakavi, L. Baskaran*

Department of Botany, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India

*E-mail address: basubotany@rediffmail.com

ABSTRACT

Chromium is one of the most common toxic metals present in the environment that induces various toxic effects in plants. A pot experiment was conducted to determine the effects of chromium on germination percentage, seedling growth, chlorophyll 'a', 'b' and proline content of *Sesbania sesban* (L.) Merr. The seedlings were treated with different concentrations of control, 0.10, 0.25, 0.50, 0.75 and 1.00 g kg⁻¹ of chromium. The parameters such as germination percentage, root and shoot length, seedling fresh weight dry weight, chlorophyll 'a', 'b' and proline content of leaves were measured. Our results indicated that a significant inhibitory effect was observed at all levels of chromium compared to control. Increasing the concentration of chromium to 1.00 g kg⁻¹ showed a significant decrease in seed germination, shoot and root length, fresh weight, dry weight and chlorophyll 'a' and 'b' content of plant. While proline, catalase and peroxidase contents were increased by increasing Cr concentration. It was also noted that accumulation of chromium in the roots was much higher than the shoots of the seedlings under treatment.

Keywords: Chromium; germination percentage; chlorophyll; enzyme; accumulation

1. INTRODUCTION

Soil is a complex mixture of mineral and organic, aqueous and gaseous components. It is a dynamic system with variations in moisture content, pH and redox potential conditions. These properties interfere with the form and availability of metals (Alloway, 1990). Soil and heavy metal interactions can be understood on the basis of ion exchange, surface adsorption and chelation reactions. Humic substances have the ability to form complexes with heavy metals due to their functional groups. Heavy metal retention by soil also depends on ionic strength, pH, type of clay minerals present, type of functional groups and competing cations (Alloway, 1990; Evangelou, 1998).

Heavy metals exist in nature, but their elevated level due to anthropogenic activities represents serious ecological, environmental, and financial issues (Sumner *et al.*, 2003). Heavy metal contaminated sites require immediate remediation and thus pose a major technological and financial problem worldwide (CEI, 2005). The problem is seriously ecological, because these metals due to their bioaccumulation can enter food chains and the

biological cycle. They can eventually affect plants and animals including humans (Kabata-Pendias *et al.*, 2001; Mertz 1987).

Chromium is the 17th most abundant element in the Earth's mantle. Cr is widely used in industry as plating, alloying and tanning of animal hides, inhibition of water corrosion, textile dyes and mordant, pigments, ceramic glazes, refractory bricks and pressure-treated lumber (Avudainayagam *et al.*, 2003). Due to this wide anthropogenic use of Cr, the consequent environmental contamination increased and has become an increasing concern (Zayed, A.M and Terry N, 2003). So the present investigation has been under taken to assess the phytotoxicity of Cr on the growth, germination, biochemical, enzymes content and accumulation of *Sesbania sesban* (Photo 1).



Photo 1. *Sesbania sesban* L.

2. MATERIALS AND METHODS

2. 1. Seed collection

The seeds *Sesbania* were purchased from Tamil Nadu seed farm Virudhachalam, Cuddalore District, Tamil Nadu, India.

2. 2. Methods of treatment

The Seeds were sterilized with 1 % HgCl₂ for 10 minutes, and then washed several times with distilled water and germination for 4 days in the green house condition. *Sesbania* were grown in unpored plastic pots in untreated soil (control) and in soil to which chromium had been applied 0.10, 0.25, 0.50, 0.75 and 1.00 g kg⁻¹. Each pot contained 5 kg of air dried soil.

2. 3. Pot experiment

Different level of chromium (K₂Cr₂O₇) was mixed with the soil and twenty seeds were sown in each pot. All pots were watered daily. Plants were thinned to a maximum of 10 per pot, after a week of germination. Each treatment including the control was replicated three times. The numbers of germinated seedlings were counted after 15 days of treatment. The plant samples were collected at 45th day, the measurement of various morphological, growth parameters like root length, shoot length, dry weight and fresh weight of root and shoot per plant were determined.

2. 4. Morphological analysis

The total length of the seedlings and fresh weight were measured immediately after removing the seedlings from the experimental pot. The dry weight of the seedlings was determined after they had been dried for 80 °C.

2. 5. Biochemical analysis

Biochemical analysis of chlorophyll 'a', 'b', proline, catalase and peroxidase contents in plant samples were estimated by the following methods. The pigment content measurement 0.5 g leaves were homogenized with 10 ml of 80 % acetone. The extract was centrifuged at 3000 g for 5 min. The upper phase was transferred into a new tube and its absorbance was measured at 663, 646 and 470 nm, respectively, for chlorophyll 'a', 'b'. The chlorophyll 'a' and 'b' content measured according to Lichtenthaler and Wellburn, 1983. Proline was measured spectrophotometrically at 520 nm according to Bates *et al.*, 1973.

2. 6. Enzyme analysis

Catalase activity was measured by the method of Maehly and Chance, 1959. Peroxidase activity was measured by the method of Kumar and Khan, 1982.

2. 7. AAS Analysis

The sample plants were removed from the pots and washed under a stream of water and then with distilled water. The collected plants were air dried, then placed in a dehydrator for 2-3 days and then oven dried for four hours at 100 °C. The dried samples of the plant were powdered and stored in polyethylene bags. The powdered samples were subjected to acid digestion. 1gm of the powdered plant material were weighed in separate digestion flasks and

digested with HNO₃ and HCl in the ratio of 3:1. The digestion on hot plate at 110 °C for 3-4 hours or continued till a clean solution was obtained. After filtering with Whatman No. 42 filter paper the filtrate was analyzed for the metal contents in AAS (More, 1974).

2. 8. Statistical analysis

The experimental datas were processed statistically by adapting the techniques of analysis of variance of standard deviation (Snedecor and Cochran, 1967).

3. RESULTS AND DISCUSSION

In the present study, at 45th day the morphological, biochemical and enzymes observation were done. Increasing the concentrations of chromium caused significant reduction in seed germination, root length and shoot length (Fig. 1 and 2). Root dry weight and shoot dry weight, were observed at 0.10, 0.25, 0.50, 0.75 and 1.00 g kg⁻¹ of chromium when compare with control (Fig. 3). Chlorophyll contents were also found to reduce at increasing levels of chromium (Fig. 4). Although proline, catalase and peroxidase contents were drastically increased at the increasing levels of same element (Fig. 5 and 6).

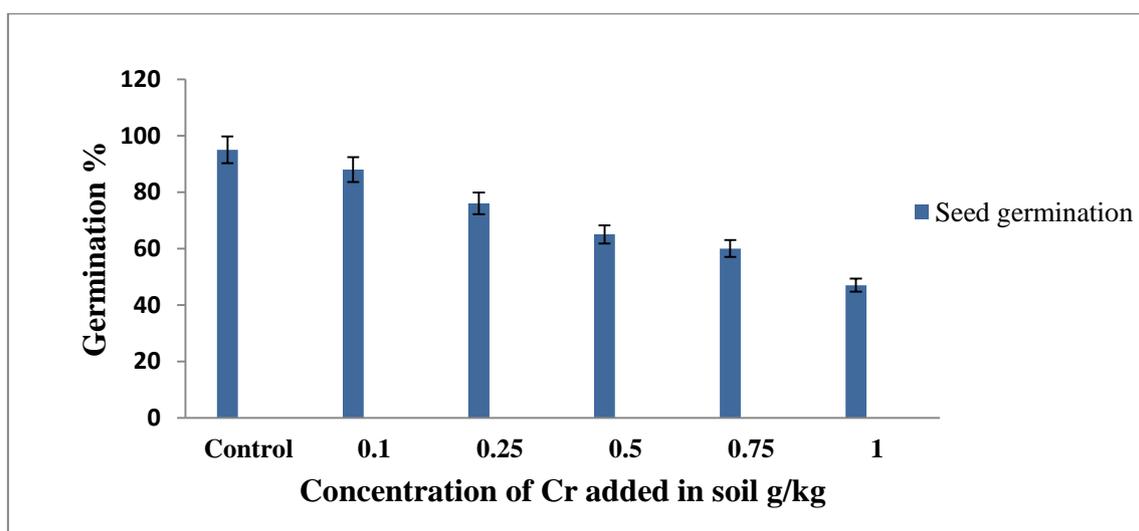


Fig. 1. Effect of different concentration of chromium on seed germination of *Sesbania sesban L.*

The present results suggested that the germination gradually decreased with increasing chromium concentrations compared to the control (Fig. 1). Since seed germination is the first physiological process affected by Cr treatment, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal (Peralta *et al.*, 2001). Seed germination of the weed *Echinochloa colona* was reduced to 25 % with 200 AMCr (Rout *et al.*, 2000). High levels (500 ppm) of hexavalent Cr in soil reduced germination up to 48 % in the bush bean *Phaseolus vulgaris* (Parr and Taylor, 1982). Peralta *et al.* (2001) found that 40 ppm of Cr(VI) reduced by 23 % the ability of seeds of lucerne (*Medicago sativa* cv. Malone) to germinate and grow in the contaminated medium.

Reductions of 32-57 % in sugarcane bud germination were observed with 20 and 80 ppm Cr, respectively (Jain *et al.*, 2000).

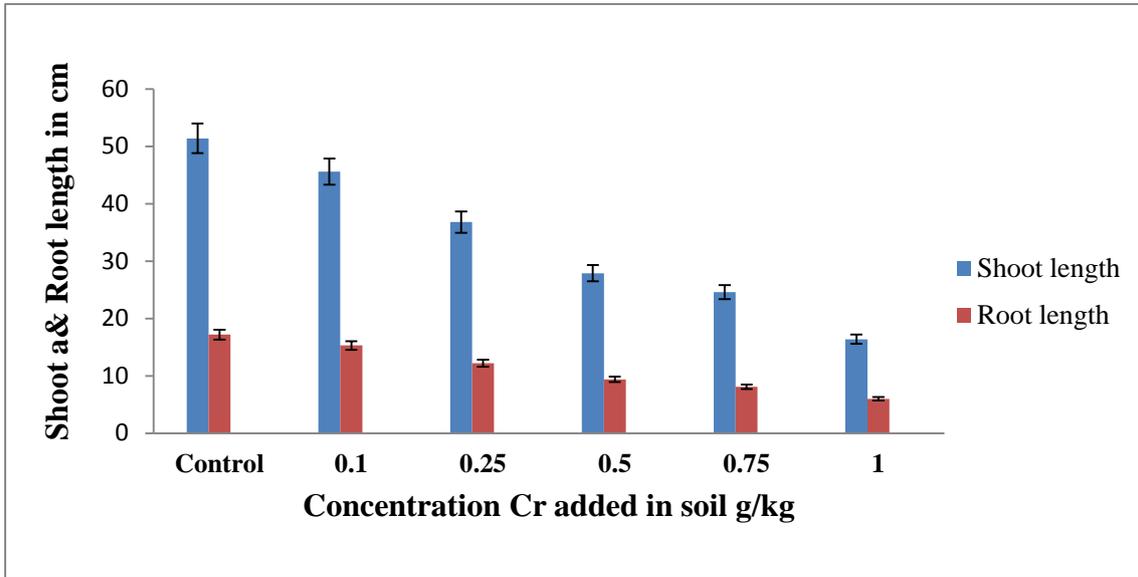


Fig. 2. Effect of different concentration of chromium on root & shoot length of *Sesbania sesban L.*

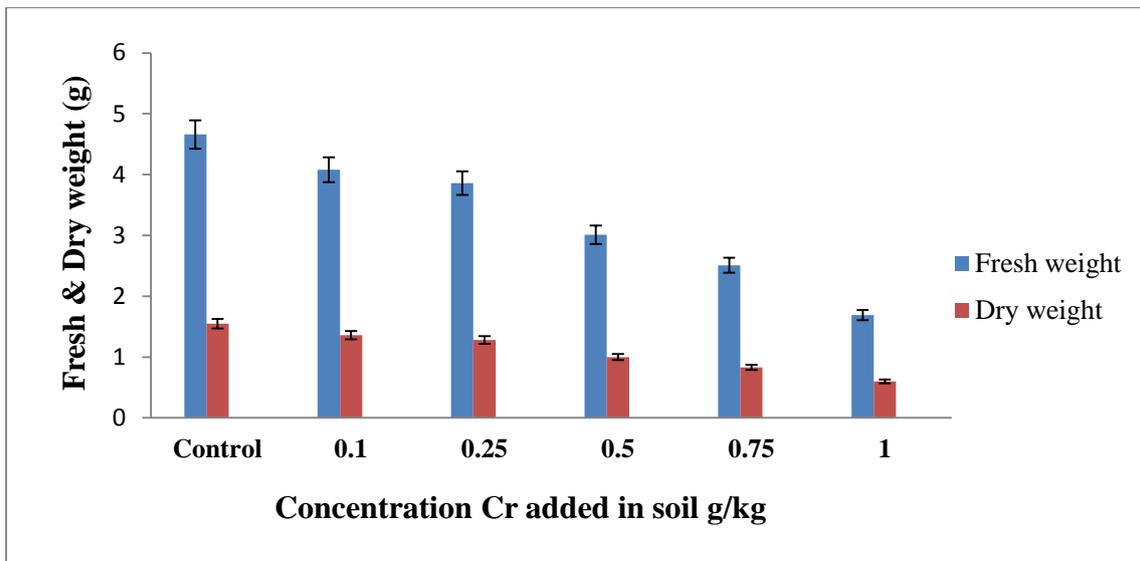


Fig. 3. Effect of different concentration of chromium on fresh & dry weight of *Sesbania sesban L.*

In the present investigation also revealed that the root, shoot growth, fresh and dry weight of the *Sesbania* plant showed significant reduction with chromium stress (Fig. 2 and 3). The reduction in seedling growth under chromium stress might be due to the poor root growth which inhibits transportation of water and nutrients to the shoot of the plants. In addition to this, chromium transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in seedling growth (Shanker *et al.*, 2005). Similarly, the decrease in the weight of the seedling is mainly due to the inhibition of water uptake.

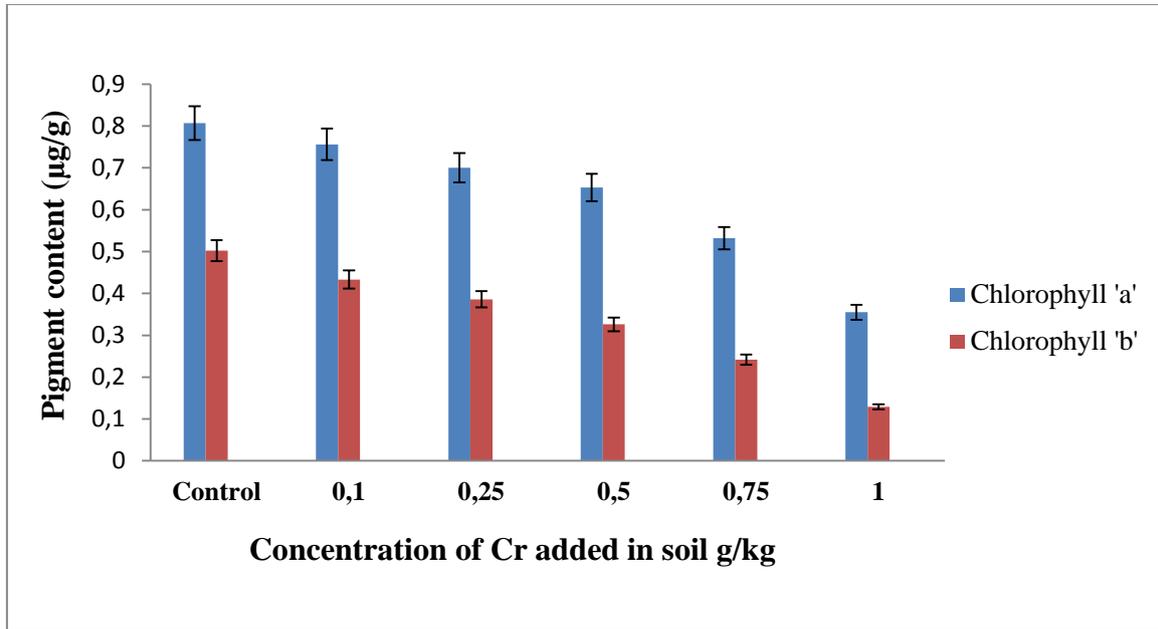


Fig. 4. Effect of different concentration of chromium on chlo 'a' & 'b' of *Sesbania sesban L.*

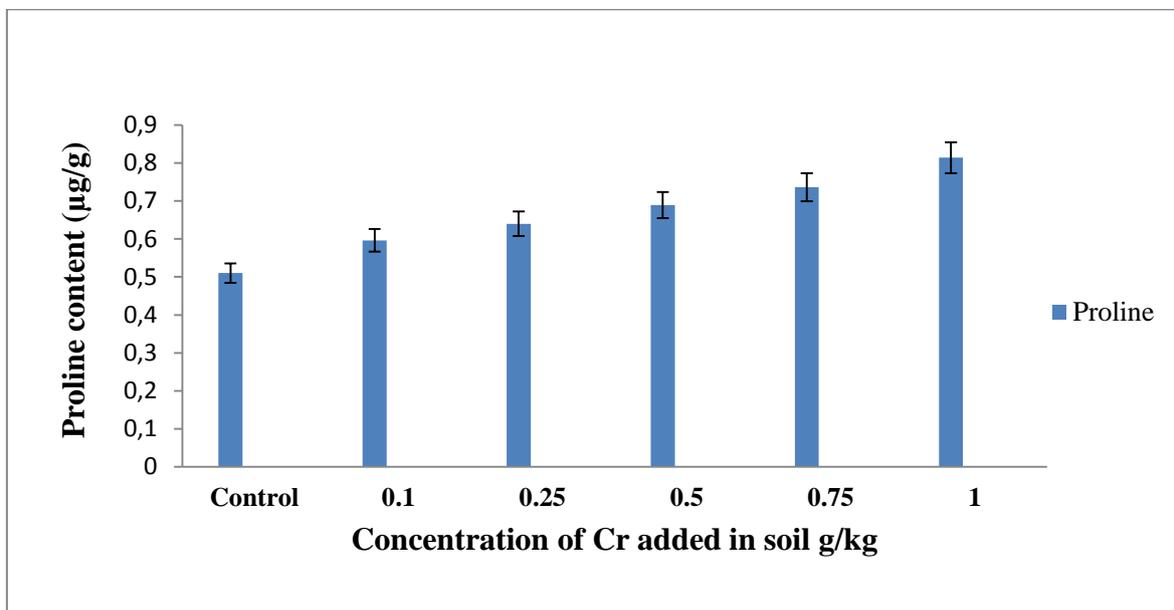


Fig. 5. Effect of different concentration of chromium on proline content of *Sesbania sesban L.*

Reduction in shoot growth could be attributed to the reduction in chlorophyll contents and activity of photosystem(I), induced by heavy metal stresses (Skorzynska-Polit & Baszynski, 1997). Similarly, metal elements transported to above ground plant part reduced height by disturbing the cellular metabolism of the shoots (Shanker *et al.*, 2005).

The decrease in shoot biomass with increasing concentration of heavy metals may be due to the sensitivity of enzymes of the photosynthetic carbon reduction cycle to cadmium (De Filippis and Ziegler, 1993). The growth of all the treatment showed inhibitory effect with

increasing concentration of Cr (VI). Chromium was found to be more toxic affecting root and shoot length. The reduction in the plant height might be mainly due to the reduced root growth and consequent lesser nutrient and water transport to the above parts of the plant. In addition to this, chromium transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction of plant height (Shankar *et al.*, 2005). Root was found to be more affected than shoot. This is due to the fact that heavy metals (Cr-VI) accumulated on root due to binding of metals (Cr-VI) on the cell wall of root and retard cell division and cell elongation (Woolhouse, 1983). General decreased root growth due to chromium toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle in the roots. The reason of the high accumulation in roots of the plants could be because chromium is immobilized in the vacuoles of the root cells, thus rendering it less toxic, which may be a natural toxicity response of plant. Also heavy metals have been reported to impair the growth of new roots and seedling establishment (Rellen-Alvarez *et al.*, 2006).

Result from the present data showed that, Chlorophyll a and chlorophyll b contents were drastically reduced under chromium treatments especially at higher level (Fig. 4). In this study, severe chlorosis on older leaves and scarce appearance on younger leaves suggested that decline in chlorophyll content in shoots of metal treated plants result mostly from its enhanced degradation or reduced synthesis (Stobart *et al.*, 1985). Moreover, chlorophyll a/b was affected under metal treatments.

In the present investigation proline, catalase and peroxidase content of the *sesbania* plant significantly increased at the increasing levels of chromium concentrations (Fig. 5 and 6). Increase of proline was occurred due to increase of chromium in root and areal part of the plant. Increase of proline in plant is a defensive mechanism proline increase tolerance of plant by several mechanisms such as elimination of hydroxyl radicals, osmosis adjustment, inhibition of destroying of enzymes and maintaining protein synthesis (Kuzentsov and Shevyakova, 1997). One of the defensive mechanisms to Cr is synthesis and accumulation of some amino acids like proline that is a osmotic alignment and reduce toxicity of heavy metals (Alia and Matysik, 1991). More accumulation of proline in root of the plant can show the importance of osmosis alignment in absorbance places.

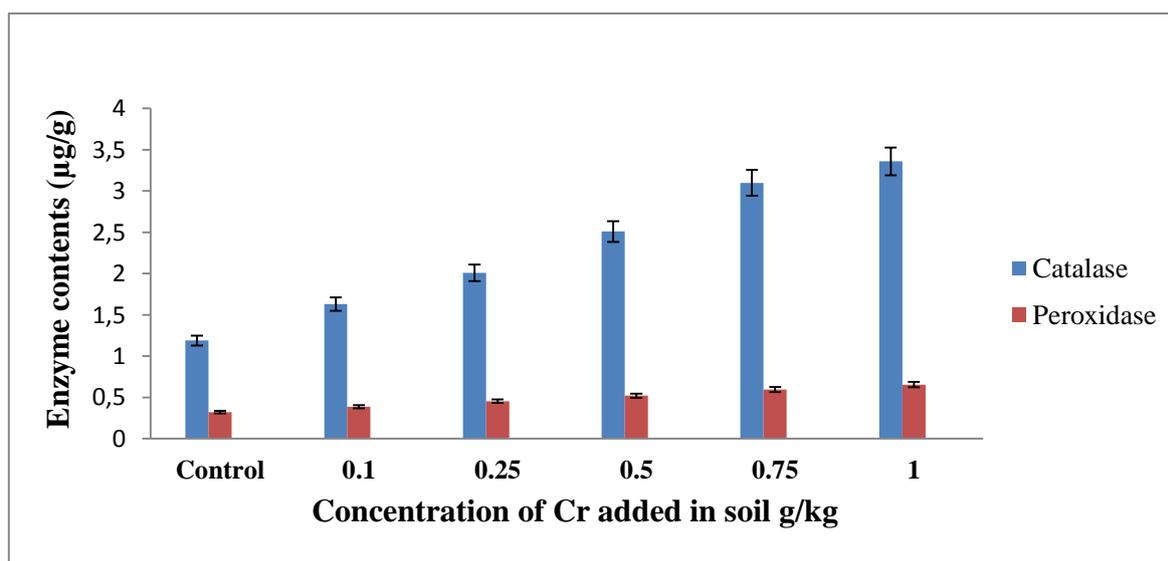


Fig. 6. Effect of different concentration of chromium on catalase & peroxidase of *Sesbania sesban* L.

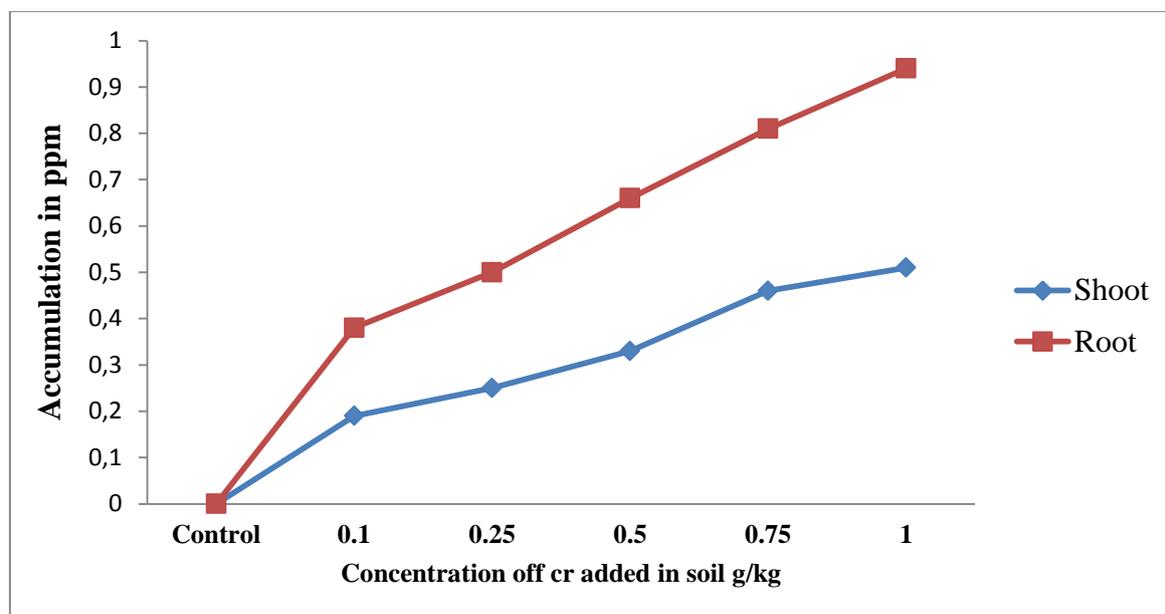


Fig. 7. Effect of different concentration of chromium on accumulation of *Sesbania sesban* L.

Peroxidase catalyzes H_2O_2 -dependent oxidation of substrate. POD activity is also considered a useful biomarker for sublethal metal toxicity in examined plant species. Previous studies in other plants have reported increases, decreases, and no changes in POD activity in response to heavy metal exposure (Shaw, 1995). In our study, the results presented show increased activities of POD activity at lower Cr concentrations and a decline with increase in Cr concentration. Previous studies in metal tolerant plant species have reported that POD activity was found to be sufficiently high to enable the plants to protect themselves against oxidative stress (Tanyolac *et al.* 2007). In our study, seedlings were able to maintain high levels of POD activity at higher Cr stress. Moreover, POD participating in lignin biosynthesis can build up a physical barrier against toxic heavy metals. Therefore, this also indicates that seedlings may be more efficient in avoiding damage from heavy metals.

Catalase is a universally present oxido-reductase that decomposes H_2O_2 to water and molecular oxygen, and it is one of the key enzymes involved in the removal of toxic peroxides. In the present study, CAT activities in seedlings significantly increased at lower Cr concentrations, while at higher Cr concentrations, it was decreased. Increase in CAT activity can be explained by an increase in its substrate, i.e., to maintain the level of H_2O_2 as an adaptive mechanism of the plants (Reddy *et al.* 2005). Decline observed at higher concentration of Pb might be attributed to inactivation of enzyme by ROS, decrease in synthesis of enzyme, or change in assembly of its subunits (Verma and Dubey, 2003). In our study, CAT activity was higher in all the Cr concentration. The higher CAT activities in seedlings indicate that its H_2O_2 scavenging mechanism is more effective.

The phytotoxicity of different concentrations of chromium on root length, shoot length and seedling dry weight after 45 days of growth is shown in Figure 7. It can be seen that as the concentration of chromium increases, it becomes phytotoxic to root length, shoot length and seedling weight. The phytotoxic effects start at 0.10 g kg^{-1} chromium and gradually increase with increase in chromium concentration. It is extremely interesting to note that chromium is more phytotoxicity to root as compared to shoot length and seedling weight.

4. CONCLUSION

The present study revealed that chromium had a toxic effect on germination, seedling growth, fresh and dry weight, pigments like chlorophyll 'a' and 'b', proline and enzymes like, catalase and peroxidase of the *Sesbania sesban*. From the results we concluded that the chromium having more toxic in nature so the industries must take care to reduce the toxicity of chromium. Then only we can able to maintain the flora and fauna.

References

- [1] Alia P., Matysik J., *J. Plant. Physiol.* 138 (1991) 554-558.
- [2] Alloway B. J., *Indian J Plant Physiol.* 5 (1990) 228-231.
- [3] Avudainayagam S., Megharaj M., Owens G., Kookana R. S., Chittleborough D., Naidu R., *Reviews of Environmental Contamination and Toxicology* 178. (2003) 53-91.
- [4] Bates L., Waldren R. P., Teare I., *Plant. Biol. Plant.* (1973) 111-115.
- [5] CEI: Soil remediation technologies: assessment, clean-up, decommissioning, rehabilitation. *Canadian Environmental Industries* (Energy and Environmental Industries Branch) (2005), available at: <http://www.ic.gc.ca/eic/site/eaae.nsf/eng/ea02201.html>.
- [6] De Filippis L. F., Ziegler H., *J. Plant Physiol.* 142 (1993) 167-172.
- [7] Evangelou V. P., *Environmental Soil and Water Chemistry Principles and Applications*. New York: John Wiley & Sons, Inc. 1998.
- [8] Jain R., Srivastava S., Madan V. K., Jain R., Joshi U. N., Rathore S. S., Arora S. H., *IJEP* 19 (1999) 745.
- [9] Kabata-Pendias A. D., Pendias H., *Trace elements in soils and plants*, CRC Press, London 2001.
- [10] Kumar K. B., Khan P. A., *Ind J Exp Bot.* 20 (1982) 412-416.
- [11] Kuzentsov W., Shevyakova N. L., *Physiol. Plantarum.* 101 (1997) 477-482.
- [12] Lichtenthaler H. K., Wellburn A. R., *Biochem Soc Trans* 11 (1983) 591-592.
- [13] Maehly A. C., Chance B., *The assay of catalase and peroxidase*. In: Methods of biochemical analysis. Vol. 1 (Glick, D.Ed.), *Inter Science Publishers. inc.*, New York 1959, pp. 357-425.
- [14] Mertz W., *Trace elements in human and animal nutrition*. San Diego, California: Academic Press, fifth ed., 1987, Vol. 1-2.
- [15] More T., 1974. Research experiences in Plant Physiology, Springer-Verlag, New York.
- [16] Parr P. D., Taylor F. G., *Environ Int.* 7 (1982) 197-202.
- [17] Peralta J. R., Gardea Torresdey J. L., Tiemann K. J., Gomez E., Arteaga S., Rascon E., *Environ Contam Toxicol.* 66(6) (2001) 727-734.
- [18] Reddy A. M., Kumar S. G., Jyonthsnakumari G., Thimmanaik S., Sudhakar C., *Chemosphere.* 60 (2005) 97-104.

- [19] Rellen-Alvarez R., Ortega-Villasante C., Alvarez-Fernandez A., del Campo F. F., Hernandez L. E., *Plant Soil* 279 (2006) 41-50.
- [20] Rout G. R., Sanghamitra S., Das P., *Chemosphere* 40 (2000) 855-859.
- [21] Shanker A. K., Cervantes C., Loza-Tavera H., Avudainayagam, S., *Environ. Int.* 31 (2005) 739-753.
- [22] Shaw B. P., *Biol Plant.* 37 (1995) 587-596.
- [23] Skorzynska-Polit E., Baszynski T., *Plant Sci.* 128 (1997) 11-21.
- [24] Snedecor G. W., Cochran W. G., *Statistical methods.* Iowa State University Press, Ames. IA. 1967, p. 593.
- [25] Stobart A. K., W. T. Griffiths, I. Ameen-Bukhari, R. P. Sherwood, *Physiol. Plantarum* 63 (1985).
- [26] Sumner M. E., Noble A. D., Soil acidification: the world story. In: Rengel Z, ed. *Handbook of soil acidity*, New York, USA: Marcel Dekker, 2003, 1-28.
- [27] Tanyolac D., Ekmekci Y., Unalan S., *Chemosphere.* 67 (2007) 89-98.
- [28] Verma S., Dubey R. S., *Plant Sci.* 64 (2003) 645-655.
- [29] Woolhouse H. W., 1983. Toxicity and tolerance in the responses of plant metals. In: *Encyclopedia of plant physiology.* Vol.12 C. (Eds: Lange *et al.*). pp. 245-300.
- [30] Zayed A. M., Terry N., *Plant and Soil.* 249(1) (2003) 139-156.
- [31] P. Unnikannan, P. Vedhanarayanan, P. Sundaramoorthy, *International Letters of Natural Sciences* 2 (2014) 35-48.
- [32] Yasabie Abatneh, Omprakash Sahu, *International Letters of Natural Sciences* 3 (2014) 44-55.

(Received 29 January 2014; accepted 03 February 2014)