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## Chromium induced changes in Soybean (*Glycine max L.*) metabolism

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### ABSTRACT

Pulses play an important role in Indian agriculture. Among the pulses, soybean (*Glycine max. L*) occupies a unique place by becoming the largest source of vegetable oil and protein. It is widely cultivated in India and occupy 5<sup>th</sup> largest production of soybean in the world. Industrialization and population explosion has caused a serious problem of pollution in the environment in all possible ways. As a result, most of the water resources are getting polluted by receiving large quantity of sewages and industrial wastewaters with heavy metals. In some places, these polluted water is being used for irrigation due to scarcity for good water. The continuous use of these wastewater containing heavy metals degraded the soil quality and reduced the growth and yield performance of agricultural crops. Aresenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, uranium, vanadium and zinc are the some of the important heavy metals found in our environment. Among the heavy metals, chromium merits a special reference for its toxic potential. It is released from the industries such as electroplating, leather tanning, textile printing and metal finishing. It is one of the main constitutions of tannery effluent. Presence of excess amount of chromium in the wastewater affect the plant growth and development when it is used for irrigation. So, Laboratory study was carried to investigate the irrigational impact of various concentrations of Cr (5, 10, 25, 50 100, 200 and 300 µg/l) on changes in morphological (germination percentage, root length, shoot length, fresh weight and dry weight), photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoid) biomolecules

(aminoacid, protein, sugar, proline) and antioxidant enzymatic activities (catalase, peroxidase, polyphenol oxidase and super oxide dismutase) were estimated. The accumulation of chromium in soybean seedlings were estimated and they were correlated with the above mentioned parameters.

**Keywords:** Chromium; morphology; biochemical contents; enzymatic activities; soybean seedlings

## 1. INTRODUCTION

Pulses play an important role in Indian agriculture because of their inherent ability to fix atmospheric nitrogen through biological nitrogen fixation. Among them, soybean (*Glycine max. L*) occupies a unique place for its use as seed and vegetable, and it is grown both as pure and mixed crop. It belongs to the family Fabaceae. It is otherwise known as a “Miracle crop” with over 40% protein and 20% oil, originated in China. As early as in 2853 BC, the Emperor Sheng – Nung of China named it as one of the five sacred grains. Now, soybean has become the largest source of vegetable oil and protein in the world and its large scale cultivation is concentrated in few countries. The world’s average soybean yield has also increased from less than 1 t/ha to 2.3 t/ha. In India, soybean cultivation was negligible until 1970, but it grew rapidly and made India as the 5<sup>th</sup> largest producer of soybean in the world today.

Water is the most vital resource for all kinds of life on this planet. The problem of water pollution due to industrial wastewater is attaining greater dimension day by day in India. To day, most of the rivers of world receive millions of liters of sewage, domestic waste and industrial and agricultural effluent and gets polluted. The wastewater discharging industries are distilleries, electroplating units, fertilizer units, iron and steel industries, paper and pulp, pharmaceuticals, pesticides and herbicide industries, textile, tannery and dye industries. These industrial effluents contain a wide variety of organic and inorganic pollutants with heavy metals which create serious physico-chemical disorders in living organisms.

Heavy metals are the metals which are having a density of five times higher than that of water (1). Arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, uranium, vanadium and zinc are the some of the important heavy metals found in our environment. Heavy metal contamination in soil and water has become a global problem that is leading to loss in crop yield and hazardous effect on human health when these metals enter the food chain (2, 3). Among these heavy metals, Chromium merits a special reference for its toxic potential. It is used in a variety of industries such as leather processing and finishing, the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacturing and the production of chromic acid and specialty chemicals (4). The higher amount of unused chromium is disposed from various industries such as steel works, electroplating, leather tanning and chemical manufacturing. Among them, tannery industry is one of the oldest industries in India. India is the largest market for hide and skin. Out of the total industrial used of Cr, 40% of Cr is used in leather processing industry and released to the environment. Majority of tanneries in India are engaged in chrome tanning processes and they use nearly 40,000 tonnes of basic chromium every year, They are releasing 75,000 m<sup>3</sup> / day of liquid effluent (5). Only a fraction of chromium is absorbed in the tanning process and the remaining major part of chromium discharged through the effluent. It has been reported that these industries release 2,000 – 3,200 tonnes of Cr into the environment through effluent

annually (6). The concentration of Cr in the tannery waste is about 20.000 ppm, about 1800 ppm in the sludges and 200 ppm in the composite effluent.

Chromium is a transition metal located in group VI B of the periodic table. It is usually exist in the environment in two forms; hexavalent chromium ( $\text{Cr}^{6+}$ ) and trivalent chromium ( $\text{Cr}^{3+}$ ). Hexavalent Cr usually occurs associated with oxygen as chromate ( $\text{CrO}_4^{2-}$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) oxyanions which are highly soluble in water. It is highly mobile, and considered as the most toxic form of chromium. On the other hand, trivalent chromium ( $\text{Cr}^{3+}$ ) is less mobile, less toxic and is mainly found bound to organic substances in the environment. (7, 8). Presence of excess amount of Cr beyond the tolerance limit makes it unsuitable for crop growth. Even, the lower concentration of chromium ( $>2$  ppm) inhibited the plant growth (9, 10).

Both forms, Cr(III) and Cr(VI) may be taken up by plants. Uptake of Cr(III) seems to be passive while that Cr(VI) is considered to be active (11). It has been reported that chromium interferes with several metabolic activities and its toxicity to plants and is exhibited reduced growth and phytomass, foliar chlorosis, stunting and finally plant death (12, 13, and 14). These untreated or partially treated effluents discharged into nearby water bodies and these polluted water is used for irrigation due to scarcity of water. The continuous use of polluted water degraded the crop productivity, soil fertility and had elevated the levels of available heavy metals in the soils of cultivated land.

In the present study, soybean was selected as test plant to investigate the impact of exogenous application of various levels of chromium on its growth metabolism. Soybean is widely cultivated for vegetable and pulse because of its high protein content. The various growth parameters like seed germination percentage, growth, accumulation of Cr, changes in biomolecules (chlorophyll, protein, aminoacid, sugar, proline) and antioxidant enzymetic activities (catalase, peroxidase, polyphenol oxidase and superoxide dismutase) was investigated.

## 2. MATERIALS AND METHODS

### 2. 1. Preparation of Chromium Solution

Chromium was given as potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ). The chromium solutions were prepared by dissolving 2.9583 g of Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) salt in 1000 ml of deionized water. From this standard solution, the various concentrations (5, 10, 25, 50, 100, 200 and 300 mg/l) of chromium solution were prepared and used for laboratory studies.

### 2. 2. Plant Materials and Culture Condition

The seeds of soybean (*Glycine max* L. Merr var. p.1) was collected from School of Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The healthy seeds of soybean (*Glycine max* L) were surface sterilized with 0.1 per cent mercuric chloride for 2 min and washed thoroughly with tap water and then with distilled water. The sterilized seeds of soybean were arranged equispacially in petriplates lined with filter paper. They were irrigated uniformly with equal volume of different concentrations (5, 10, 25, 50, 100, 200 and 300 mg/l) of chromium solution. The seeds irrigated with distilled water were maintained as control. They were allowed to grow for 15 days. Three replications were maintained for this experiment. When the seedlings were 7 and 15 days old, seedlings

from control and treated harvested and different parameters were analysed. Germination percentage of seeds was recorded by calculating the number of seeds germinated with respect to control. For measurement of seedling growth, twenty seedlings were randomly selected from both control and treated sample. Their root and shoot length were measured by using cm scale and expressed in cm/seedling. The seedlings were weighed and their fresh weight were expressed in g/seedlings. Their dry weight were taken by keeping the plant materials in an hot air oven at 80 °C for 24 hrs. The morphological growth parameters such as germination percentage, root length, number of root nodules, shoot length, fresh weight and dry weight were taken on 7<sup>th</sup> and 15<sup>th</sup> day old seedling.

### **3. ESTIMATION OF PHOTOSYNTHETIC PIGMENTS**

#### **3. 1. Chlorophyll (15)**

The chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid contents were estimated and expressed in mg/g fresh weight basis. Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 ml of 80 per cent acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved and the absorbance values were read at 645 nm and 663 nm in UV-Spectrophotometer (Hitachi) and the same acetone extract was read at 480 nm in UV-Spectrophotometer. The carotenoid content of soybean was estimated by the methods given by Kirk and Allen (16).

#### **3. 2. Estimation of sugars (17)**

Five hundred mg of plant materials (root and shoot) were weighed and macerated in a pestle and mortar with 10 ml of 80 per cent ethanol. One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 min at 100 °C in a boiling water bath, cooled and added 1 ml of arsenomolybdate reagent. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in UV-Spectrophotometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

#### **3. 3. Estimation of protein (18)**

Five hundred mg of plant materials (root and shoot) were weighed and macerated in a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. One ml of the extract was taken in a 10 ml test tube and 5 ml of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in UV-Spectrophotometer. The protein contents were expressed in mg/g fresh weight basis.

#### **3. 4. Estimation of amino acids (19)**

Five hundred mg of plant materials (root and shoot) were weighed and macerated with a pestle and mortar with 10 ml of 80 per cent ethanol. One ml of the extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of Ninhydrin reagent was added and mixed

thoroughly. The content of the test tube was heated for 20 min in a boiling water bath. Five ml of the diluent solution was added and heated in water bath for 10 min. The absorbance was read at 570 nm in UV-Spectrophotometer. The amino acid contents were expressed in mg/g fresh weight basis

### **3. 5. Estimation of proline (20)**

0.5 g of plant material (root and shoot) was homogenized using 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered by Whatman no.1 filter paper. 2 ml of acid ninhydrin (1.25 g of Ninhydrin + 30 ml of glacial acetic acid + 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid were added. The sample was heated for an hour at 100 °C in water bath and 4 ml of Toluene was added. This solution was mixed well and read at 520 nm in UV – Spectrophotometer.

### **3. 6. Estimation of Chromium (21)**

500 mg of Oven dried (80 °C) plant tissues of each treatment was digested in tri-acid mixture (HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and HCl 5:1:1 ratio v/v), filtered and brought upto 100 ml with distilled water. The solution was read at 540 nm in an Atomic Absorption Spectrophotometer. The digested sample were filtered by using whatmann No.42 filter papers and the filtrate was read at 540 nm in an Atomic Absorption Spectrophotometer. The instrument was calibrated using standard stock solution of Cr.

## **4. ASSAY OF ANTIOXIDANT ENZYMES**

### **4. 1. Catalase (22)**

One gram of leaf sample was homogenized in 10 ml of 0.1 M phosphate buffer (pH 7) and centrifuged at 4 °C for 10 min at 10,000 rpm. An aliquot of 1 ml of the supernatant of the enzyme extract was added to the reaction mixture containing 1 ml of 0.01 M H<sub>2</sub>O<sub>2</sub> and 3 ml of 0.1 M sodium phosphate buffer. The reaction was stopped by adding 10 ml of one per cent H<sub>2</sub>SO<sub>4</sub> and it was incubated at 20 °C for 5 min. The acidified medium with or without the enzyme extract was titrated against with 0.005 N KMnO<sub>4</sub> and catalase activity was expressed as nmoles of H<sub>2</sub>O<sub>2</sub> utilized (units min<sup>-1</sup> mg<sup>-1</sup> protein).

### **4. 2. Peroxidase (22)**

One gram of fresh plant material was homogenized with 20 ml of ice-cold extraction medium containing 2 mM MgCl<sub>2</sub>, 1 mM EDTA, 10 mM β-mercapto ethanol, 7 per cent PVP and 10 mM sodium meta bisulphate. The homogenate was stained through two layers of cheese cloth and centrifuged at 10,000 rpm for 15 min. The supernatant was made upto 20 ml with the same buffer and it was used as the source of enzyme. Assay mixture of peroxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.001 M pyrogallol, 12 ml of 0.005 M hydrogen peroxide and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25 °C, after which the reaction was terminated by adding 1 ml of 2.5 N sulphuric acid. The amount of purpurogallin formed was determined by reading the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N sulphuric acid. The activity was expressed in unit = 0.1 absorbance min<sup>-1</sup> mg<sup>-1</sup> protein.

## **5. POLYPHENOL OXIDASE (PPO, EC 1.10.3.1)**

The assay polyphenol oxidase was (PPO) carried out by the method of Kumar and Khan (23). Assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer (PH 6.0) 1 ml of 0.1 M catechol and 0,5 ml of enzyme extract. This was incubated for 5 min at 25 °C, after which adding 1ml of 2.5 N H<sub>2</sub>SO<sub>4</sub> stopped the reaction. The absorbancy of the purpurogalolin formed was read at 495 nm. To the blank, 2.5 N H<sub>2</sub>SO<sub>4</sub> was added of the zero time of same assay mixture. PPO activity is expressed in U mg<sup>-1</sup> protein (U = Change in 0.1 absorbance min<sup>-1</sup> mg<sup>-1</sup> protein). For all the enzymatic calculations, protein was determined by the method of Bradford (24), using Bovine Serum Albumin (BSA, Sigma, USA) as the standard.

## **6. SUPEROXIDE DISMUTASE (SOD, EC 1.15.1.1)**

Curde enzyme extract was prepared, for the assay of superoxide dismutase (SOD) by the method of Hwang et al., (25).

## **7. EXTRACTION**

One gram of fresh tissue was homogenized with 10 ml of ice cold 50 mM sodium phosphate buffer containing 1mM PMSF. The extract was filtered through a double layered cheese cloth. The extract was centrifuged at 12,500 rpm for 20 minutes at 4 °C, The supernatant was saved and made upto 10 ml with extraction buffer and used for estimation of the SOD enzyme activity, The enzyme protein was determined by Broadford (24) method.

## **8. ESTIMATION**

Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich (26). The reaction medium was prepared and 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17 x 10<sup>-6</sup> M riboflavin, 0.1 μ methionine, 2 x 10<sup>-5</sup> potassium cyanide and 5.6 x 10<sup>-5</sup> M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by 2 sets of Philips 40 W florescent tubes. Illumination started to initiate the reaction at 30°C for one hour. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the Spectrophotometer against blank. SOD activity was expressed in units. One unit is defined as the amount of changes in the absorbance by 0.1 per milligram protein under the assay condition. All the experiments were performed at least for three times with three replicates in each time. The mean values are presented in figures.

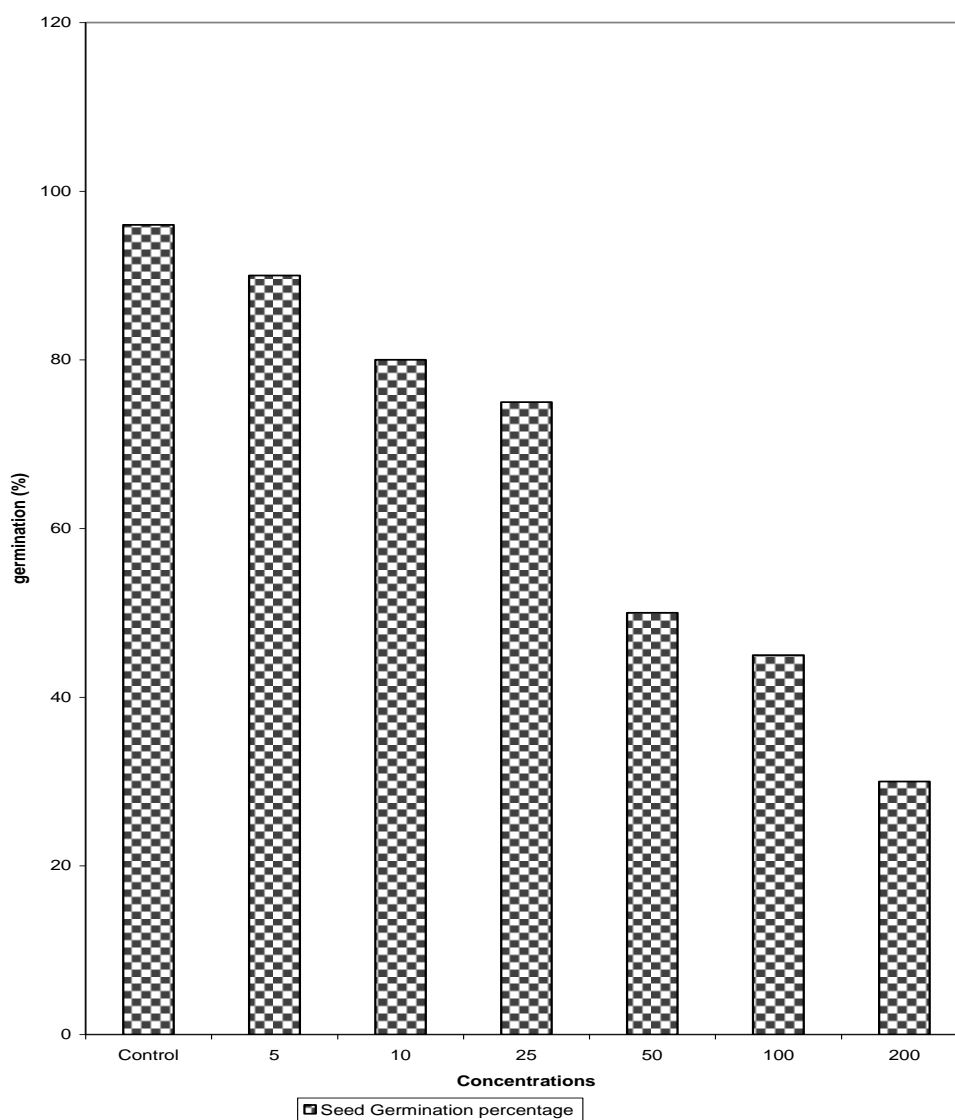
## **9. DISCUSSION**

Heavy metals are one of the most important groups of pollutants of aquatic environment and it is considered to be a serious environmental problem facing the modern world (27). Addition of heavy metals like Al, Cu, Hg, Fe, Cr, Cd and Pb etc., into the environment and

their subsequent toxic and carcinogenic effects on flora and fauna cause a great ecological crisis at global level. By this way, it is gaining importance due to its obvious impact on human health through food chain. Among the heavy metals, chromium is considered to be toxic to living organisms. It is released into environment along with their effluents from various industries such as leather tanning, electroplating units, iron and steel industries, dyeing industries, metal cleaning and processing, textile, food processing units and ceramics and polluted nearly water bodies. When these wastewater containing heavy metals used for irrigation it affects the growth performance of crop plants. In the present study, the varying levels of Cr on soybean metabolism discussed in this chapter.

## 10. SEED GERMINATION PERCENTAGE AND GROWTH

**Fig.1 Effect of Different concentrations of Chromium on seed germination of soybean**



Germination, the critical phase in the life cycle of a crop plant, is subjected to numerous environmental stresses. Any disturbance in the environment in which the seed germinates affects the germination and ultimately the growth, dry weight and yield of crop (28). Seed germination and growth are of vital importance for continuation of plant life. Seed germination is defined as the resumption of metabolic activity. The growth of an embryo starts with the rupture of the seed coat and the emergence of the young plant. The time between the seed sowing and seedling establishment is considered to be the crucial period of any plant. (29). The most common response of plants to metal phytotoxicity is growth inhibition. Plants tolerance to heavy metal phytotoxicity is usually estimated on the basis of the degree of inhibition in growth by the metal. Seed germination is considered as a sensitive process compared to other stages of plant development and represents a limiting stage of plant life cycle under heavy metal toxicity.

The percentage of germination decreased with the increase of chromium concentrations. No germination was recorded beyond 300 mg/l concentration of chromium. Similar inhibition of germination percentage at higher concentrations of chromium was observed in blackgram (30), greengram (31) and paddy, blackgram and soybean (32, 33, 34).

The reduction in germination percentage of plants at higher chromium concentrations may be attributed to the interference of metal ions, which may inhibit seed germination by exerting unfavourable effect on the activities of hydrolytic enzymes involved in the mobilization of major seed reserves such as starch, protein, RNA and phytin (35, 36, 37). The reduced germination of seeds under chromium stress could be a depressive effect of chromium on the activity of amylases and on the subsequent transport of sugars to the embryo axes (38, 39).

## **11. SEEDLING LENGTH**

Seedling stage is the most sensitive stage in the life of a plant and hence it is more susceptible to physical and chemical adversities. Presence of chromium in the irrigation water results the changes in the growth and development pattern of the plant (40). Decrease in root and shoot length are a well documented effect due to heavy metal toxicity in plants (41).

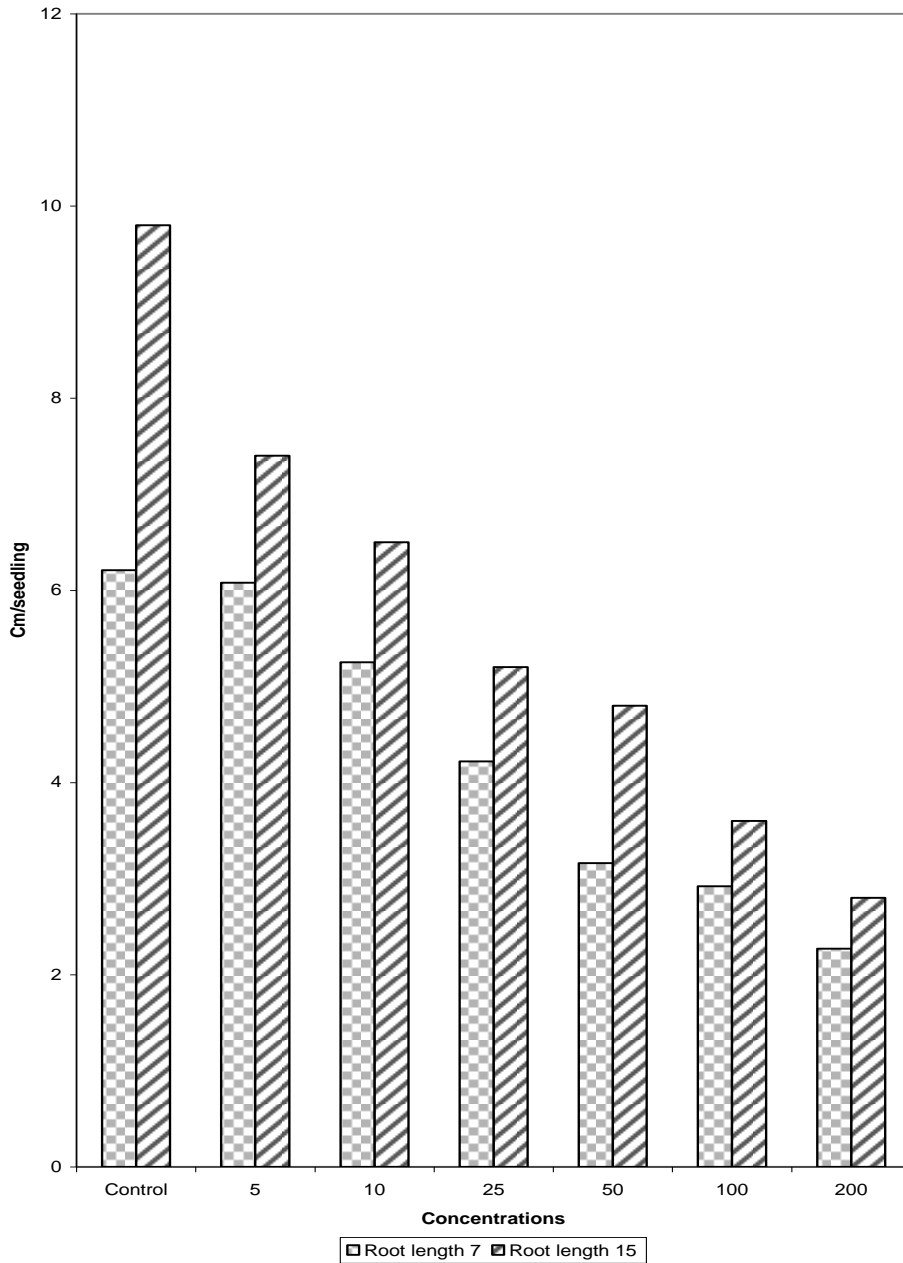
In this experiment, the seedling length gradually decreased with the increase in chromium concentrations. The seedling growth parameters like shoot length and root length declined with the increase in chromium concentrations. The seedling growth was totally affected at 300 mg/l concentration.

The reduction in seedling length at higher chromium concentrations was recorded in various crops such as cowpea (42), mungbean (43, 31) and greengram (44). It has been already reported that chromium toxicity can reduce radicle growth of plants (45, 40). It may also be due to the direct contact of seedling roots with chromium in the medium. It caused a subsequent inability of roots to absorb water from medium (11). Inhibition in growth of roots leads to the reduction in water and mineral elements absorption. (2, 3). The altered root growth also affected the shoot growth since there was limited nutrient supply to shoot tissues. Besides, the Cr transported to the aerial parts could have also affected the physiological processes contributing to the reduction in the plant height. (6). Chromium at higher concentrations may inhibit the shoot and root growth directly by inhibition of cell division or cell elongation or combination of both (46). The poor seedling growth in chromium treatment may be due to the poor breakdown of starch by amylase activity. The reduction in seedling



length may also be due to the deleterious effects of heavy metals on the hydrolytic enzymes present in the storage organs as observed in other crops (47, 48, 49).

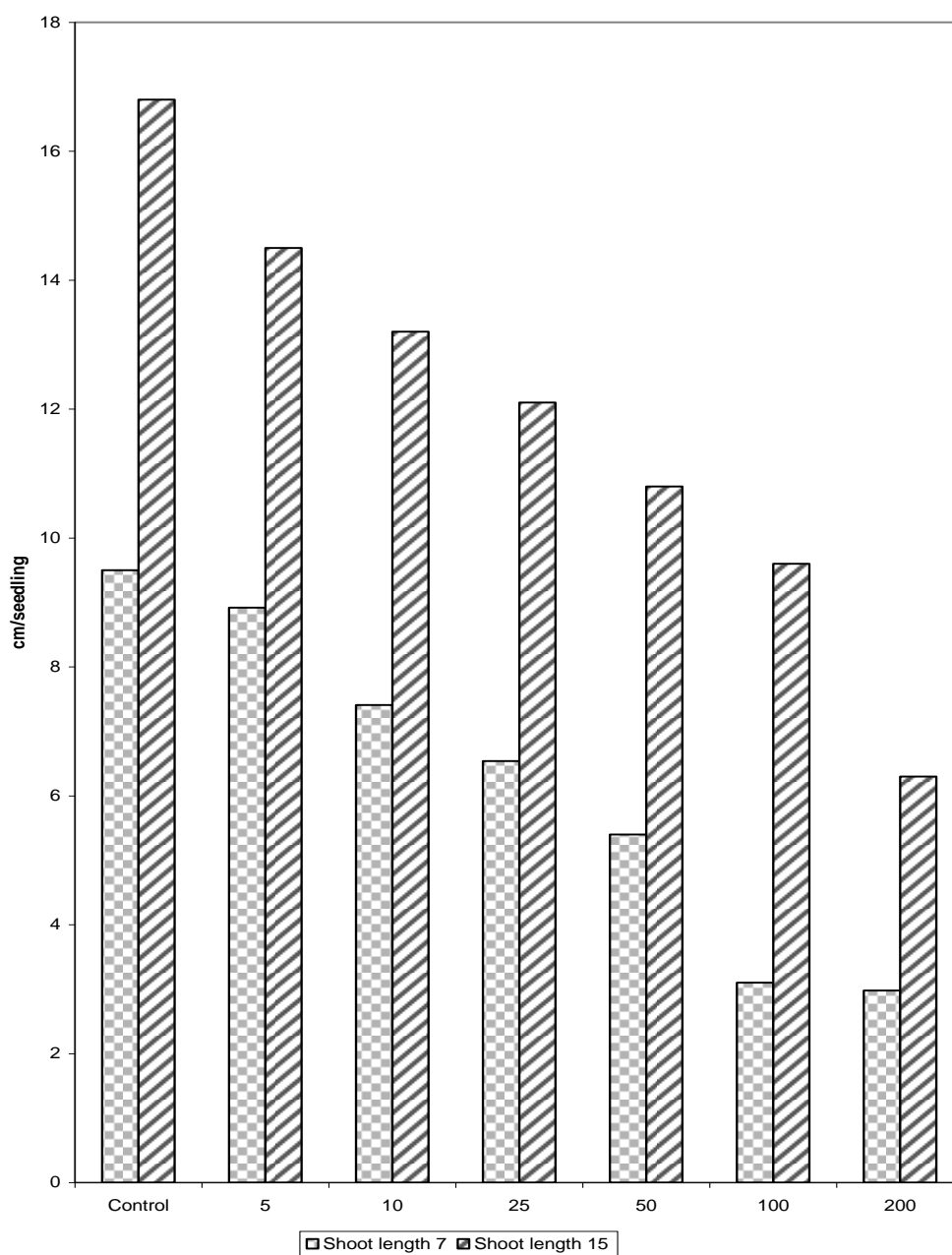
**Fig.2 Effect of Different concentrations of Chromium on root length (cm/seedling) of soybean**



There was a gradual decrease in seedling fresh weight and dry weight with the progressive increase in chromium concentrations. Chromium interfered with the seedlings biomass accumulation from lower concentrations to higher concentrations. Similar type of reduction in fresh weight and dry weight of seedlings were reported due to increasing

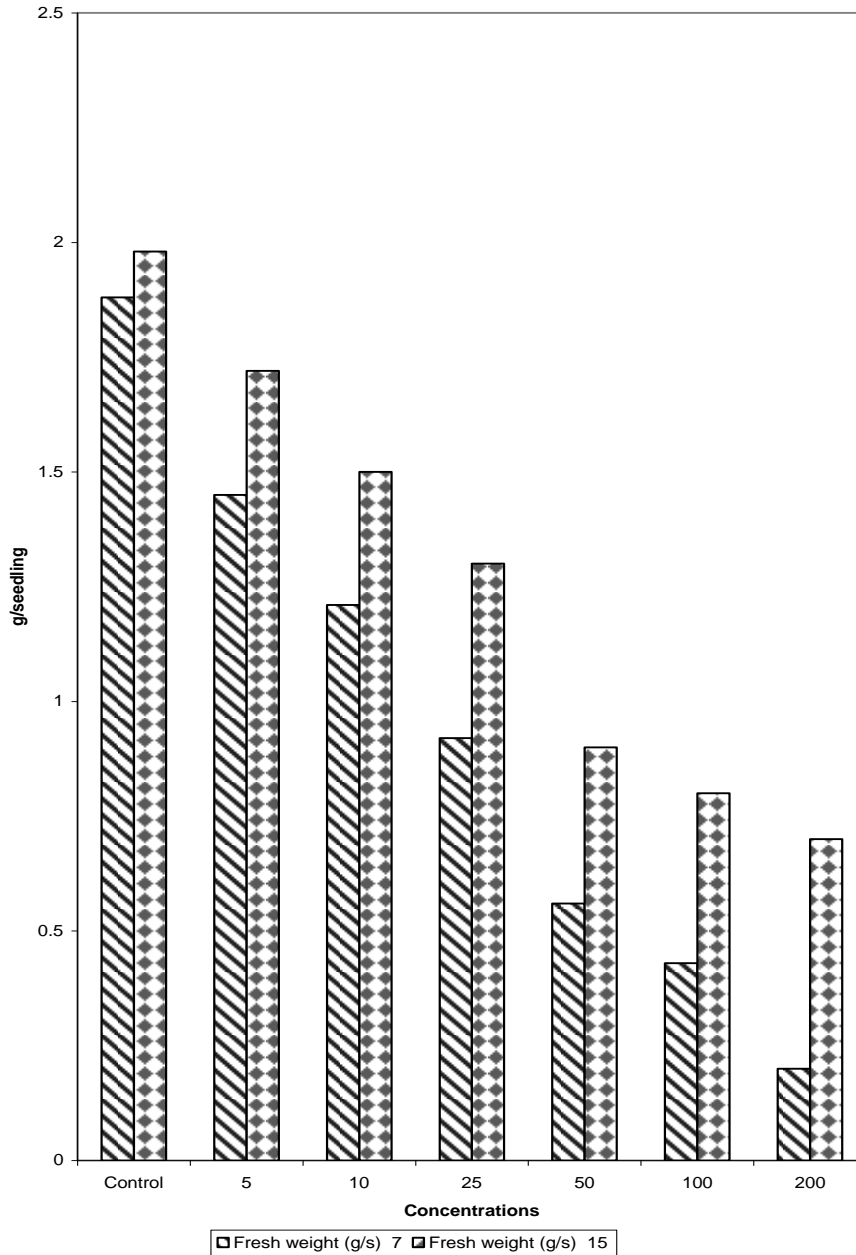
chromium concentrations (50, 32). Higher uptake of chromium by roots resulted into its reduced fresh weight and dry weight (51, 52, 53). The observed reduction in dry weight of seedlings under chromium stress was due to poor growth of seedlings in agreement with the result of Subramani *et al.* (36); Lakshmi and Sundaramoorthy (30) and Nath *et al.* (10). A decrease in biomass productivity might be attributed to a disruption in nitrogen metabolism of seedlings under chromium stress (54). The decrease in fresh weight and dry weight of seedlings is mainly due to the inhibition of water uptake and enlargement of root cells (55, 56).

**Fig.3 Effect of Different concentrations of Chromium on shoot length (cm/seedling) of soybean**



### 11. 1. Fresh weight and dry weight of seedling

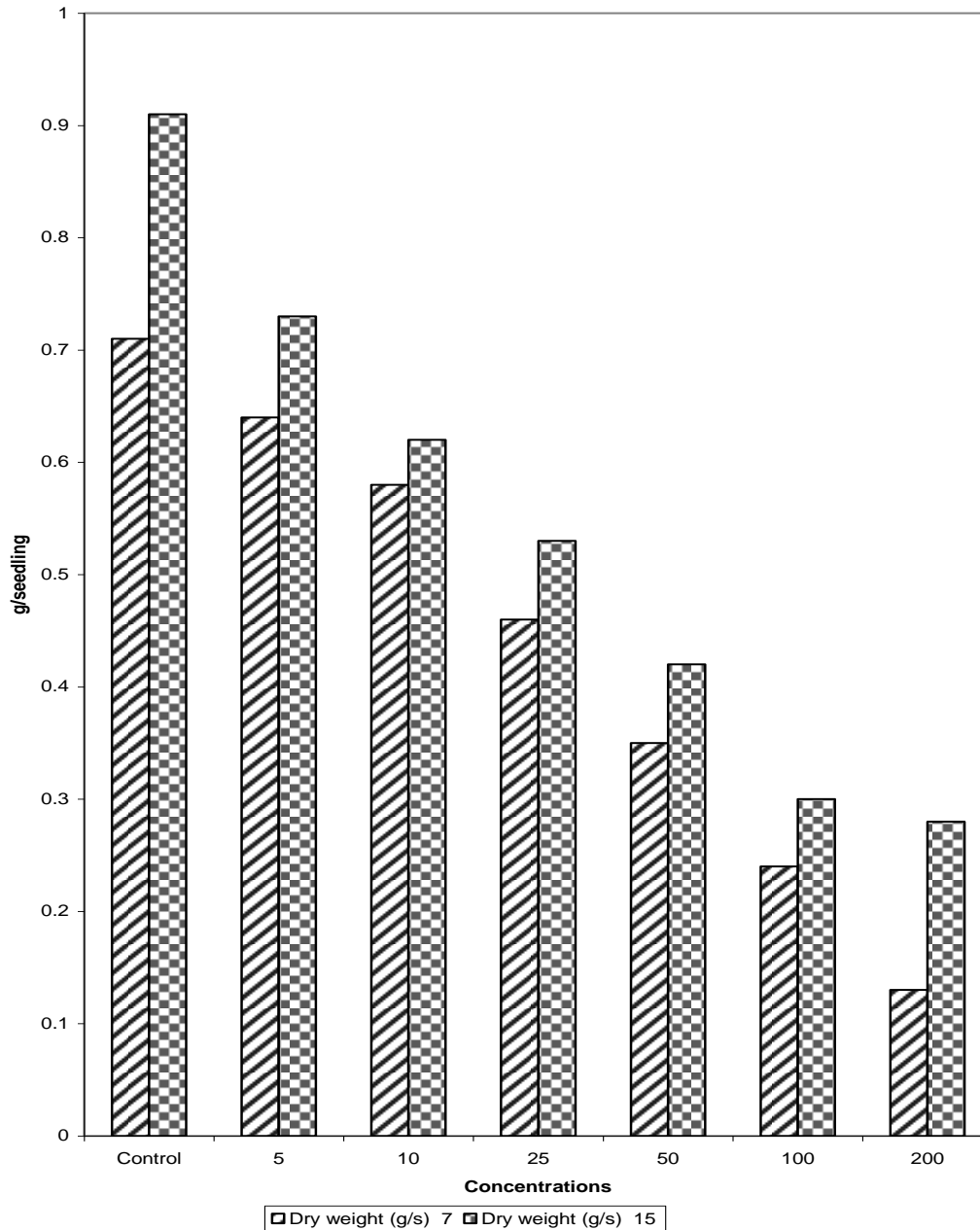
Fig.4 Effect of Different concentrations of Chromium on fresh weight (g/seedling) of soybean



There was a gradual decrease in seedling fresh weight and dry weight with the progressive increase in chromium concentrations. Chromium interfered with the seedlings biomass accumulation from lower concentrations to higher concentrations. Similar type of reduction in fresh weight and dry weight of seedlings were reported due to increasing chromium concentrations (50, 32). Higher uptake of chromium by roots resulted into its reduced fresh weight and dry weight (51, 52, 53). The observed reduction in dry weight of

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**Fig.5 Effect of Different concentrations of Chromium on dry weight (g/seedling) of soybean**

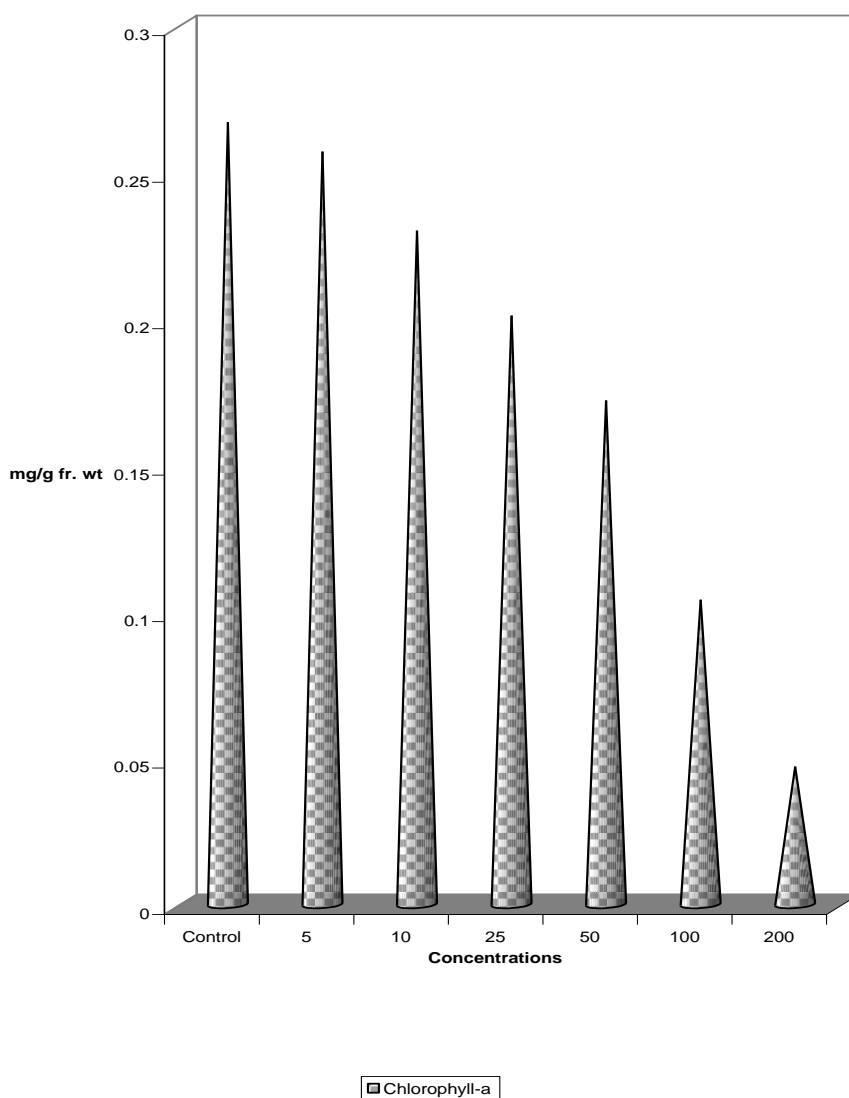


## 12. BIOMOLECULES

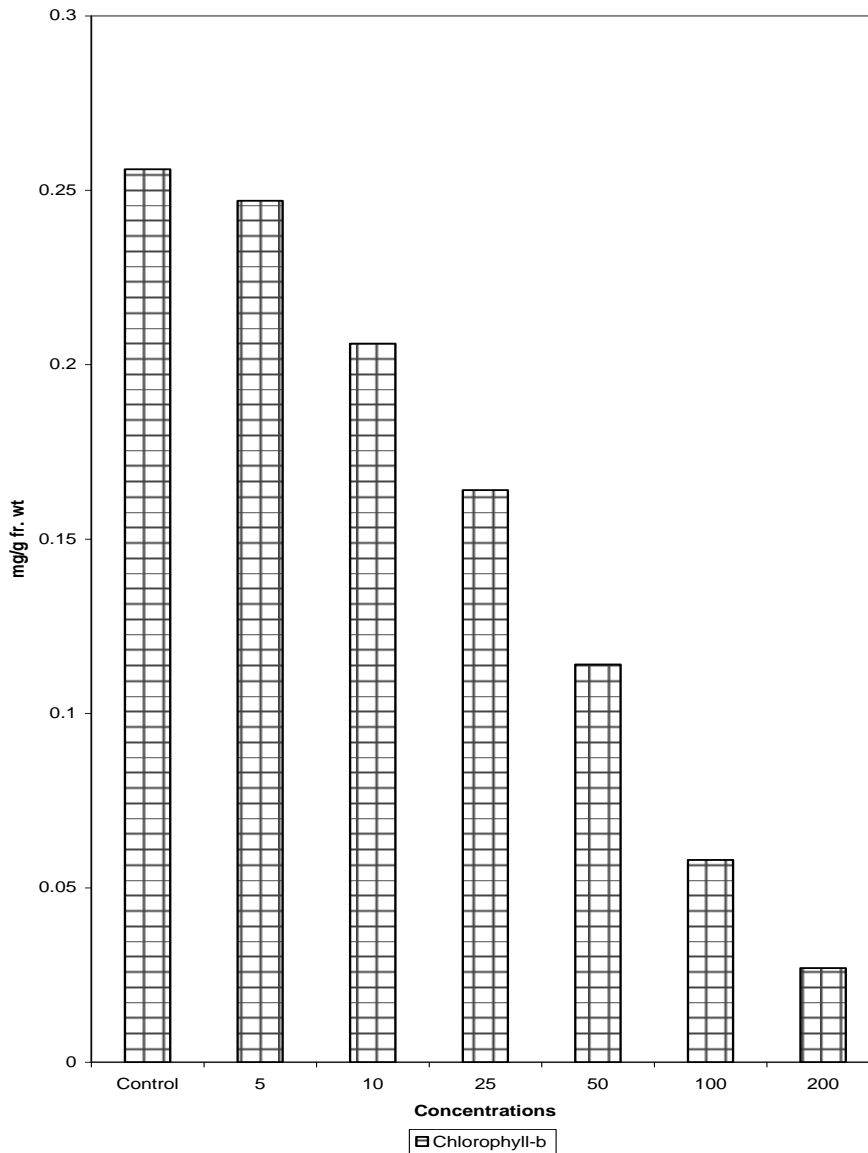
The seed germination and growth of crops are more vulnerable to pollution stress. Reduction in germination and growth of crops grown under chromium treatments ultimately led to impairment in various biochemical constituents. So, it was planned to investigate some biochemical constituents of soybean seedlings grown under various concentrations of chromium. In order to obtain a better understanding of the biochemical effect, the changes in chlorophyll, sugar, amino acids and protein contents have been discussed under chromium stress.

### 12. 1. Photosynthetic pigments Chlorophyll

**Fig.6 Effect of Different concentrations of Chromium on Chlorophyll-a content (mg/g fr. wt.) of soybean**

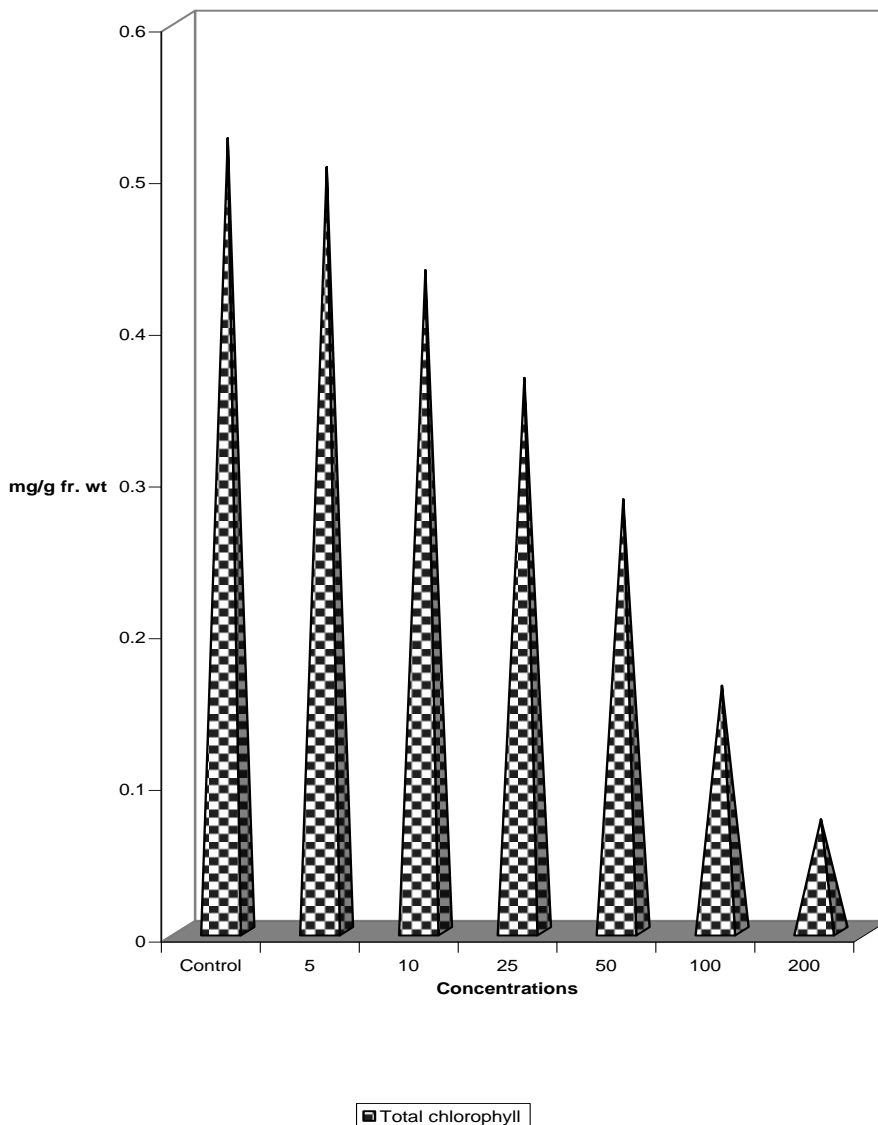


**Fig.7 Effect of Different concentrations of Chromium on Chlorophyll-b content (mg/g fr. wt.) of soybean**



Chlorophyll is an integral component of plant pigments and plays a vital role in the process of photosynthesis. In the present study, the effect of various concentrations of chromium on chlorophyll 'a', chlorophyll 'b' and total chlorophyll of soybean seedlings were estimated in the laboratory studies. The reduction in chlorophyll content was observed from 5 to 200 mg/l chromium concentrations. Chromium induced plants showed similar changes in pigment contents of various plants such as *Lycopersicam esculentum* (57), *Cuscuta reflexa* (58) and *Pistia stratiotes*, *Eichhornia crassipes*, *Lemna minor* (59). Similar findings of the reduction in chlorophyll contents of various crops treated with chromium were already reported in mungbean (9,31) and cauliflower (54).

**Fig.8 Effect of Different concentrations of Chromium on Total Chlorophyll content (mg/g fr. wt.) of soybean**

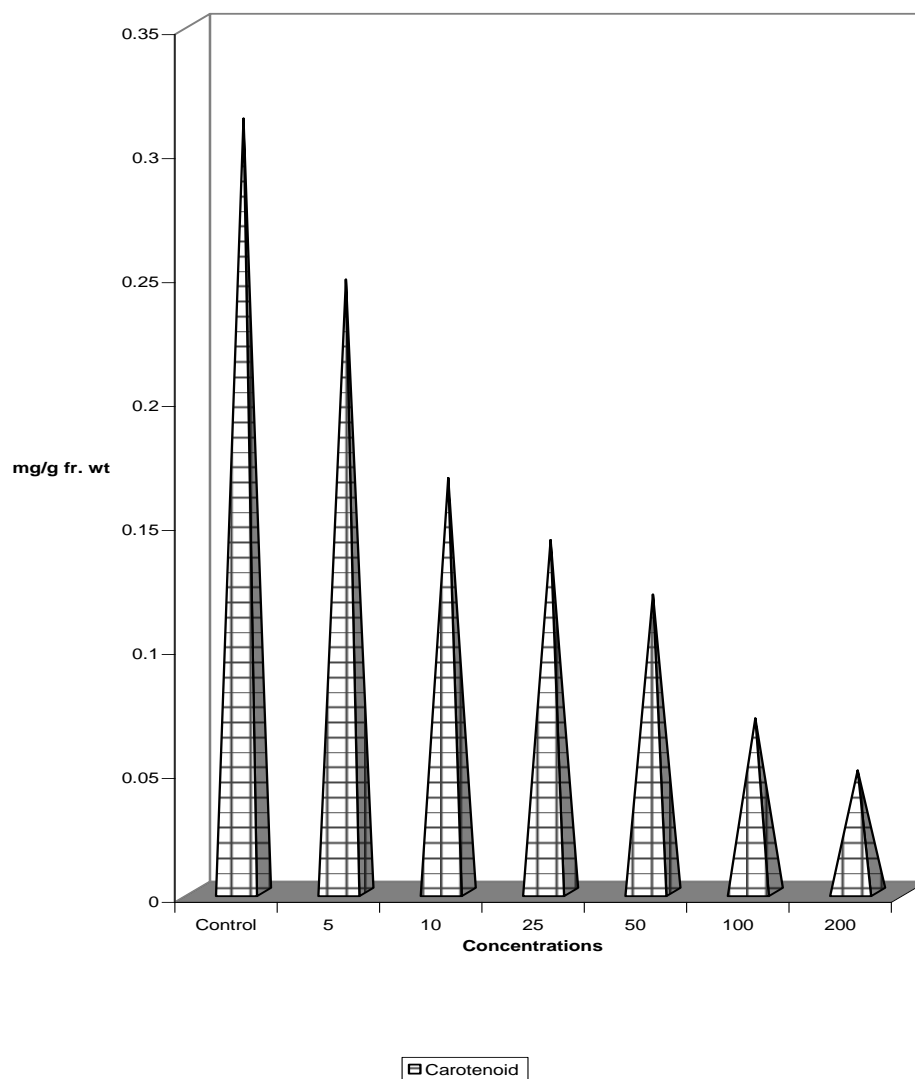


The decrease in chlorophyll content may be due to the interference of Cr with pigment metabolism. Cr absorbed by plants caused inhibition of important enzymes in chlorophyll biosynthesis. Impaired  $\alpha$ -aminolaevulinic acid dehydrogenase activity and photochlorophyllide reductase leading to reduced photosynthetic pigments has been observed in chromium treated *Nymphaea alba* (12). The decrease in chlorophyll content may be due to destabilization and degradation of proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll content in most plants under chromium stress was reported (40).

## 12. 2. Carotenoid

Carotenoid is an accessory pigment on photosynthesis. The gradual decline in carotenoid biosynthesis was observed in soybean seedlings with the increasing chromium concentrations. The carotenoid content was found to decrease with increase in Cr Concentration as compared to control. The reduction in carotenoid content was observed from 2.5 to 200 mg/l concentrations.

**Fig.9 Effect of Different concentrations of Chromium on Carotenoid content (mg/g fr. wt.) of soybean**

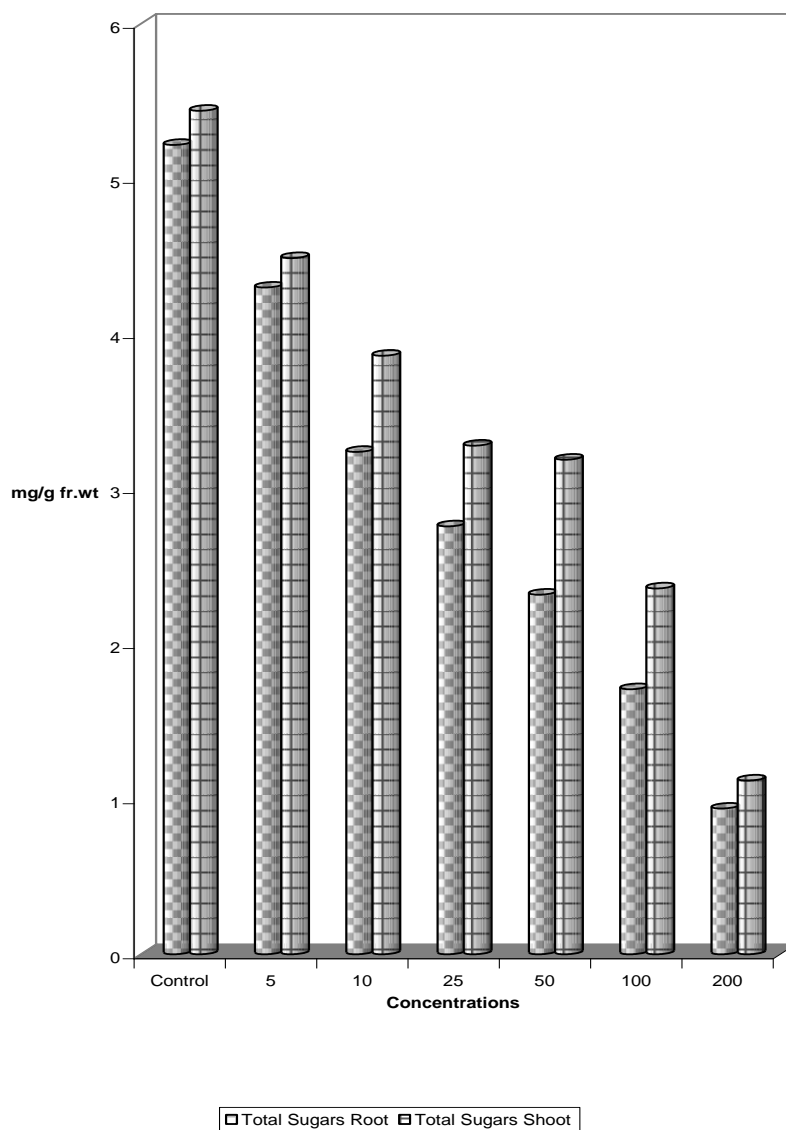


The higher concentrations of chromium severely affected the carotenoid biosynthesis. Similar findings of the decline in carotenoid content were reported in various test crops (60, 61, 31, 62, 40). Hexavalent chromium can replace magnesium ions from the active sites of many enzymes and deplete chlorophyll and carotenoid content (12)



### 12. 3. Total Sugars

**Fig.10 Effect of Different concentrations of Chromium on Total Sugar content (mg/g fr. wt.) of soybean**

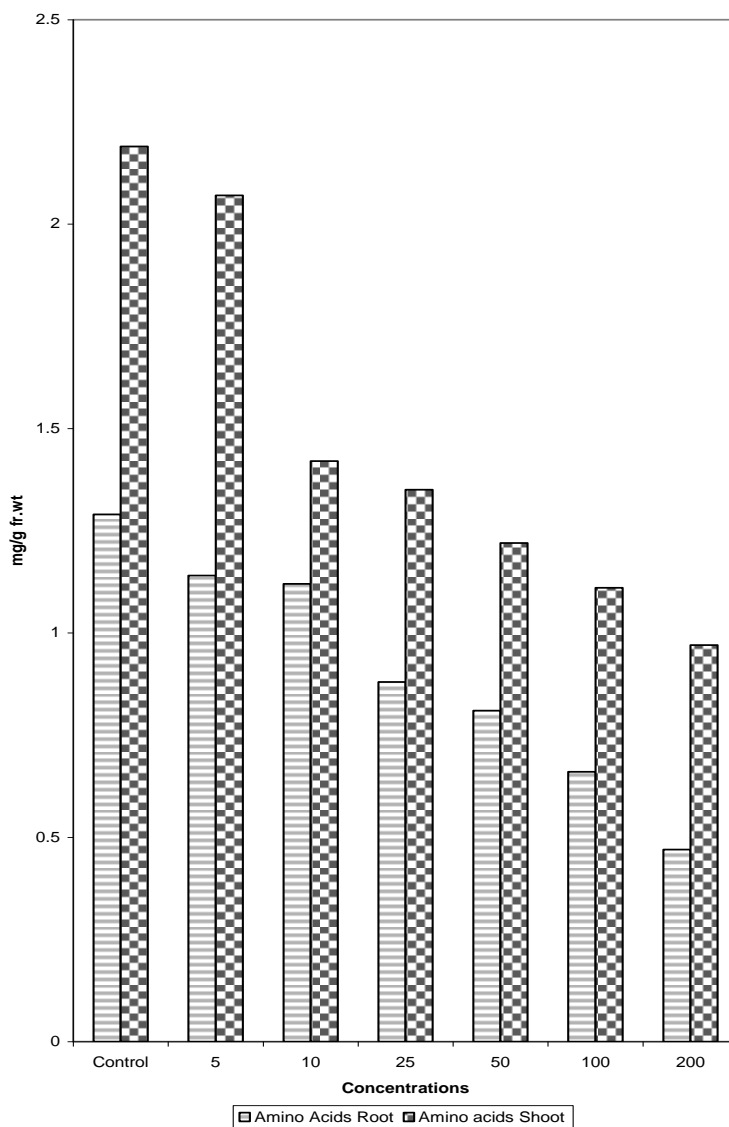


Sugar, an important constituent source of energy is needed for all living organisms. The concentration of soluble sugars is indicative of the physiological activity of a plant and it determines the sensitivity of plants to pollution. The sugar contents decreased gradually with the increase of chromium concentrations. Similar findings of sugar content were reported in various crop plants such as *Phaseolus mungo* and potato (63), sugarcane (64), blackgram (30) and water lilies (65). The lesser sugar content in the root and stem at high concentrations of chromium treatments implies the deranged metabolism and poor translocation of sugars and

starch metabolites to the growing parts. The decline in sugar formation may be associated with reduced rates of photochemical activities and chlorophyll formation. Loss of sugar formation may also be due to the conversion of sugar into energy when the plants were stressed (66s).

#### 12. 4. Amino acids

**Fig.11 Effect of Different concentrations of Chromium on Amino Acids content (mg/g fr. wt.) of soybean**

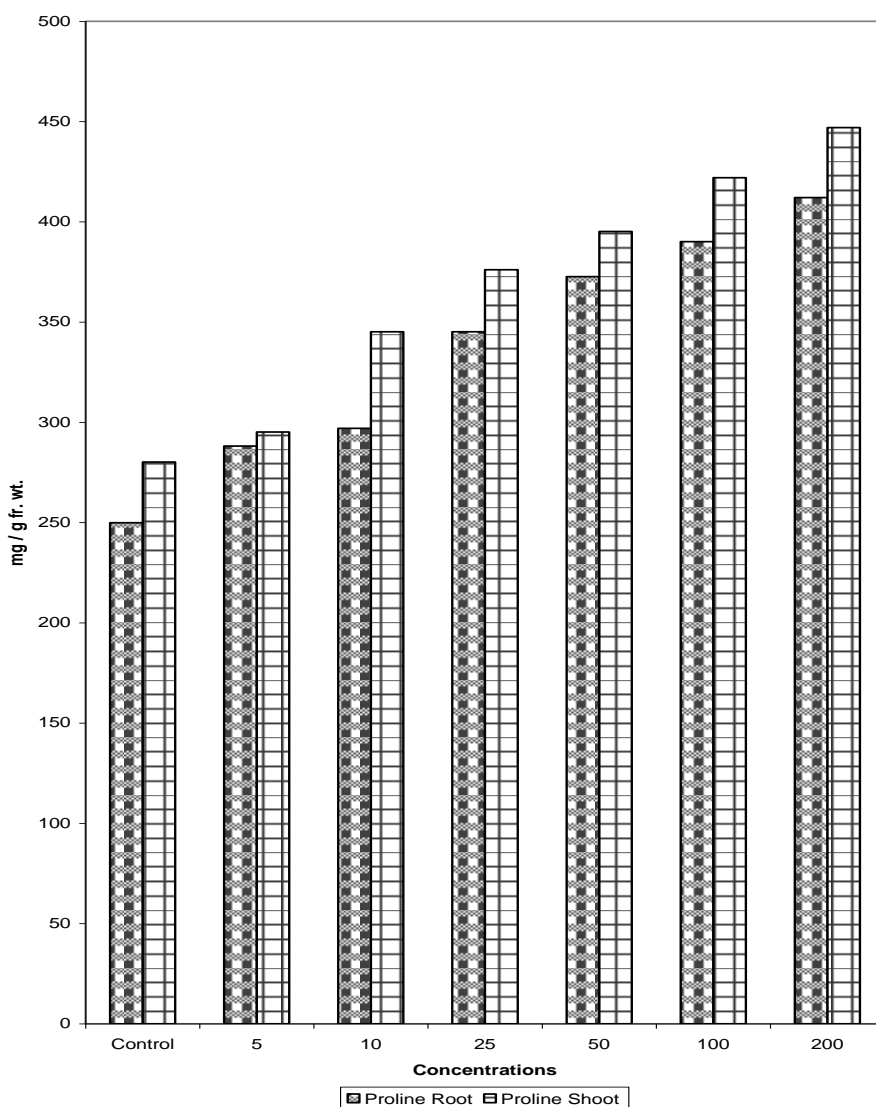


Amino acid is the monomer of protein, the common reserve food material manufactured by plants. There was a gradual decline in amino acid content with a progressive increase in chromium concentrations. A decrease in amino acids content at higher concentrations of metal has been reported in rice (67). Similar findings of changes in amino acid content in potato

(63) and soybean (68) were already reported under chromium treatment. The decrease in amino acid content may be due to the inhibitory act of the metal on protease activity. This tends support to the reports that the major amount of enzyme appeared to be synthesized could be stopped by an inhibitory action of metals on protein synthesis (69, 70). It is well known that nitrogen is a precursor for the synthesis of amino acids (71). Since nitrogen content of plants reduced by metal stress ultimately the amino acid content of plants also got reduced (72, 73).

### 12. 5. Proline

**Fig.12 Effect of Different concentrations of Chromium on Proline content (mg/g fr. wt.) of soybean**



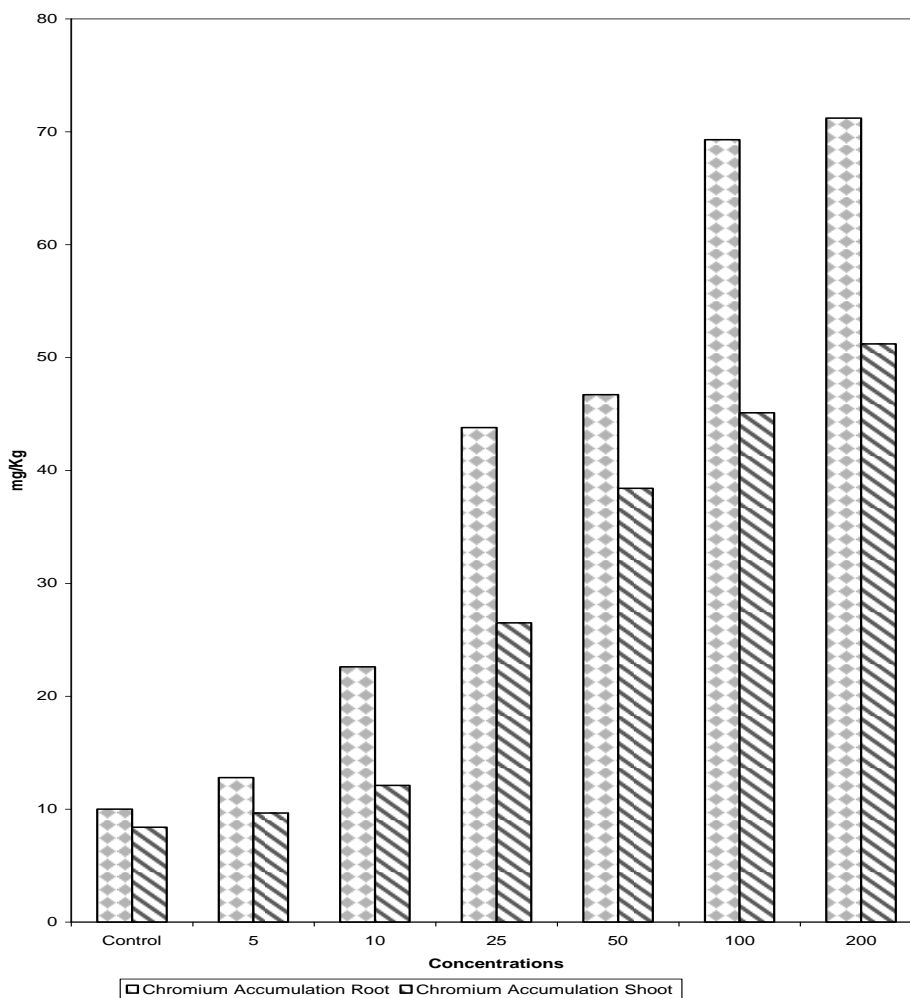
Proline, an aminoacid is well known to get accumulated in wide variety of organisms on exposure to abiotic stress. Chromium significantly increased proline content with increasing concentrations in soybean seedlings. The maximum proline content was recorded in the

seedlings grown in 300 mg/l concentrations of chromium. Higher accumulation of proline in chromium treated seedlings might be attributed to the strategies adopted by plants to cope with or toxicity. It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organells. Increase in proline content may be either due to denovo synthesis or decreased degradation or both. Proline increases the stress tolerance of plants through such mechanism as osmo-regulation, protection of enzymes against denaturation, and stabilization of protein synthesis. Similar results were observed by Rai *et al.*, (62).

Increased levels of heavy metals are known to affect permeability of membrances which may lead to a water stress like condition and inducing the production of proline (74). As proline has multiple functions such as osmoticum, scavenger of free radicals, protector role of cytoplasmic enzymes, sources of nitrogen and carbon for past stress growth, stabilization of membrances, machinery of protein synthesis and a sink for energy to regulate redox potential any impairment in proline content will reflect in the growth of plant.

## 12. 6. Cr uptake and accumlaion

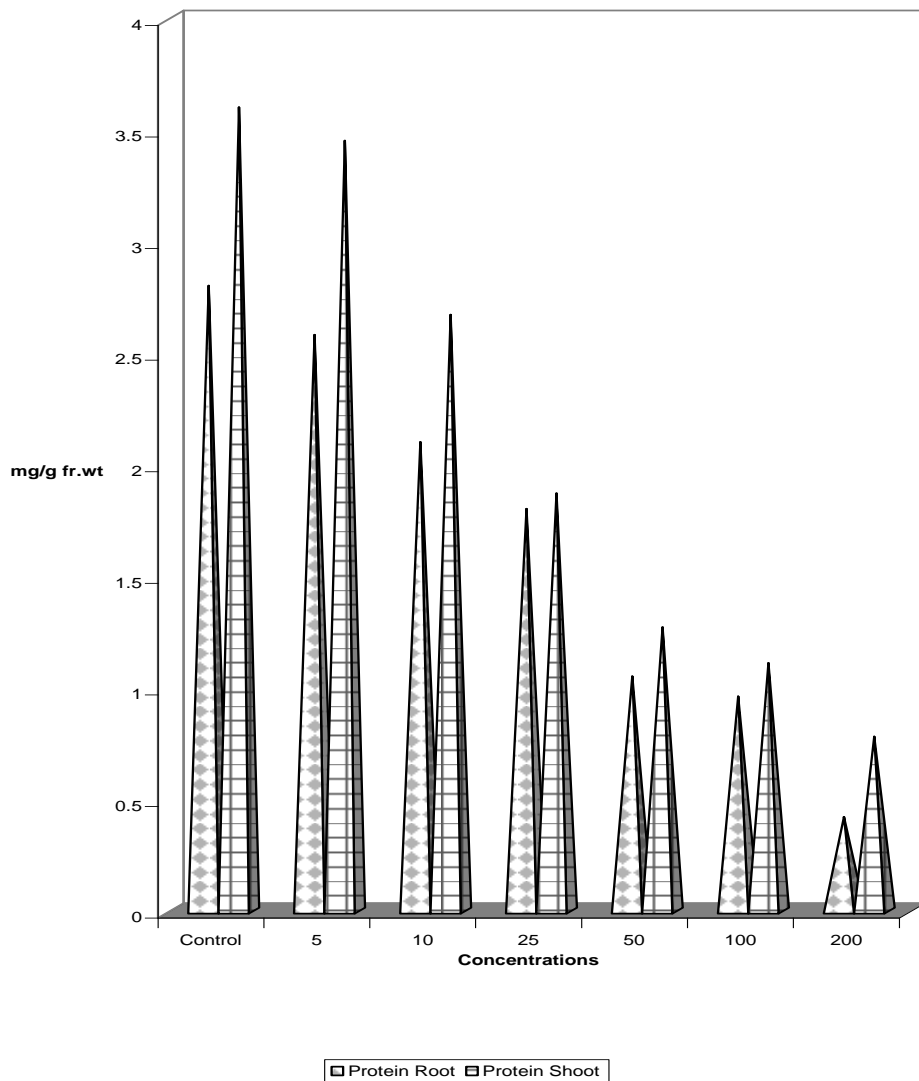
**Fig.13 Effect of Different concentrations of Chromium on Chromium Accumulation (mg/Kg) in soybean**



Cr is a toxic, non-essential element to plants. It is also taken by carrier used for essential metals. Cr uptake and translocation in different plant parts were variable with respect to genus and species. In the present study, the highest and lowest accumulation of Cr was recorded in seedlings treated with 200 mg/l and 5 mg/l concentration respectively. Similar observations was made in various plants were recorded. (75, 76, 77, 78, 79, 62). Cr uptake by plants is mainly non-specific, probably as a result of uptake of essential nutrients and water (80). It has been reported that Cr which absorbed by roots poorly translocated and retained in the roots. Shanker *et al.*, (40) reported that poor translocation of Cr to shoots could be due to sequestration of roots of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant.

## 12. 7. Protein

**Fig.14 Effect of Different concentrations of Chromium on Protein content (mg/g fr. wt.) of soybean**



Protein is one of the reserved food materials, which is utilized for the growth of seedlings and further growth of plants. It may be considered as important indicators to assess the growth performance of plants under stress conditions.

The protein content of soybean gradually decreased with increase in chromium concentrations. This could be due to the transportation of the nitrogen absorbed by the plants at various stages of its growth (81). The shoot portion of soybean contains higher protein content than the root. Significant differences were observed in soybean grown in 200 mg/l concentrations of chromium. Similar findings were recorded in *Vigna unguiculata* (82), *Triticum aestivum* (83), *Zea mays* (84), *Eichhornia* and *Pistia* (75), *Ocimum tenuiflorum* (62) and soybean and *Pistia* (37). Reduction in protein content at higher chromium concentration may be due to the enhanced rate of protein denaturation as suggested by Prasad and Inamdar (85).

### 13. ANTIOXIDANT ENZYMATIC ACTIVITY

The enzyme activities are the most sensitive parameters in evaluations and stress and plant systems. Heavy metal stress induced alteration in the activities of several representative of the enzymatic antioxidant defense system. Plant enzymes such as catalase and peroxidase play key roles in several metabolic pathways.  $H_2O_2$  produced either directly or indirectly in plants is metabolized by the catalases and peroxidases of plants (86, 87).

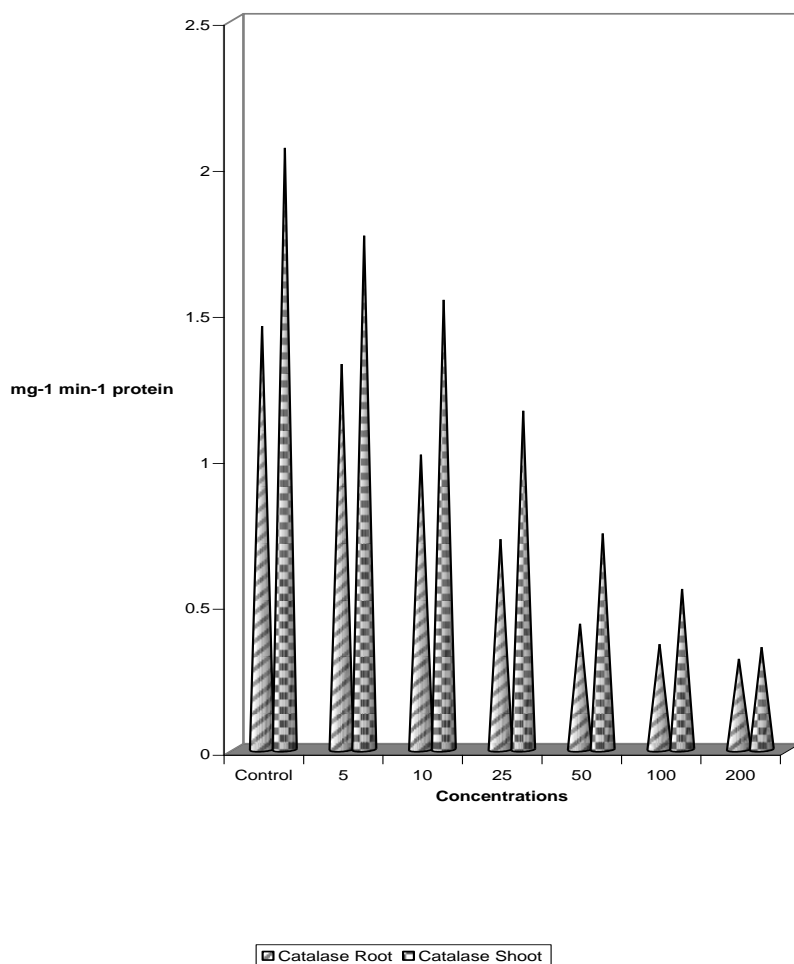
#### 13.1. Catalase

Catalase play a role in the protection against environmental stress (88). It seems that synthesis of catalase is initiated in early stages of seed germination and increased towards the later stage of seedling growth. A gradual decline in catalase activity of root and shoot was observed with increasing chromium concentration. The shoots contain higher catalase activity than in roots. The reduction of catalase was observed from 5 mg/l chromium concentrations onwards and it was severely affected at 200 mg/l chromium concentration. Activity of catalase in response to chromium has been studied in many crops like rice, wheat, greengram and even in lower plants like mosses (89, 90, 91, 92, 45, 93, 94, 95). The decrease in the enzyme activity may be due to accumulation of some inhibitory effect of chromium on the protein synthesis pathway (40, 10). Panda and Patra (90) reported that chromium ions increased the catalase activity in younger leaves while the activity decreased in older ones. It seems that synthesis of catalase is initiated in early stage of seed germination and increased towards the latter stage of seedling growth. But, the chromium in early stage enhances the catalase levels but in latter stage it causes adverse effect (96).

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**Fig.15 Effect of Different concentrations of Chromium on Catalase content (mg-1 min-1 protein) of soybean**

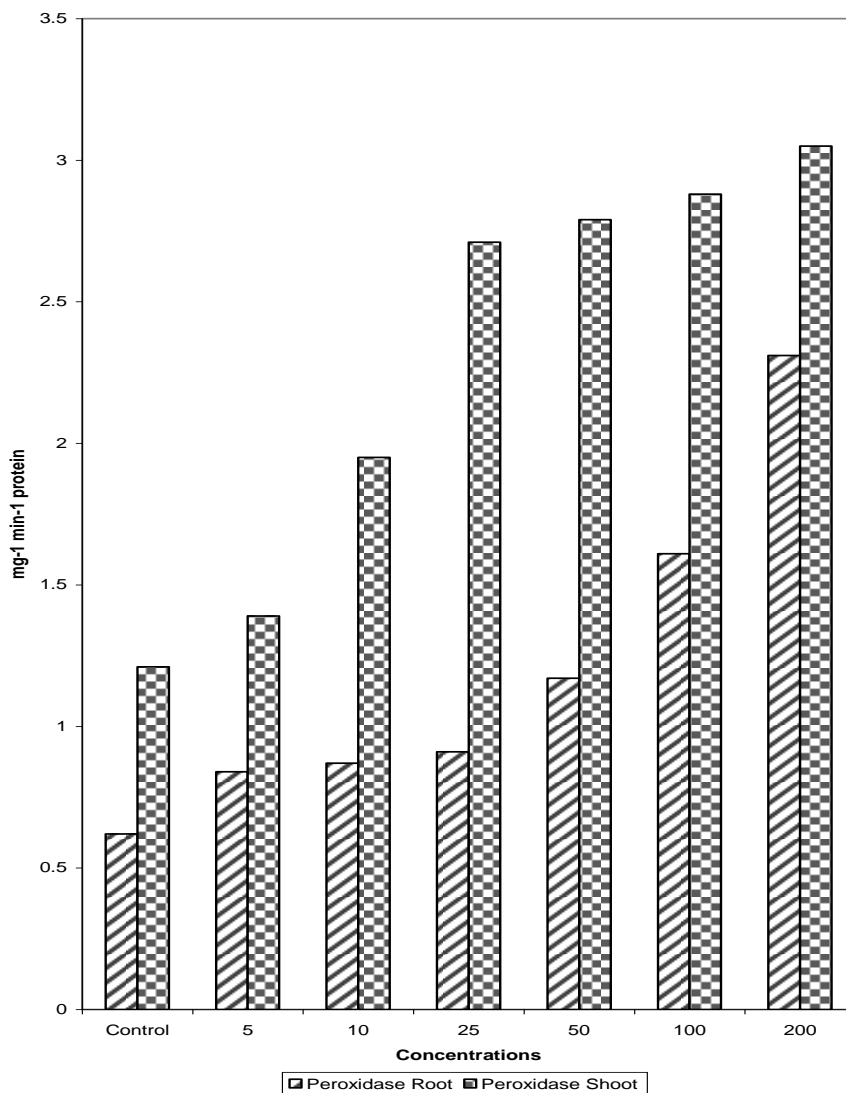


### 13. 2. Peroxidase

Peroxidase is one of the important enzymes which play a major role in a variety of cellular functions (86). The increase in peroxidase activity in plant cells under a variety of stresses such as mechanical injury and attack by pathogen or an influence of environmental pollution was reported. The peroxidases activity showed that the greatest response to the

pollutants and the increase in enzyme activities might be the indication of a stress situation in plant. It has been reported that the peroxidase activity was induced due to increase of metal stress. The increase in peroxidase activity varies with the plant species and the concentration of pollutants (97). Increased peroxidase activity might be linked to a decline in growth rate and biomass as found in plants treated with various metals because peroxidase have various physiological role in plant cells and participate in many reactions (98, 99).

**Fig.16 Effect of Different concentrations of Chromium on Peroxidase content (mg-1 min-1 protein) of soybean**



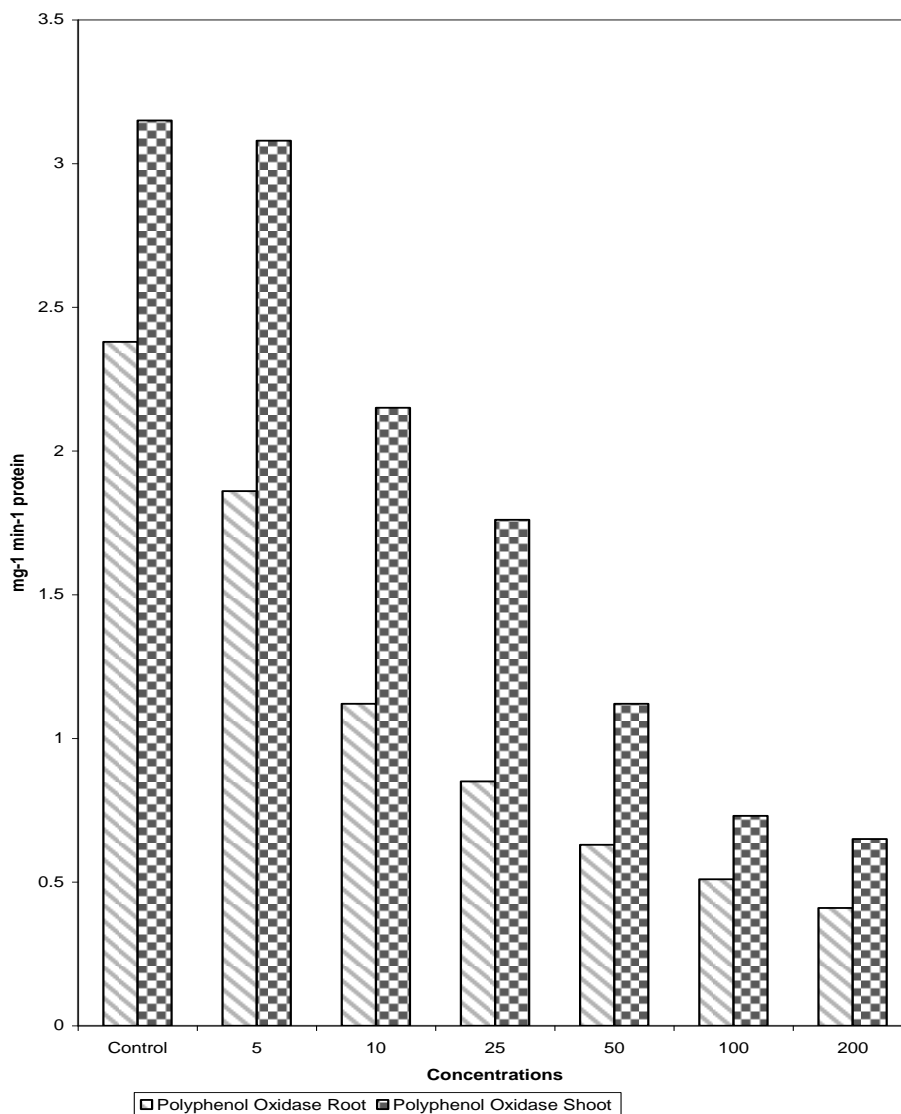
The peroxidase activity gradually increased with the increase in chromium concentrations. A higher peroxidase activity was observed in 200 mg/l chromium treated plants and the lower peroxidase activity was recorded in control plants. A higher peroxidase activity was observed in hypocotyls of *Phaseolus vulgaris* seedlings treated with both



mercury and chromium heavy metals. Sen *et al.* (100) observed an increase in peroxidase activity in higher concentrations (above 10 mg/l) of chromium, whereas, the enzyme activities were least affected by lower concentrations of chromium. It is well known that catalase and peroxidase play an important role in preventing oxidative stress by catalyzing the reduction of hydrogen peroxidase (101). Both catalase and peroxidase activities increased by heavy metal treatment (102). The observed increase in peroxidase activity might have been in direct response to the generation of Superoxide radical by Cr induced blockage of the electron transport chain in the mitochondria (40).

### 13. 3. Polyphenol Oxidase

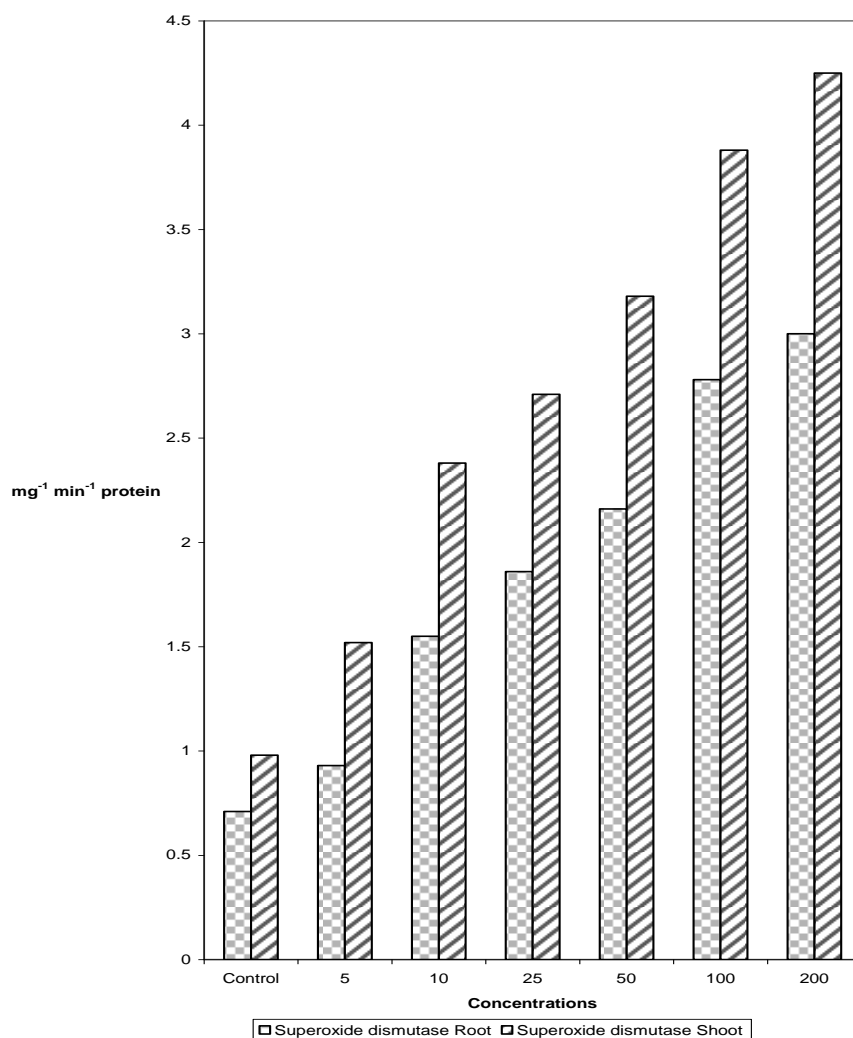
**Fig.17 Effect of Different concentrations of Chromium on Polyphenol Oxidase content (mg-1 min-1 protein) of soybean**



Polyphenols, especially tannins, are well known for their ability to chelate heavy metals such as Fe and recently they have received much attention as potential antioxidants. Thus interactions of polyphenols with heavy metals can involve chelation, antioxidative activity against active oxygen species caused by heavy metals. In this experiment the polyphenol oxidase contents are gradually decreased with the increase of chromium concentrations. Higher polyphenol oxidase was recorded in control and the lower content was recorded in 200 mg/l Cr concentration. Similar results were observed already in *camellia sinensis* (103) and cowpea (104) under heavy metal stress. It is possible that the mechanism for increasing ppo activity occurs in a latent form in plants. The changes activity of ppo in the plant tissue after metal stress could have resulted from the activation of latent phenolase or from solubilising phenolase from the cellular structure as reported by Robby *et al.*, (105) for diseased plants under pathogen stress or due to the novo synthesis of polyphenol oxidase under metal stress (106).

### 13. 4. Super oxide dismutase

**Fig.18 Effect of Different concentrations of Chromium on Superoxide dismutase content (mg-1 min-1 protein) of soybean**



Super oxide dismutase is an important anti oxidant enzyme. It is considered to be plant's antioxidative defense system which plays an important role in dismutation of free hydroxyl radicles by the formation of hydrogen peroxide. A gradual increase was observed in all the antioxidant levels with increase of Cr concentrations. The SOD activity increased in *Pisum sativum* (107) and *Vigna radiata* (40) under Cr stress. The phenomenon of varied levels of antioxidant enzymes can be explained as redundancy in ROI – scavenging mechanism (108). The increase of SOD activity can be considered as an indirect evidence for enhanced production of free radicles. It has been reported that higher concentrations of chromium increased the activity of SOD in higher plants during Oxidative damage (109, 110).

#### 14. CONCLUSION

It is concluded that chromium is toxic to germination, growth and biochemical constituents of the test crop soybean. It accumulates considerable amount of chromium and affects various metabolic activities. Chromium induced oxidative stress was tolerated by this plant through the hyperactivity of antioxidant defence system. Suitable ameliorative measures should be taken to reduce the toxic effects of these metals by adding fertilizers and biofertilizers in contaminated agricultural fields. In addition, Interaction of Cr with uptake and accumulation of other inorganic nutrients can be managed in the soil by adding suitable surface medium which control the Cr Mobility. Further research should be encouraged for removing the heavy metals from medium by phytoremediation technology and also to control the mobility of heavy metals from soil to aerial portion of the plant system.

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