PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ASPECTS OF CHROMIUM TOXICITY AND TOLERANCE IN SELECTED CROPS AND TREE SPECIES

Thesis submitted in part fulfillment of the requirements for the Degree of ***Doctor of Philosophy (Agriculture)*** *in* ***Crop Physiology*** *to the Tamil Nadu Agricultural University, Coimbatore*

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TAMIL NADU AGRICULTURAL UNIVERSITY

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CERTIFICATE

This is to certify that the thesis entitled "**PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ASPECTS OF CHROMIUM TOXICITY AND TOLERANCE IN SELECTED CROPS AND TREE SPECIES"** submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN CROP PHYSIOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a *bonafide* record of research work carried out by **Mr. ARUN KUMAR SHANKER** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

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BY

ARUN KUMAR SHANKER

Degree	:	Doctor of Philosophy (Agriculture) in Crop Physiology
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A series of experiments were conducted in the laboratory, glass house and farmers field to understand and elucidate the mechanism of tolerance and toxicity to Cr in nutrient medium, soil added Cr and Cr from tannery effluent. VAM as a biological ameliorant and $FeSO_4$ spray as a chemical ameliorant was tried in soil added Cr and Cr from tannery effluent treatments. A cereal (rice), a pulse (green gram), an oilseed (sunflower) and a coarse cereal (sorghum) were taken as experimental materials. *Tectona grandis, Leucaena luecocephala, Albizia amara,* and *Casuarina equisetifolia* were evaluated for their phytoaccumulatory potential of soil added and tannery effluent added Cr.
In addition, biological amendment in the form of VAM and chemical amendment in the form of citric and oxalic acid were tried to enhance Cr phytoaccumulation.

Treatments in the laboratory study consisted of various concentrations of Cr(III) and Cr(VI) alone and in combination, in the nutrient medium. Treatments in the pot culture studies consisted of soil added Cr(III) and Cr(VI) in addition to Cr from 10 and 15 per cent tannery effluent source. VAM and $FeSO_4$ were used as an ameliorant in the Cr stress imposed treatments. Both the speciation of Cr reduced germination percentage, vigour index, germination stress index and promptness index. Cr(VI) affected the above parameters more than Cr(III). Root length decreased in a concentration dependant manner. Anthocyanin content increased with the initial increase in concentration of Cr in the medium and was more in sorghum and sunflower and particularly in varieties CO 27 and CO 4 of sorghum and sunflower respectively.

Lipid peroxidation in terms of malondialdehyde formation was seen due to Cr in the medium by all the crops studied. There was a progressive concentration dependant increase in Cr content in the seedlings. Highest Cr content was observed in rice var.

ADT 37 (342.0 μg g^{-1}) as against the lowest observed in sunflower var. CO 4 (282.8 μg g^{-1}) at the high concentration treatment. MT 3 gene expression was seen in CO 27 sorghum as a response to Cr(VI)100 μM exposure for 10 days in nutrient medium. Sunflower var. CO 4 distinctly developed stress protein of 19 kDa. Root protein profile showed that there were distinct proteins formed due to Cr(III) 200 μM of molecular weight ~28, ~32 and ~40 in sunflower var. CO 4. SOD was induced and its activity increased as the concentration of the Cr the nutrient medium increased, but at higher concentration and mixed speciation there was drastic reduction in the activity of the enzyme. In the pot culture studies with soil added Cr, tannery effluent added Cr and ameliorants, plant height, root length, leaf area and total dry matter accumulation was reduced in the Cr(VI) treated plants.

The overall efficiency of the photochemical reaction was reduced as evidenced by decline in the fluorescence parameters observed. There was decline in the chlorophyll *a*, *b* and *a*/*b* ratio due to Cr stress. The antioxidative enzyme SOD increased in response to Cr addition but a similar increase was not seen in the case of catalase activity. VAM as an ameliorant was very effective in the Cr(III) and 10 per cent effluent treatments in increasing growth which in turn reflected in the yield of the plants. The antioxidative enzyme SOD increased in response to Cr addition but a similar increase was not seen in the case of catalase activity. The predominant antioxidative quenching of free radical was by the ascorbate- glutathione pathway as evidenced by the differential activity of the enzymes (APX, MDHAR, DHAR and GR) and metabolites (GSH, GSSG and AA) of the pathway. Glutathione increased in response to stress. Phytochelatin was deduced from metabolic assay of glutathione pathway and there was a presence of increased PC-SH in sunflower as against sorghum. $FeSO_4$ as an ameliorant increased the thiol pathway enzymes and metabolites. There was an increase in free amino acids and ascorbic acid due to Cr stress indicating the possible role of these metabolites in detoxification of tissue Cr. Ethylene increase was brought about by Cr in the roots.

There was a significant reduction in seeds per head due to Cr treatments. Cr(III) treated plants recorded 432 seeds per head and Cr(VI) treated plants recorded 392 seeds per head. There was a significant decrease in yield due to Cr addition. Nutrients (N,P and K) and micronutrients (S and Fe) reduced due to the presence of Cr. B content was not influenced by stress. Cr content was 10 times more in roots than in shoots. Sorghum accumulated lesser Cr than sunflower. VAM was effective in reducing the Cr translocation to the shoots by increasing the root accumulatory capacity of these plants. *Albizia amara* was found to accumulate more Cr than the other species studied in terms of bio magnification ratio and accumulation factor. Organic acids *viz*., oxalic and citric acid were effective in increasing the uptake of Cr from soil added and tannery effluent added form. The results of the present studies indicated that Cr(VI) was more toxic than Cr(III) to plant growth and development. Ten per cent and 15 per cent tannery effluent were also detrimental to plant growth, development and yield. VAM effectively ameliorated the deleterious effect of soil added Cr(III) and 10 per cent tannery effluent. Sorghum variety CO 27 inoculated with VAM can be effectively cultivated in the tannery effluent affected lands. *Albizia amara* can be used as an effective phtoremediator species in view of the fact that the Cr accumulated by the tree will not get into the food chain since it is mainly used as a timber source.

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CHAPTER I
INTRODUCTION

Leather industry is one of the important industries in India as it earns a high proportion of foreign exchange. This industry, which employs more than 2.5 million people, exported about US$ 1970.98 million worth of goods during 2000-01 (Tiwari, 2001). Along with the positive features of this industry there also exists negative aspects which has come to fore in this era of environmental concerns. Soil and water ecosystems have been contaminated to an overwhelming extent in the vicinity of leather industry and this has rendered arable land unproductive and underproductive. This negative feature is ballooning to uncontrollable proportion due to indiscriminate disposal of untreated and under treated leather industry effluent.

This problem has worsened with the evidence of contamination of wells used for agriculture and drinking water (Mahimairaja *et al*., 1997). With special reference to Vellore district in Tamil Nadu nearly 35,000 hectares of agricultural land in the tanneries belt has become either partially or totally unfit for cultivation (Kennedy, 1999). The damage due to tannery effluent assessed for a period between August 19, 1991 and December 31, 1998 indicated that 29,193 families have been affected due to underproductive nature of 15,164 ha of agricultural lands in 186 villages of Vellore district in Tamil Nadu. The economic loss alone has been estimated to the tune of Rs. 26.82 crores (Warrier, 2001). The same fate is envisaged in the Kanpur leather belt in Uttar Pradesh (Rajamani, 2001).

Chromium (Cr) is the chief heavy metal contaminant found in the tannery effluent. Cr used by the leather industry to tan hides is not taken up completely by leather and relatively large amounts escapes into the effluent. Due to chrome leather tanning processes, large quantities of Cr compounds are discharged through liquid, solid, and gaseous wastes into the environment and can have significant adverse biological and ecological effects. Several reports have shown that the values for Cr in tannery effluent are considerably higher than the safe limits prescribed by National and International standards in Tamil Nadu and various other tanneries in the world (Khan, 2001).

Cr is a toxic element to higher vascular plants and is detrimental to its growth, development and reproduction (Cervantes *et al*., 2001). The physiological impact of Cr contamination in soil and water is dependant on the speciation. These factors are responsible for the mobilisation of the metal, subsequent uptake and resultant toxicity in the plant system. The action of Cr is seen at the whole plant level as reduced growth, and at the organ level through leaf symptoms. On a smaller scale, the effects of Cr can be seen as cellular symptoms. Symptoms, both macro, cellular and growth effects are side effects of the direct mode of action. The two common oxidation states of Cr present in the environment, *viz*., Cr(III) and Cr(VI), are drastically different in charge, physicochemical properties as well as chemical and biochemical reactivity.

The toxicological impact of Cr(VI) originates from the action of this form itself as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr(VI) to Cr(III) occurring inside the cell. Cr(III) on the other hand if present in significant concentration can cause further adverse effects because of its high capability to coordinate various organic compounds resulting in inhibition of some metallo-enzyme systems (Kotas and Stasicka, 2000).

Cultivated crops if examined for their ability to tolerate Cr in soil added form or from tannery effluent source can offer prospects for cultivation of these crops in tannery affected areas. Crops tolerate Cr toxicity by their ability to exclude translocation of Cr from roots to aerial parts, detoxification by compartmentalization in vacuoles and complexation with organic ligands, phytochelatins and metallothionein (Toppi *et al*., 2002). Neutralisation of Cr(VI) generated free radicals by antioxidant enzymes and metabolites and protection of Cr(III) inhibition of metallo- enzymes are the basic biochemical and molecular level detoxification mechanisms observed in crops (Samataray, 2002).

Mycorrhizal fungi have greatest impact on elements with narrow diffusion zones around plant roots, including heavy metals and phosphorus. An important arbuscular mycorrhizal genus is *Glomus*, which colonize a variety of host species, including crops and tree species (Bagyaraj *et al*., 1998). Mycorrhizal fungi are a direct link between soil and roots, and consequently of great importance in phytoremediation. Little is known of the ability of mycorrhizal fungi to enhance plant tolerance or phytoaccumulation of Cr. Furthermore, information is lacking on the influence of mycorrhizae on the uptake of other essential plant macro- and micronutrients in soils contaminated with Cr.

Since ascorbate–glutathione pathway plays a major role in the antioxidant response of plants under abiotic environmental stress and that increased sulphur enhances the components of the pathway, it is possible that supplemented sulphur could enhance the antioxidant response of stressed plants (Fitzgerald *et al*., 2001). Chromium is a known competitive inhibitor of iron uptake in plants due to its similar atomic configuration, hence it is possible that supplementation of iron can suppress Cr induced toxicity (Clara *et al*., 1998).

Cleaning up of the Cr contaminated sites is a challenging task. Phytoremediation is an emerging technology that can be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable; at sites with low concentrations of contaminants, where treatment is required over long periods of time. Phytoextraction refers to the use of metal accumulating plants that translocate and concentrate metals from the soil in roots and above ground shoots or leaves (Cunningham and Ow, 1996). Tree species in association with mycorrhizae have shown promising prospects for phytoremediation of Cr contaminated lands in and around tannery industrial areas (Khan, 2001). Organic acids have been used to enhance extraction of immobile metals from soils due its ability to complex with metals and increase its availability (Wu *et al*., 2003).

The above scenario emphasises the need for research efforts to unravel the complex nature of Cr speciation and concentration effects on crop growth and development and its accumulation potential in tree species. Keeping in view the above concepts and facts the present study was undertaken with the following objectives.

- To understand the physiological, biochemical and molecular mechanism of chromium toxicity and tolerance in selected crops.
- To screen crops and identify varieties of selected annual crop species suitable for tannery effluent contaminated soils in and around tannery industrial areas.
- To induce tolerance to Cr added through soil and tannery effluent in selected crops by biological and chemical ameliorants.
- To explore the possibilities of phytoremediation or phytomining by tree seedlings for ecological sustenance in tannery waste contaminated areas.

CHAPTER II

REVIEW OF LITERATURE

Chromium compounds are highly toxic to plants and are detrimental to its growth and development. Chromium is reported to be potentially toxic to higher plants at total tissue concentration of 0.1mM kg^{-1} dry weight (Davies 2002); although some studies have indicated that at low concentration (1 μM) Cr stimulated plant growth (Bonet *et al*., 1991). It is also known that growth of tomato, lettuce, wheat and bean was unaffected by very low concentration of Cr (3.8×10^{-4} μM) (Huffman and Allaway, 1973).

Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquein. It is a transition element located in the group VI-B of the periodic table. It has a ground state electronic configuration of [Ar] $3d^5 4s^1$. The stable forms of chromium are the trivalent Cr(III) and the hexavalent Cr(VI) species although there are various other valence states, which are unstable and short-lived in biological systems. Cr(VI) is considered the most toxic form of Cr, which usually occurs with oxygen as chromate ($Cr\ O_4^{2-}$) or as dichromate ($Cr\ O_7^{2-}$) as oxyanions. Cr(III) on the other hand is less mobile and less toxic and is mainly found in bound form with organic matter in soil and aquatic environments (Becquer *et al*., 2003).

The most important environmental source of chromium is the leather industry effluent. Indiscriminate disposal of tannery effluent facilitates the movement of chromium into arable soil and irrigation water through ground water contamination. A thorough review of chromium and its physiological effects in plants, biological and chemical ameliorative measures to overcome chromium toxicity and phytoremediation of heavy metal contaminated land is presented hereunder.

2.1 Chromium in the natural environment

Chromium is found in all phases of the environment, including air, water, soil and virtually all biota. Naturally occurring soil chromium ranges from 10 – 50 mg kg^{-1} depending on the parent material and in ultramafic soils (serpentine), it can reach up to 125,000 mg kg^{-1} or more (Adriano, 1986). Fresh water chromium concentrations generally range from 0.1 to 6.0 μg L^{-1} while values for seawater range from 0.2 to 50 μg L^{-1}.Chromium levels vary widely within the atmosphere. Background concentration from 5.0×10^{-6} to 1.2×10^{-3} μg m^{-3} in air samples from remote areas such as Antarctica and Greenland. By comparison, chromium content of air samples collected over urban areas averages from 0.015 to 0.03 μg m^{-3} (Nriagu, 1988).

2.2 Chromium as an environmental contaminant

Chromium and its compounds have multifarious industrial uses and used extensively in leather processing and finishing (Nriagu, 1988), these anthropogenic activities have led to the wide spread contamination of the environment by chromium and has increased its bioavailability and bio mobility.

2.2.1 Chromium and the leather industry

The leather industry is the major reason for the environmental influx of chromium. Forty per cent of total industrial chromium use is by the leather industry (Barnhart, 1997). The effluent and sludge disposed from these industries into rivers and onto land has led to extensive degradation of productive land (Ramasamy, 1997). There are two main types of leather tanning processes followed by the leather industry. The vegetable tanning process, which does not involve the use of toxic chromium, is relatively safe to environment. On the other hand, chrome-tanning process uses chromium compounds and sulphuric acid to produce high quality finished leather.

To obtain good quality leather, it is necessary to use a quantity of chromium salts representing 2 to 2.5 per cent (calculated as Cr_2O_3) of the mass of skins to be tanned. This implies that for every kilogram of leather tanned 250 mg of chromium is used. The excessive and unabsorbed chromic acids and chromium compounds which amounts to 10-20 percent of the used chromium is washed out and let into effluent, thus approximately 25 mg of chromium is let out in the effluent for every kilogram of leather tanned (Sandro, 1989). This forms the major source of toxic chromium in land and water ecosystems. The Government of India discouraged vegetable tanned leather goods exports due to its poor market quality and low foreign exchange earnings. This policy forced the leather tanners to go for chrome tanning which was profitable to both the industry and the Government, but was extremely toxic to the environment in the long run (Teekaraman and Farooque, 1982).

Tamil Nadu is a leading finished leather producer in India. Over 250 tanneries had been functioning in the past decade and were actively involved in chrome tanning process. Presently there are 6000 tanneries out of which a sizable percentage is actively involved in chrome tanning process (Asha, 1995).

2.2.2 Chromium in tannery effluent

Chromium is one of the important contaminants in the tannery effluent. Stepniewska and Bucior (2001a) studied the water quality mixtures obtained from tannery effluents and found that it contained up to 250 μM of total chromium.

Ma-Hongrui *et al*. (2001) reported that $2ML^{-1}$ HCl extractable Cr could be the major fraction of soil Cr available to the plants from tannery effluent. Thangarajan (1999) modelled pollutant migration in the Upper Palar River Basin in North Arcot district of Tamil Nadu and noted the possibility of chromium migration and bioavailabity to arable activities. Balasubramanian and Pugalenthi (1999) determined chromium by digestion of samples in a HNO_3/H_2SO_4 mixture followed by $KMnO_4$ oxidation, inductively coupled plasma-atomic emission spectrometry (ICP-AES), flame atomic absorption spectrometry (FAAS) and UV-visible spectrophotometry (1,5-diphenyl carbazide method) and found that total chromium in the tannery effluent had a wide range of 200-2400 mg L^{-1}. In a chromium speciation study in tannery effluent by Walsh and O'Halloran (1996) the speciation of chromium in the final effluent was dominated by Cr(III) particulates with a mean concentration of 72.4 mg Cr L^{-1}, 80-90 per cent of these particulates were inorganic while 10-20 per cent was bound to organic ligands.

Iqbal Muhammad (1996) found that there were zootoxic and phytotoxic amounts of chromium in sediments of moving stream of tannery wastewater near tannery industrial area in Karachi, Pakistan. Chuan and Liu (1996) studied the release behaviour of chromium from tannery waste and found that total Cr content was 17,200 mg kg^{-1}.

Mahimiaraja *et al*. (1997) reported a wide variation in the chromium concentration of effluent collected from different sites in North Arcot District in Tamil Nadu, the total Cr concentration ranged from 620-26,200 μm L^{-1}.

2.2.3 Chromium in soil and ground water due to tannery effluent influx

The discharge of tannery effluent into river bodies and land leads to the accumulation of toxic levels of chromium in soil and ground water. The mean values of Cr in surface and subsurface around some tanneries in North Arcot district of Tamil Nadu was found to be 3,791 and 20,164 mg Kg^{-1} in Ambur and Vaniambadi respectively by Mahimiaraja *et al*. (1997). They reported that at both locations Cr content did not show a definite pattern with soil depth but accumulated in the soil.

The speciation of soil Cr as determined by sequential fractionation indicated that 85-99 per cent was HNO_3 extractable and only 0.5 to 15 per cent was organic and organics plus iron oxide bound (Mahimiaraja *et. al*., 1997). Stepniewska and Bucior (2001) studied the presence of chromium forms on the area contaminated by tannery wastes and reported that concentrations of different species of chromium were found in water and soils ranging from 5-10 per cent of Cr_2O_3. They also reported that conditions favourable for oxidation of Cr (III) to Cr (VI) existed in the area. Stuart and Milne (2001) in the conclusions of their study clearly warned about the risk to groundwater from wastewater irrigation using high chromium tannery effluent.

Khwaja *et al*. (2001) monitored river Ganga for chromium accumulation due to tannery effluent discharge at two sites and reported that the leakage of chromium into the Ganga was evident. Beg *et al*. (1990) in a study of pollution due to tannery effluents in the Korangi Industrial Area, Karachi, concluded that wells located in the tannery area contained high dissolved solids exceeding 5000 ppm and chromium ion ranging between 0.048 and 0.39 ppm.

2.2.4 Chromium in tannery effluent and its effect on agriculture

Agriculture has been reported to be severely affected by pollution of ground water and soil by tannery effluent. Arable land contamination has shown to increase chromium concentration in plants in the first year of the crop. The possibility of oxidation of Cr(III) to

Cr(VI) in soils where C(III) in the form of tannery effluent is added was found to be a threat to agriculture (James and Bartlett, 1983). Beg *et al.* (1990) reported the adverse effect of tannery effluent on cultivation of various crops near the Korangi tannery area in Karachi, Pakistan. Teekaram and Farooque Ahmed (1982) studied the extent of damage to agriculture by tannery effluent in North Arcot district of Tamil Nadu and summarised the effect on various crops based on farmer's response (Table 2.1).

TABLE 2.1 EFFECT OF TANNERY EFFLUENTS ON AGRICULTURAL CROPS
(Teekaram and Farooque Ahmed 1982)

Crop	Effects
Paddy	Poor germination, stunted vegetative growth, poor grain growth, chaffy ear heads resulting in reduced yield
Sugarcane	Decrease in internode length, thin stem girth, low cane weight and jaggery with poor consistence and black in colour
Finger Millet	Yield reduction of about 10-20 per cent
Coconut	Tender coconut water is salty in taste, reduced size of nuts and increased button fall
Groundnut	Leaf drying, root formation hampered, low pod yield
Vegetables	Stunted growth, poor flowering and fruit formation and drying of leaves
Sorghum	Stunted growth and moderate yield reduction of fodder and grain
Pearl Millet	Moderate yield reduction, growth stunted
Maize	Stunted growth and moderate yield reduction

2.3 Effect of chromium in plants

2.3.1 Growth and development

Plant growth and development are essential processes of life and propagation of the species. They are continuous and mainly depend on external resource present in soil and air. Growth is chiefly expressed as a function of genotype and environment, which consists of external growth factors and internal growth factors. Presence of Cr in the external environment leads to changes in the growth and development pattern of the plant.

2.3.1.1 Germination

The ability of a crop to germinate and establish under heavy metal stress is an early indicator of tolerance of the plant. Seed germination was the first physiological process affected by chromium and other heavy metals when present in the culture medium (Peralta *et al*., 2001). Rout *et al*. (2000) studied the effect of chromium on the germination and growth of *Echinochloa colona* and reported that the percentage of seed germination reduced up to 25 per cent under high chromium concentrations (200μM). Parr and Taylor (1982) reported that high levels (500 μg g^{-1}) of hexavalent chromium in soil affected germination and growth in *Phaseolus vulgaris*.

Peralta *et al.* (2001) investigated the ability of lucerne cv. Malone seeds to germinate and grow in media containing Cr(IV) ions at 0, 5, 10, 20, and 40 ppm concentrations, and found that 40 ppm concentration of Cr(VI) significantly reduced the ability of the seed to germinate and grow in the contaminated medium. Jain *et al.* (2000) observed significant reductions in bud germination at 20 to 80 ppm Cr (by 32 to 57%) in sugarcane.

Britto-de *et al*. (1998) saw a concentration dependant deleterious effect on seed germination by chromium in *Vigna radiata* and *Cicer arietinum*, wherein higher concentrations (200μM) inhibited germination up to 50 percent. Bera *et al*. (1999) reported that effluent concentrations up to 10 per cent did not significantly reduce seed germination, but at 50 per cent concentration germination was 64 per cent compared with 96 per cent in controls in *Vigna radiata* cv Pusa Baisakhi.

2.3.1.2 Root growth

Tolerance to toxic elements and other abiotic stress depends largely on well-branched and extensive root systems. Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Breckle, 1991; Godbold and Kettner, 1991; Tang *et al*., 2001).

Prasad *et al*. (2001) reported that the order of metal toxicity to new root primordia in *Salix viminalis* is Cd > Cr > Pb whereas root length was more affected by Cr than by other heavy metals studied. Iqbal *et al*. (2001) found that root length and dry weight of an important arid tree *Caesalpinia pulcherrima* was inhibited by 100 ppm Cr. Total root weight and root length of wheat was affected by 20 mg Cr(VI) kg^{-1} soil as $K_2Cr_2O_7$ (Chen *et al*., 2001). Panda and Patra (2000) reported that 0.001 mM of chromium increased the root length in seedlings growing under nitrogen (N) nutrition, higher chromium concentrations decreased root length in all the N treatments. Samantaray *et al*. (1999) in a study with chromite mine spoil soil, in five cultivars of mung bean noted that root growth was significantly affected 28 days after root emergence.

Lubben *et al*. (1991) reported marked decreases in root dry weight, length and surface and an increase in root diameter in a study on bush beans grown under compacted densities of 1.2, 1.36 and 1.49 g cm^3 concentration of Cr in the soil. Scanning electron microscope studies of root affected by chromium showed that there was increased growth of root hairs, and increased relative proportion of pith and cortical tissue layers (Suseela *et al*., 2002).

2.3.1.3 Plant height

Shoot growth is considered as an important morphological parameter related to growth and development of the whole plant. Realisation of yield potential of crops depends a great deal on plant height of the crop. Several workers have reported the adverse effect of Cr and other heavy metals on plant height and shoot growth (Mukhopadhyay and Aery, 2000; Britto-de *et al*., 1998; Rout *et al*., 1997).

When Cr was added at 2, 10 and 25 ppm concentration to nutrient solutions in sand cultures in oats, Anderson *et al*. (1972) observed 11, 22 and 41 per cent reduction in plant height respectively over control. Fresh and dry weight of shoot showed significant decrease with increase in concentrations of Cu and Cr in the nutrient solution, Cr at 2.0-ppm reduced shoot dry weight by 77 per cent (Khan *et al*., 2000).

Joseph *et al*. (1993) reported reduction in plant height due to Cr(VI) on *Curcumas sativus*, *Lactuca sativa* and *Panicum miliaceum*. Barton *et al*. (2000) reported that Cr(III) addition inhibited shoot growth in Lucerne cultures. Sharma and Sharma (1993) reported that after 32 and 96 days, plant height reduced significantly in wheat cv. UP 2003 in a glasshouse trial when sown in sand and 0-0.5 µM sodium dichromate was applied. Hanus and Tomas (1993) reported reduction in plant height in *Sinapsis alba* when Cr was given at the rates of 0, 200 or 400 mg kg^{-1} soil along with N, P, K and S fertilizers.

2.3.1.4 Leaf growth

Leaf growth, area development and total leaf number decisively determines the yield of crops. Leaf number per plant reduced by 50 percent in wheat when 0.5 mM Cr was added in nutrient solution (Sharma and Sharma, 1993). Tripathi *et al*. (1999) found that leaf area and biomass of *Albizia lebbek* seedlings was severely affected by high concentration of Cr(VI). The authors opined that leaf growth traits might serve as suitable bio indicators of heavy metal pollution and in the selection of resistant species. Primary and trifoliate leaves of bush bean plants grown in 1.0, 2.5, 5.0, and 10 µg cm^{-3} Cr showed marked decrease in leaf area, trifoliate leaves were more affected by Cr than the primary leaves (Barceló *et al*. 1985). Dry leaf yield of bush bean plants was found to reduce up to 45 per cent when 100 ppm of Cr was added to soil (Wallace *et al*., 1976). Karunyal *et al*. (1994) studied the effect of tannery effluent (25, 50 75 and 100%) on leaf area and biomass and reported that above 25 per cent all the concentrations decreased leaf area and leaf dry weight in *Oryza sativa*, *Acacia holosericea* and *Leucaena luecocephala*.

In a study on the effect of trivalent and hexavalent chromium on spinach, Singh (2001) reported that chromium applied at 60 mg kg^{-1} and higher levels reduced the leaf size, caused burning of leaf tips or margin and slowed leaf growth rate. Jain *et al*. (2000) reported leaf chlorosis at 40 ppm Cr that turned to necrosis at 80 ppm Cr. Leaf number reduced due to Cr(VI) addition, in seedlings of *Vigna radiata* and *Cicer arietinum* (Britto-de *et al*., 1998). In a study with several heavy metals, Pedreno *et al*. (1997) reported that Cr had a pronounced effect on leaf growth and preferentially affected young leaves in tomato plants. Poschenrieder *et al*. (1993) reported that reduction

in leaf biomass was correlated with the acid oxalate extractable Cr in *Phaseolus vulgaris*. Excessive rates of Cr in a liquid medium negatively affected the mesostructure of the leaves (Bessonova, 1991).

2.3.1.5 Total dry matter production

The first prerequisite for higher yields is an increase in biomass production in terms of dry matter. Carbon compounds account for 80-90 per cent of the total dry matter produced by plants. Higher source size and increased photosynthetic process was found to be the basis for the building up of organic substances and dry matter production under heavy metal stress in general, and chromium in particular (Bishnoi *et al*., 1993).

In a study conducted on *V. spiralis* to evaluate the Cr accumulation and toxicity in relation to biomass production by Vajpayee *et al*. (2001) it was found that dry matter production was severely affected by Cr concentrations of 2.5, 5.0 and 10.0 µg mL^{-1} in nutrient medium. Zurayk *et al*. (2001) reported that salinity and Cr interaction caused a significant decrease in the dry biomass accumulation of *Portulaca oleracea*. Cauliflower (cv. Maghi) when cultivated at 0.5 mM Cr restricted dry biomass (Chaterjee and Chaterjee, 2000). Kocik and Ilavsky (1994) studied the effect of Cr (0-200 mg kg^{-1}) on quality and quantity of biomass in sunflower, maize and *Vicia faba* and reported that dry matter production was not markedly affected by Cr, but uptake of Cr into plant tissue was positively correlated with their contents in the soil.

The dry matter yield of water hyacinth shoots grown in ^{115}Cd labelled nutrient solution was significantly lower than that of ^{51}Cr in a radio tracer study by Ramachandran and D'-Souza (1998). Hanus and Tomas (1993) reported reduction in dry biomass at flowering stage in *Sinapsis alba* when Cr was given at the rates of 0, 200 or 400 mg kg^{-1} soil along with N, P, K and S fertilizers. *Phaseolus vulgaris* and maize plants exposed to 1µM Cr(III) showed higher root and leaf dry weight (DW) than controls and this increase in DW was more pronounced in Fe-deficient conditions (Barceló *et al*., 1993). Cabbage plants water cultured under Cr level (0, 0.2,2,10 ppm) exhibited marked reduction in dry weight of whole plant from 88.4 g $plant^{-1}$ in control to 28.4 g $plant^{-1}$in 10 ppm Cr (Hara and Sonoda, 1979).

2.3.1.6 Yield

Most physiological and biochemical processes are severely affected by heavy metals in general and chromium in particular and as a logical consequence the yield and productivity of the crops is equally affected (Barceló *et al*., 1993).

Kadar and Prokisch (2000) reported 50 per cent reduction of fresh tuber yield when chromium was applied to the soil at 0, 90, 270 or 810 kg ha^{-1} in compound form and 100 kg each of N, P_2O_5 and K_2O fertilizers. In pot trials with soil amendment of Cr at the rate of 0, 100 or 300 mg kg^{-1}, Golovatyj and Bogatyreva (1999) reported reduction in yield of barley and maize. Richter *et al*. (1997) studied the productivity of a crop rotation in soil with a natural and increased content of Cr and found that it reduced crop production and productivity.

No harvestable yield was obtained where Cr was applied at 270 or 810 kg ha^{-1} in carrot (Biacs *et al*., 1995). Radish (cv. Pusa Chetki) grown in pots with Cr mixed in the soil {as $K_2Cr_2O_7$ for Cr(VI) and $CrCl_3$ for Cr(III)} at

concentrations ranging from 0 to 135 ppm markedly reduced the yield with Cr(VI) showing greater effect than Cr(III) (Singh *et al*., 1993).

Kalarani *et al*. (2002) reported a decrease in yield of finger millet due to soil added Cr. Sharma and Sharma (1993) reported that the number of flowers plant^{-1} decreased by >50 per cent at 0.05µM compared with the control and the number of flowers plant^{-1} decreased more with increase in Cr concentration from 0.05 to 0.5 µM. The number of grains plant^{-1} decreased 59 per cent from the control in 0.05 µM Cr. Grain DW was highest in the control and was reduced by 58, 73, 77 and 92 per cent with increase in Cr rate. Tillering was reduced and seed deformities increased with increase in Cr rate.

Phaseolus vulgaris grown in pots of calcareous soil (pH 7.3) compacted to densities of 1.2, 1.36 and 1.49 g cm^3 Cr in the soil, reduced pod weight significantly (Lubben *et al*. 1991). Sharma and Mehrotra (1993) reported seed DW yield was 2.11 g plant^{-1} without chromium, and 0.39 g and 0.16 g with the highest and lowest rates of chromium, respectively.

2.3.2 Physiological processes

2.3.2.1 Photosynthesis

Photosynthesis is most important and possibly the only mechanism of energy input into the living world. Heavy metal stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Clijsters and Van Assche, 1985). Photosynthetic CO_2 fixation capacity of isolated chloroplasts was inhibited by 50 per cent after 5 min incubation in reaction mixtures containing various heavy metals (Hampp *et al*., 1973). In higher plants and trees, the effect of Cr on photosynthesis is well documented (Foy *et al*., 1978; Van Assche and Clijsters, 1983).

However, it is not well understood to what extent Cr-induced inhibition of photosynthesis is due to increased stomatal resistance (Gunse, 1987), disorganisation of chloroplasts ultrastucture (Vazquez *et al*., 1987), inhibition of electron transport or the influence of Cr on the enzymes of the Calvin cycle.

Chromate is used as a Hill reagent by isolated chloroplast (Demest *et al*., 1975). The more pronounced effect of Cr(VI) on PS I than on PS II activity in isolated chloroplasts has been reported by Bishnoi *et al*. (1993). Nevertheless, in whole plants both the photosystems were affected (Dixit *et al*., 2002). Zeid (2001) reported that Cr at the highest concentration (10^{-2} M) decreased photosynthesis drastically. Krupa and Baszynski (1995) explained some hypotheses concerning the possible mechanisms of heavy metals toxicity on photosynthesis and presented a list of key enzymes of photosynthetic carbon reduction, which were inhibited in heavy metal treated plants (mainly cereal and legume crops).

Yagdi *et al*. (2000) opined that toxicity of Cr decreases Calvin cycle enzyme activities, which in turn reduced photosynthesis in plants. In 64-day old pea plants 0.05, 0.10, 0.2 and 0.5 mM Cr(VI) diminished the rate of whole plant photosynthesis by 42, 68, 78 and 85 per cent respectively. It was also noticed that the 40 per cent inhibition in

52 day old plants at 0.10 mM Cr(VI) was further enhanced to 65 and 95 per cent after 76 and 89 days of growth respectively (Bishnoi *et al*., 1993a).

2.3.2.2 Water relations

Several authors have reported wilting of various crops and plant species due to Cr toxicity (Turner and Rust, 1971; Foroughi *et al*., 1976; Barceló *et al*., 1978), but little information is available on the exact effect of Cr on water relations of higher plants. Inhibition of water uptake in germinating seedlings of different species has been reported (Barceló *et al*., 1978). Mukherji and Roy (1977) observed inhibition of water uptake by Cr salts in potato slices and proposed that reduced water uptake due to impermeability as a possible cause of wilting. Barceló *et al*. (1985) observed a decrease in leaf water potential in Cr treated bean plants. Excess Cr decreased the water potential and transpiration rates and increased diffusive resistance and relative water content in leaves of cauliflower (Chatterjee and Chatterjee, 2000).

Britto *et al*. (1998) reported phytotoxicity percentage and relative water content increase as the concentration of Cr (VI) increased in seedlings of *Vigna radiata* and *Cicer arietinum*. Decreased turgor and plasmolysis was observed in epidermal and cortical cells of bush bean plants exposed to Cr (Vazquesz *et al*., 1987). Toxic levels of Cr in beans were found to decrease tracheary vessel diameter thereby reducing longitudinal water movement. (Vazquesz *et al*., 1987). Studies on whole plants exposed to Cr(VI) through roots revealed that the effects on transpiration was complex, *Phaseoulus vugalris* when exposed to 3.6 μM Cr(VI) for 14 days showed a transpiration rate of 3.3 dm^{-2} day^{-1} as against 0.8 dm^{-2} day^{-1} when exposed to 26.8 μM Cr(VI) for the same number of days (Gunse , 1987).

2.3.2.3 Chlorophyll fluorescence

Chlorophyll fluorescence is very useful to study the effects of environmental stresses on plants since photosynthesis is often reduced in plants experiencing adverse conditions, such as water deficit, temperature, nutrient deficiency, polluting agents, and attack by pathogens (Van kooten and Snel, 1990). Fluorescence is an easily measured signal, and different components of fluorescence, including photochemical and non-photochemical quenching, can be measured with the appropriate instrumentation. Chlorophyll fluorescence also allows investigating specific components of the photosynthetic apparatus (Krause and Weis, 1991; Schreiber *et al*., 1998).

Over the last decade, the measurement of chlorophyll fluorescence kinetics has provided considerable information on the organization and function of the photosynthetic apparatus. With the development of instruments that are capable of rapidly resolving the differences in photochemical and non-photochemical quenching, the use of the chlorophyll fluorescence signal as an intrinsic probe of photosynthetic function of plants under stress has become essential. Furthermore, the use of a far-red background light, which oxidizes the plastoquinone pool, now allows the correct determination of the minimum fluorescence (Fo) signal in pre-illuminated leaves (Maxwell and Johnson, 2000).

Dan *et al.* (2000) studied the effects of cadmium and nickel on chlorophyll fluorescence kinetics of scented geranium and opined that metal tolerance was correlated to higher photochemical quenching. Enhanced tolerance to heavy metals accompanied higher chlorophyll fluorescence parameters F_v/F_m and photochemical quenching in *Brassica juncea* (Smits *et al.*, 2000). The DF/Fm' chlorophyll-*a* fluorescence induction parameter, which indicates the quantum efficiency of photosystem II (PSII) (Genty *et al.*, 1989), was measured to detect the damaging effect of 0.5 mM Cd treatment after 1 day, this parameter decreased from 0.7 to 0.5 in Cd-treated plants (Pal *et al.*, 2002). By use of a fluorescence quenching analysis during steady-state photosynthesis Krupa *et al.* (1992) reported that heavy metals reduced photochemical quenching (qP) and stimulated non-photochemical quenching (qN) in been leaves.

Dan *et al.* (2000) investigated the effects of toxic concentrations of heavy metals on the fast induction kinetics of fluorescence from PSII. The authors reported not only a different time course of the changes in fluorescence related parameters for different metals, but also different effects on the fluorescence induction kinetics, which could possibly be linked to different mechanisms of action of the metals.

2.3.2.4 Mineral Nutrition

Minerals are an essential part of the life cycle of the plant. Plants require adequate supply of all the essential and micronutrients for optimum growth and realisation of full yield potential. Heavy metals in general affect mineral nutrition of plants, which in turn affects the development of the plant in different ways. Cr(III) and Cr(VI) was taken up by the plants by different mechanisms (Zaccheo *et al.*, 1985). It has been opined that both speciation can interfere with uptake of several other ionically similar elements like Fe and Sulphur (S) (Skeffington *et al.*, 1976).

Nutrient solution concentration of 9.6 μM Cr(VI) decreased the uptake of Potassium (K), Magnesium (Mg), Phosphorus (P), Fe and Mn in roots of soybean (Turner and Rust, 1971). Excess Cr interfered with the uptake of Fe, Molybdenum (Mo), P and N (Adriano, 1986). Barceló *et al.* (1985) described the inhibition of P, K, Zinc (Zn), Copper (Cu) and Fe translocation within the plant parts when bean plants were exposed to Cr in nutrient solutions. Sujatha *et al.* (1996) reported that tannery effluent irrigation caused micronutrient deficiencies in agricultural crops.

In soil grown plants the influence of Cr on mineral nutrition was highly variable and depended on the source of Cr and soil properties (Ottabbong, 1989a) wherein it was reported that differences in soluble Mn fractions, interactions with P and critical effects on the uptake of Mn, Cu, Zn, Fe and Aluminium (Al) was influenced by Cr (Ottabbong 1989b). Cr induced chlorosis was observed whereas there was no clear correlation between leaf Fe levels and chlorosis. (Ottabbong, 1989c). Cr (VI) induced decrease in calcium (Ca), K, Mg, P, boron (B) and Cu concentrations in soil grown soybean tops was observed, but Fe, Mn, and Zn uptake was not affected. In non-calcareous soils amended with Cr(III), the translocation of Fe, Zn and Mo to bean plants was decreased (Wallace *et al.*, 1976). In contrast, other workers who supplied Cr in the form of Cr(VI), Cr(III) or in the form of tannery waste to soils have found an enhancement of Fe availability and Fe uptake by plants (Cary *et al.*, 1977; Barceló *et al.*, 1993).

Barceló *et al.* (1985) found high correlations between chlorophyll pigments and Fe and Zn uptake by Cr stressed plants. del Castilhos *et al.*(2001) in an experiment with five Cr(VI) concentrations (0, 5, 10, 20 and 40 mg L^{1}) in the nutrient solution observed that Cr(VI) above 5 mg L^{1} affected P, K, Ca and Mg uptake in soybean. Shanjida Khan *et al.* (2001) observed that threshold values of the concentrations of N, P and K in dry weight of rice plants showed significant decrease at 0.5 ppm Cr concentration. Krstic *et al*. (1991) reported that 50, 100 or 200 ppm. of Cr (III) increased the N concentration in sunflower, barley and wheat. Excess of Cr (0.5mM) caused a decrease in concentrations of Fe and affected the translocation of P, S, Mn, Zn and Cu from roots to tops (Chaterjee and Chaterjee, 2000). Gupta *et al*. (2000) reported in sunflower hull that total P was greatest with Cr (0.5 ppm) 30 days after flowering. Sharma and Sharma (1996) reported that leaf P concentration decreased with 0.25 mM Cr in wheat cv. UP2003.

Singh *et al*.(1993) found that the root contents of K, Fe and Mn decreased with increasing Cr concentration [0 to 135 ppm, Cr(VI) as $K_2Cr_2O_7$ and Cr(III) as $CrCl_3$] in Radish (cv. Pusa Chetki). Biddappa and Bopaiah (1989) reported the inhibitory role of Cr on the incorporation of P, K, Ca, Mg, Fe, Mn, Zn and Cu in different cellular constituents in 1-year-old West Coast Tall coconut plants growing in pots.

Moral *et al*.(1995) reported that in general, the nutrient elements N, P, K, Na, Ca and Mg concentrations in stems and branches were significantly affected by the Cr treatments (0, 50 and 100 mgL^{-1}) in tomato. Later Moral *et al*. (1996) conducted a detailed study on the mineral nutrition of tomatoes under Cr stress and noted that Cr had a negative effect on Fe absorption. Competitive interaction between Cr and Cu in the roots, stems and leaves was confirmed. Mn was not clearly affected; B and Cr had synergistic interactions in roots, but an antagonistic effect in the stems and leaves. In the fruits, Cr treatment had no effect on Fe, Mn, Cu and Zn contents. B increased with Cr concentration in the nutrient solution.

In maize (cv. Ganga 5), the effects of Cr on Fe concentration varied with plant organ and Cr rate. Mn and Cu concentrations generally decreased with increasing Cr rate, while Zn concentration decreased in leaves and flowers but increased in stem and roots (Sharma and Pant, 1994). In a study on chromium III-Fe, interaction Bonet *et al*. (1991) reported that Cr enhanced growth of both Fe-control and Fe-deficient plants. However, Cr concentration was correlated neither to changes of Mn, P or Fe tissue concentration nor to Cr-induced alterations of the Fe/Mn and P/Fe ratios.

2.3.2.5 Chromium uptake, translocation and accumulation

The sum total of all the toxic effects of Cr in plants is solely dependant on the uptake, translocation, accumulation and speciation of Cr in plant parts. Uptake and accumulation of Cr by various crops is well documented (Table 2.2).

Evidence for independent uptake mechanisms of Cr(VI) and Cr(III) in barley has been reported. The use of metabolic inhibitors reduced Cr(VI) uptake whereas Cr(III) uptake was unaffected indicating that Cr(VI) uptake depends on metabolic energy and Cr(III) does not (Skeffington *et al*., 1976). In contrast to the above report, Ramachandran *et al.* (1980) reported an active uptake of both Cr species, slightly higher for Cr(III) than for Cr(VI) in the same crop. In seven out

of ten crops analysed, more Cr accumulated when plants were grown with Cr(VI) than with Cr(III) (Zayed *et al*., 1998). Skeffington *et al*. (1976) from radioactive tracer studies reported that Cr mainly moved in the xylem of the plants. Golovatyj *et al*.(1999) reported that Cr distribution in crops had a stable character, which did not depend on soil properties and concentration of this element in it; the maximum quantity of element-contaminant was always contained in roots, and a minimum in the vegetative and reproductive organs. In bean, only 0.1 per cent of the Cr accumulated was found in the seeds as against 98 per cent in the roots (Huffman and Allaway, 1973a). Kadlec *et al*. (2000) reported only 0.20 mg kg^{-1} Cr in soybean seeds when cultivated in Cr containing soils.

2.3.3 Metabolites

Plants are energy dependant chemical factories that follow chemical laws. The chemical reaction that makes life possible is collectively called as metabolism and the products of metabolism are called metabolites. The process of normal metabolite production is affected severely when the plants are exposed to abiotic stress in general and heavy metal stress in particular (Hall, 2002).

Chromium stress induced response can be of three main types- i) Alteration in the production of metabolites which are involved in the life sustenance of plants ii) Increased production of metabolites as a direct response to Cr stress which may cause damage to the plants iii) Alterations in the metabolic pool to channelise the production of new bio chemically related metabolites which may have the property to confer resistance or tolerance to chromium stress. Furthermore, Cr stress response is typically complex, and is exhibited by almost all the parts of the plant. Enzymes mediate the production and utilization of theses metabolites by catalysing the various steps in the metabolic pathway.

2.3.3.1 Chlorophyll content

Bioaccumulation of chromium and its toxicity to photosynthetic pigments in various crops and trees is well documented (Barceló *et al*., 1986; Vajpayee *et al*., 1999; Sharma and Sharma, 1996). Bera *et al*. (1999) studied the effect of Cr present in tannery effluent on chloroplast pigment content in mungbean and reported that irrespective of concentration, chlorophyll-*a*, chlorophyll-*b* and total chlorophyll decreased in 6-day-old mung bean seedlings as compared to control.

Chlorophyll content was high in tolerant calluses in terms of survival under high chromium concentration in a study of chromium and nickel tolerance in *E. colona* by Samantaray *et al*. (2001). Chlorophyll content decreased as a marked effect of various concentrations of different chromium compounds {Cr (III) and Cr (VI)} in *Triticum aestivum*. Cauliflower (cv. Maghi) grown in refined sand with complete nutrition (control) and at 0.5 mM each of Co, Cr and Cu showed drastic decrease in chlorophylls *a* and *b* in leaves in the order Co > Cu > Cr (Chaterjee and Chaterjee, 2000).

Chlorophyll *a*/*b* ratio gradually decreased, with increasing treatment concentration and time due to addition of Cr(VI) (AnZhi *et al*., 2000). The influence of Cr(VI) concentrations of 1 and 2 mg L^{-1} on *Salvinia minima* decreased chlorophyll *a* and *b* and carotenoid concentrations significantly (Nichols *et al*., 2000).

2.3.3.2 Anthocyanin

Anthocyanins belong to the group of flavaniods and are red, purple and blue coloured pigments that occur commonly in various plant parts. Anthocyanins are typical characteristics of higher vascular plants. There are several different types of anthocyanins and usually more than one type is present in a particular organ. Of late, the role of anthocyanins in relation to stress resistance has drawn considerable attention. Krupa *et al.* (1996) reported that these pigments can be used as a simple and reliable indicator of heavy metal toxicity for higher plants. Kumar and Kumar (1992) reported the changes in anthocyanin content and its relationship with petal senescence under heavy metal enrichment.

Glutathione S-transferase performs the last genetically defined step in anthocyanin biosynthesis, which is, tagging of anthocyanin precursors with glutathione; this process was reported to be induced by heavy metals (Marrs and Walbot, 1997). In an interesting finding Chengbin *et al*. (2001) reported that the plants with low glutathione levels were able to accumulate only low levels of anthocyanins, supporting a role for glutathione S-transferases for anthocyanin formation or for the vacuolar localization and therefore accumulation of these compounds in heavy metal hypersensitive *Arabidopsis thaliana* .

2.3.3.3 Lipid Peroxidation

Lipid Peroxidation is the oxidative degradation of polyunsaturated fatty acids and is a basic membrane damage process. It has been reported that the permeability of the membrane depends on the degree of lipid peroxidation (Dhinsa *et al*., 1981). The lipid peroxidation process is initiated by free radicals, such as the hydroxyl radicals generated in the Haber Weiss reaction, and peroxyl and alkoxyl radicals, which are produced during the decomposition of organic hydroperoxides, heavy metals catalyse these reactions very effectively (Aust *et al*., 1985).

Behra *et al*. (1999) reported an increase in lipid peroxidation in terms of malondialdehyde (MDA) in chromium treated developing wheat seedlings. Epidermal cells, root hairs and outer cortex of roots of *Phaseolus vulgaris* and *Salvia sclarea* were extremely damaged by long-term exposure to 96 μM and 17 μM Cr(VI), respectively (Corradi *et al*., 1993). Transmission Electron Micrographs (TEM) revealed membrane damage, especially of the tonoplast in roots and the basal parts of the stem. Lipid peroxidation due to Cr has also been described in fronds of *Lemna minor* and leaves of *Pistia stratiotes* (Bassi *et al*., 1990).

Ying *et al*. (2000) reported a significant induction of lipid peroxidation in response to oxidative stress from days 2 to 8 following imposition of metal stress in rice seedlings. Jita and Panda, (1998) compared the biochemical response to oxidation and metal stress in seedlings of barley and found a significant induction of all antioxidant enzymes along with an increase in the levels of lipid peroxidation.

De Vos *et al*. (1992) opined that toxic concentration of metals induced oxidative stress, which was effectively quantified by measuring the products of lipid peroxidation. Gallego *et al*. (1996) reported an increase in lipid peroxidation due to heavy metals in sunflower leaves.

2.3.3.4 Ethylene production

Heavy metal induced differential ethylene response is well documented (Tani *et al*., 1997; Lin *et al*., 2000). Cr(VI) induced inhibition of ethylene evolution was seen aquatic in plants (Bassi *et al*., 1990). Hou and Kao (1993) reported that polyamine accumulation might partly regulate ethylene

production in heavy metal treated rice leaves. Vangronsveld *et al*. (1992) studied the role of heavy metal induction of ethylene production, stress enzymes, and reported that ethylene reversed the damage caused to membrane permeability in heavy metal treated plants.

Ethylene production in leaves increased within 2 hours of application of heavy metals (Weckx *et al*., 1992). In the trifoliate leaves of plants exposed to 10 µM Cr(VI), a significant decrease of ethylene production from endogenous amino cyclopropane carboxylic acid (ACC) was observed by Poschenrieder *et al*. (1993).

Pei *et al*. (2002) reported that ethylene in combination with metals played a regulatory role in the differential expression of three metallothionein-like genes in Cavendish banana (*Musa acuminata*). Ji *et al*. (1989) reported that the increase in ethylene production in shoots mainly resulted from the direct action of heavy metals transported from roots to shoots and not from the conversion of ACC synthesized in roots, transported to shoots and converted to ethylene.

2.3.3.5 Free amino acids and organic acids

Free amino acids and organic acids such as citric, malic and free histidine are potential ligands for heavy metals and so could play a role in detoxification and tolerance (Clemens, 2001). Several reports have suggested a differential response of organic acids in inducing tolerance to various heavy metals in different plants (Mathys, 1977; Thurman and Rankin, 1982). A 36-fold increase in free histidine content in xylem sap on exposure to heavy metal was reported by Kramer *et al*. (1996). Different metals have shown variable induction of amino acid synthesis in plants (Costa and Spitz, 1997; Shah *et al*., 1998).

Treatment with Nickel (Ni) induced an accumulation in roots of all free amino acids associated with a decrease in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Treatment with Cd decreased the amount of all free amino acids. Cysteine, which is the main amino acid in the phytochelatin complexes, constituted about 17.5 per cent of total free amino acids (Shintinawy and Ansary, 2000).

2.3.3.6 Glutathione

Glutathione represents the major pool of non-protein sulphur in plants. The reduced form of glutathione (GSH) is a tripeptide that exists interchangeably with the oxidized form (GSSG). Certain plants contain tripeptide homologs of GSH, in which the carboxy terminal glycine is replaced by other amino acids; these are homoglutathione and hydroxymethylglutathione, and are related forms.

In plants, the physiological significance of glutathione has been divided into two categories: sulphur metabolism and defence. GSH was found to be the predominant nonprotein thiol and which regulated sulphur uptake at root level (Herschbach and Rennenberg, 1994). It was used by the GSH S-transferases in the detoxification of xenobiotics (Lamoureux and Rusness, 1993) and was reported to be precursor of the phytochelatin, which are crucial in controlling cellular heavy metal concentrations (Grill *et al*., 1989).

GSH is important as an antioxidant and redox buffer (Law *et al*., 1983). Under conditions of heavy metal stress the level of glutathione has been reported to change biphasically from normal levels, this phenomenon has been attributed to phytochelatin synthesis (Grill *et al*., 1989). It is a precursor for phytochelatin synthesis, which binds with supraoptimal concentration of heavy metals. Owing to its redox active thiol group, reduced glutathione has often been considered to play an important role against oxidative stress. Stimulation of GSH biosynthesis was observed in stress conditions (Noctor *et al*., 1998). Similarly, GSH accumulation was found to compensate for decrease in the capacity of other antioxidants such as catalase and superoxide dismutase (Smith, 1985).

As a physiological parameter, the induction of thiol-containing peptide glutathione under heavy metal contamination was investigated by Bruns *et al*. (1997). A positive correlation was found between glutathione and heavy metal levels in plant samples. The suitability of this biochemical response to stress as a biomarker for heavy-metal pollution at field locations was recommended by the authors.

In a study on comparison of physiological responses to oxidative and heavy metal stress in seedlings of rice paddy, Ying *et al*. (2000) reported a significant induction of glutathione along with other antioxidant enzymes. Olena *et al*. (2000) opined that blocked thiols are sufficient for phytochelatin synthase-catalyzed transpeptidation of glutathione and related thiol peptides in a study on the mechanism of heavy metal ion activation by phytochelatins.

Liang *et al*. (1999) reported that over expression of glutathione synthase and subsequent production of glutathione offers a promising strategy for the production of plants with superior heavy-metal tolerance capacity. Gupta *et al*.(1998) reported that the induction of phytochelatins was accompanied by a decline in cellular levels of glutathione in aquatic plants.

Toppi *et al*. (2002) reported that glutathione levels ranged from about 30 nM SH g^{-1} fresh weight (FW) of root extracts to 300 nM SH g^{-1} FW of leaf extracts in maize, tomato and cauliflower plants following a Cr(VI) treatment at concentrations of 5 and 10 mg L^{-1}. ChengBin *et al*. (1998) reported that *Arabidopsis thaliana* plants treated with cadmium or copper responded by increasing transcription of the genes for glutathione synthesis, and glutathione reductase. Heavy metal treatments (30-250 µM) in pedunculate oak (*Quercus robur*) resulted in increased non-protein thiol levels after 3 days, but decreased GSH contents (Gullner *et al*., 1998).

Howden *et al*. (1995) isolated a heavy metal sensitive, glutathione-deficient mutant of *Arabidopsis thaliana* and concluded that the deficiency in phytochelatin biosynthesis can be explained by a deficiency in glutathione. Observations on the recovery of the level of glutathione, the precursor of phytochelatins, after arresting the heavy metal exposure, suggested that tolerant plants did not exhibit a decreased capacity to synthesize glutathione in *Silene vulgaris* (Knecht *et al*., 1995).

2.3.3.7 Phytochelatins

Phytochelatins are cysteine-rich non-protein metal-binding peptides produced by plants with glutathione as its precursor. It has been suggested that they function in the regulation of essential metals and in the detoxification of all toxic metals (Oven *et al*., 2002). *Zea mays* has been found to have phytochelatins, which bind to excess Cu and Cd (Galli *et al*., 1996). However, this was not tested against a control or sensitive cultivar. Phytochelatin synthesis was initiated in *Rubia tinctorum* root cultures upon exposure to a number of metals including Zn, Cu and Cd, and no phytochelatin production was observed in controls plants (Maitani *et al*., 1996). Exposure of *Nicotiana tabacum* seedlings to Cd resulted in the production of phytochelatins (Vogeli-Lange and Wagner, 1990).

Computer simulation studies with *N. tabacum* suggested that phytochelatins could have a major role in tolerance to Cd assuming that they were produced upon exposure to Cd (Wang *et al*., 1991). Oven *et al*. (2001) reported that homo-phytochelatins are formed when adzuki beans are challenged with heavy metals. Clemens *et al*. (1999) reported tolerance to toxic metals by a gene family of phytochelatin synthases from plants.

In a study on *Rauvolfia serpentina* suspension cells with sub-phytotoxic levels (100 μM) of $CdCl_2$ containing ^{109}Cd, it was concluded that heavy metal ions entering cells at sub-lethal concentrations were totally complexed by phytochelatins and to a much lesser extent by some high molecular weight proteins (Kneer and Zenk, 1992).

2.3.4 Enzymes

2.3.4.1 Nitrate reductase (NR)

Since nitrate is the most significant source of nitrogen in crop plants, understanding the role of NR in higher plants has potential economic importance, especially in light of recent studies illuminating the enzyme as one focal point for integration of control of carbon and nitrogen metabolism. Nitrate reductase in crops and their role in nitrate assimilation are well documented (Campbell, 1988; Barber and Solomonson, 1990). Nitrate reductase activity is severely affected by heavy metals in general and chromium in particular (Rai and Dubey, 1989) hence it is a suitable
bio indicator of heavy metal pollution and in the selection of resistant species.

Nitrate reductase activity of leaves were significantly increased over control values and negatively correlated with root and shoot length, leaf area and biomass of the plants, indicating stress due to Cr (VI) in *Albizia lebbek* (Tripathi *et al*., 1999). Saxena *et al*. (1999) studied the heavy metal accumulation and *in vivo* nitrate reductase activity in the *Sphagnum* sp. and found that the metal accumulation inhibited *in vivo* nitrate reductase activity, which could be mitigated to some extent by the supply of glutathione.

Nelumbo nucifera when grown in different chromium concentrations (50 to 200 μM) resulted in significant inhibition of *in vitro* nitrate reductase activity (Vajpayee *et al*., 1999). Plants of *Nymphaea alba* grown at various levels of Cr(VI) ranging from 1 to 200μM exhibited severe impairing of nitrate reductase activity (Vajpayee *et al*., 2000). Seedlings treated with chromium at 0.001 mM resulted in increased nitrate reductase activity, whereas higher

chromium concentrations were toxic and reduced the enzyme activity significantly in wheat (Panda and Patra, 2000).

2.3.4.2 Root Fe(III) reductase

Reduction of Fe(III) to Fe(II) is necessary for uptake of adequate Fe by the roots of dicots and many graminaceous monocots and is carried out by a plasma membrane bound Fe(III) reductase located at root soil interface (Beinfait, 1988). Not only heavy metals other than Fe were reduced as part of Fe stress response, but also metal toxicity other than Fe- deficiency induced Fe reductive activity of roots (Norvell *et al*., 1993).

Heavy metal induced chlorosis has been generally correlated with low plant Fe contents suggesting effects of heavy metals on Fe mobilisation and uptake. Under Fe–deficient conditions, dicotyledonous plants enhanced root Fe (III) reductase activity, thus increasing the capacity to reduce Fe(III) to Fe(II), the form in which roots absorb Fe (Alcantara *et al*., 1994). Welch *et al*. (1993) studied the induction of Fe (III) reductase by heavy metals and opined that root cell plasmalemma Fe(III) reductase may perform a general role in regulating cation uptake.

Cr is reported to affect uptake of Fe in dicots either by inhibiting reduction of Fe (III) to Fe(II) or by competing with Fe(II) at the site of absorption. Landsberg (1982) found that an excess of heavy metal in the nutrient solution blocked the differentiation of root transfer cells, where Fe(III) reductase seems to be located and inhibited the acidification of the medium. Olsen and Brown (1980) obtained a large inhibition of root Fe(III) reduction when heavy metals at 100μM concentration was applied to the Fe(III) reduction assay solution. Chromium application to iron-deficient *Plantago lanceolata* roots increased the activity of root associated Fe(III) reductase. This effect was evident only with acceptors of the turbo reductase and was not observed in iron-sufficient plants (Wolfgang, 1996).

In split-root experiments, which allowed only a part of the root system to receive chromium, while the other portion was grown in iron-free medium, roots subjected to either treatment showed an intermediate FeEDTA reductase activity with respect to non-split control plants (Wolfgang, 1996). The addition of Cr(III) at 2.0 μM slightly inhibited ferric chelate reductase in roots of plants grown under iron-limited conditions, Cr(III) at 10 μM stimulated ferric chelate reductase in roots from both iron-limited and iron-sufficient media (Barton *et al*., 2000).

2.3.4.3 Plasma membrane (PM) H^+ ATPase

Metal uptake by plants is regulated by the electrochemical potential gradient for each metal ion that exists across the plasma membrane of root cells. Most plants are reported to have a plasma membrane potential between -120 and -180 mV, hence a large electrical gradient exists, that powers metal uptake (Kochian, 1991). The plasma membrane H^+ ATPase plays a significant role in many of the life functions of the plant cells. By mediating ATP dependant H^+ extrusion to the cell exterior the plasma membrane H^+ ATPase sets up the driving force for solute transport in terms of an inwardly directed proton electrochemical gradient in the plasma membrane (Sanders and Slayman, 1990).

Plasma membrane H^+ ATPase is believed to be responsible for the regulation of fluxes of several solutes across the PM (Serrano, 1989). ATPase plays a significant role in the adaptation to heavy metal conditions and it is regulated at the molecular and biochemical level (Dietz *et al*., 2001). Obata *et al*. (1996) opined that the effect of heavy metals on plasma membrane ATPase from plant roots could be used to study differential tolerance to heavy metals by plants.

A toxic effect of Cr on the transport activities of plant cell plasma membrane has been suggested by Zaccheo *et al*. (1982). After a short term exposure to 2µM Cr(VI), a strong inhibition of both H^+ and K^+ uptake in maize root segments was observed, while the transmembrane electric potential was unchanged (Zaccheo *et al*., 1985). Pillay (1994) reported that ATPase activity increased at higher treatment concentrations in a study on the effects of soil chromium treatment (0-750 ppm) on different metabolites and certain enzymes of *H. suaveolens* and *H. annuus* leaves.

2.3.4.4 Anti oxidant enzymes

Life in oxygen has led to the evolution of biochemical adaptations that exploit the reactivity of active oxygen species (AOS). The term AOS is generic, embracing not only free radicals such as superoxide (O°) and hydroxyl radicals but also H_2O_2 and singlet oxygen.

It is generally assumed that, the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized (Jacob and Heber, 1996). The chief toxicity of O and H_2O_2 is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radical and other destructive species such as lipid peroxides. These cascades are prevented by efficient operation of the cell's antioxidant defences.

The term antioxidant can be considered to describe any compound capable of quenching AOS without itself undergoing conversion to a destructive radical (Nishikimi and Yagi, 1996). Antioxidant enzymes are considered as those that either catalyze such reactions or are involved in the direct processing of AOS. Hence, antioxidant enzymes function to interrupt the cascades of uncontrolled oxidation by scavenging activated oxygen.

2.3.4.4.1 Superoxide dismutase, catalase and peroxidase

Induction and activation superoxide dismutase and of antioxidant catalase has been reported to be the metal detoxification mechanisms in plants (Prasad, 1998). Bessonova *et al*. (1992) reported enhanced activities of superoxide dismutase and catalase in sweet pea leaves when an excess of heavy metals [Fe (II), Mn(II) and Cr(III)] in modified Knop nutrient medium was given to the plants.

Pea plants exposed to environmentally relevant (20 µM) and acute (200 µM) concentrations of chromium for 7 days, affected total SOD activity of root mitochondria differently. At 20 µM Cr(VI), SOD activity was found to increase by 29 per cent, whereas 200 µM Cr(VI) produced a significant inhibition (Dixit *et al*., 2002).

In *E. colona* plants achieved through callus culture derived from leaf base, leaf tip and mesocotyl explants supplemented with Cr at 1.5 mg L^{-1}, activities of peroxidase, catalase were more in tolerant calluses than non-tolerant ones (Samantaray *et al*., 2001). Jain *et al*. (2000) observed a decline in the specific activity of catalase with increase in chromium concentration from 20 to 80 ppm. Excess of Cr (0.5 mM) restricted activity of catalase in leaves of cauliflower as reported by Chatterjee and Chatterjee (2000). Samantaray *et al*. (1999) used peroxidase and catalase activities as enzyme markers for identifying Cr tolerant mung bean cultivars. In wheat cultivar cv. UP2003, the application of 0.05-0.5 mM Cr decreased activities of catalase and peroxidase (Sharma and Sharma, 1996).

Sen *et al*. (1994) observed a decrease in catalase activity and increase in peroxidase activity at concentrations above 10 µg Cr(VI), whereas the enzyme activities were least affected by Cr(VI) at concentrations below 10 µg. The calli derived from *L. leucocephala* growing on contaminated soil when supplemented with 0.015 mM
Cr exhibited higher catalase and peroxidase activities than those from the uncontaminated soil. This provided evidence that plant material from the contaminated sources were physiologically distinct from the uncontaminated ones (Rout *et al*., 1999).

The activity of catalase in young leaves was impaired by an increase in Cr(VI) supply from 0.05 to 1.0 mM in wheat cv. HD 2204 (Sharma *et al*. 1995). Gwozdz *et al*. (1997) reported that at lower heavy metal concentrations, activity of antioxidant enzymes increased, whereas at higher concentrations, the superoxide dismutase enzyme activity did not increase further and catalase activity decreased.

2.3.4.4.2 Ascorbate peroxidase (APX) and Monodehydroascorbate reductase (MDHAR)

In plant cells, the most important reducing substrate for H_2O_2 detoxification is ascorbate. The enzyme APX has two cytosolic forms with a purely defensive role and a membrane bound form, which has a functional role in addition to hydrogen peroxide scavenging. It is reported to be involved in modulation of quantum efficiency and control of electron transport in conjunction with the ascorbate glutathione cycle (Nakano and Asada, 1987). Ascorbate peroxidase (APX) uses two molecules of ascorbate to reduce H_2O_2 to water, with the concomitant generation of two molecules of monodehydroascorbate (MDHA). MDHA is a radical with a short lifetime that, if not rapidly reduced, disproportionates to ascorbate and dehydroascorbate.

Within the cell MDHA can be reduced directly to ascorbate, at the plasmalemma or at the thylakoid membrane. The electron donor for MDHA reduction may be *b*-type cytochrome, reduced ferredoxin, or NAD(P)H. The latter reaction is catalyzed by MDHAR, which is found in several cellular compartments (Asada, 1997). Ascorbate peroxidase is a prominent membrane protein in glyoxysomes of oilseed crops like sunflower (Bunkelmann and Trelease, 1996).

APX and MDHAR were found to be enhanced several fold by heavy metals (Gupta *et al*., 1999). APX was induced much later (14 -16 hours) as against only 5 hours for the induction of MDHAR when 50 µM of heavy metals were present in the rot nutrient medium in the primary leaves of *Phaseolus vulgaris* (Cuypers *et al*., 1999).

Schutzendubel *et al*. (2001) reported cadmium-induced changes in APX activity and hydrogen peroxide content in Scots pine roots.

Weck and Clijsters (1997) showed that some heavy metals preferentially increase the ascorbate peroxidase rather than superoxide dismutase or catalase; however, they observed that increased APX activity did not seem to succeed in completely removing active oxygen species accumulated in the leaves. Cuypers *et al*. (2000) observed a copper stress induced increase in the activities of APX and MDHAR.

2.3.5 Molecular Studies

2.3.5.1 Metallothionein (MT) gene expression

MTs are cysteine-rich polypeptides encoded by a family of genes. Metallothionein proteins, products of mRNA translation, are characterized as low molecular weight, cysteine-rich, metal-binding proteins (Liu *et al*., 2000). MT proteins and genes have been found throughout the animal and plant kingdoms as well as in the prokaryote *Synechococcus species*.

The large number of cysteine residues in MTs binds a variety of metals by mercaptide bonds. MTs typically contain two metal-binding, cysteine-rich domains that give these metalloproteins a dumbbell conformation. MT proteins are classified based on the arrangement of Cys residues (Palmiter, 1998). Class I MTs contain 20 highly conserved Cys residues based on mammalian MTs and are widespread in vertebrates. MTs without this strict arrangement of cysteines are referred to as Class II MTs and include all those from plants and fungi as well as nonvertebrate animals. In this MT classification system, PCs are, somewhat confusingly, described as Class III MTs.

Type 1 MTs contain a total of six Cys-Xaa-Cys motifs (where Xaa represents another amino acid) that are distributed equally among two domains. Type 2 MTs also contain two cysteine-rich domains separated by a spacer of approximately 40 amino acid residues. Type 3 MTs contain only four Cys residues in the N-terminal domain. The consensus sequence for the first three is Cys-Gly-Asn-Cys-Asp-Cys (Kagi, 1993.)

The flood of information about plant genes and cDNAs encoding MT proteins has not been accompanied by a corresponding increase in knowledge about the expression or distribution of MT proteins. Consequently, there has been a trend to describe these as “metallothionein like genes,” for fear that they were in fact not translated into bona fide metal-binding proteins (Cobbet and Goldsbrough, 2002). Difficulties in identifying MTs in plants may arise from instability of these proteins in the presence of oxygen.

In attempting to shed light on their function, investigators have relied primarily on RNA blot hybridization to study the expression of MT genes during development and in response to various environmental factors (Giordani *et al*., 2000). More detailed localization of MT mRNAs or MT gene promoter activity has been obtained in a small number of cases through *in situ* hybridization and reporter gene expression studies. Many MT genes are expressed at very high levels in plant tissues, at least in terms of transcript abundance (Andrews, 2000).

The Arabidopsis genome sequence has provided information on how the seven members of the MT gene family are organized. The MT1a and MT1c genes lie within 4 kb as an inverted repeat on chromosome 1; MT2a and

MT3 are at distinct positions on chromosome 3; both MT4a and MT4b lie on chromosome 2 but are not closely linked; finally, MT2b is positioned on chromosome 5. One pseudogene, MT1b, has been identified in Arabidopsis and is also found on chromosome 5. Mapping and genome sequencing have demonstrated that MT genes are also distributed across different chromosomes in the tomato and rice genomes. However, evidence of MT gene clustering has been found in cotton where three MT genes were identified within a 10-kb fragment of genomic DNA (Hudspeth *et al*., 1996; Giritch *et al*., 1998).

Expression of other plant MT genes was not restricted to a single organ and cannot be categorized simply as that of Type 4 MTs. However, a number of general observations have been made about the expression of these genes. RNA expression of Type 1 MT genes tended to be higher in roots than shoots, whereas the reverse was observed generally for Type 2 MTs (Rauser, 1999). Although MTs are expressed ubiquitously and conserved in plants, determining their function remains a future challenge.

Quantitative assay based on competitive reverse transcription polymerase chain reaction (RT-PCR) was developed for MT by Butler and Roesijadi (2001). RT-PCR was evaluated as an alternative to Northern blot analysis in quantifying levels of MT mRNA and it was reported that sensitivities of both techniques were similar in detecting induced levels of MT mRNA. These results suggested that RT-PCR may provide a sensitive and quantitative method to evaluate MT gene expression (Kaplan, 2000).

2.3.5.2 Stress proteins

Several stress proteins characteristically show increased expression in response to the presence of toxic amounts of heavy metals in the growth medium of plants (Lewis *et al*., 2001). They are classified according to molecular size and are known to act as molecular chaperones in normal protein folding and assembly but may also function in the protection and repair of proteins under heavy metal stress condition (Hall, 2002).

There are several reports of increase in stress protein expression in plants in response to heavy metal stress. Tseng *et al*. (1993) showed that in rice, both heat stress and heavy metal stress increased the levels of mRNAs for low molecular mass stress proteins (16-20 kDa), while Neumann *et al*. (1995) indicated that a 17 kDa stress protein is expressed in roots of *Armeria maritime* plants grown on heavy metal rich soils.

Smaller molecular weight stress proteins are also known to increase in *Silene vulgaris* and *Lycopersicon peruvariium* plants grown in metaliferous soils and in response to a range of heavy metal concentration (Wollgien and Neuman, 1999).

Working with cell cultures of *Lycopersicon peruvariium* Neumann *et al*. (1994) found that a larger stress protein was formed in response to heavy metals and was found to be localised in the nucleus and the cytoplasm. Interestingly it was reported that a short heat stress given prior to heavy metal stress induced a tolerance effect by preventing membrane damage as judged by ultrastructural studies (Neumann *et al*., 1994).

Samantaray *et al*. (2001) conducted SDS-PAGE studies, which indicated that specific proteins (20.8, 27.6, 30.8, 45.2, 57.6, 63.4, 74.2, 80.6 and 95.2 kDa) appeared in chromium and nickel-tolerant calluses compared with the control in *E. callus* culture derived from leaf base, leaf tip and mesocotyl.

2.3.5.3 Glutathione reductase (GR) isozyme

Glutathione reductase is ubiquitous NADPH dependant enzyme which catalyses the reduction of oxidised glutathione present in both the chloroplast and the mitochondria. The enzyme is extensively purified in corn, pea and spinach (Halliwel and Foyer, 1978) and its molecular weight ranges from 135-290 kDa depending on the source, and the enzyme is composed of several subunits of approximately 60-30 kDa in weight.

Lower molecular weight isoforms has been reported from proteolytic digestion of the native enzyme (Mahan and Bruke, 1997). Anderson *et al.* (1990) purified, characterized and described the immunological properties for two isoforms of glutathione reductase from eastern white pine needles. There is dearth of literature available on the study of glutathione reductase isozymes due to metal stress although it has been studied widely in other abiotic stresses.

Anderson *et al*. (1990) reported minor changes in total GR activity observed in response to acclimation and chilling. GR isozymes were identified in the leaf, mesocotyl and root wherein roots had thick bands in response to stress, examination of GR isozyme profiles in plastid, mitochondrial, and cytosolic fractions revealed that three bands observed in the cell-free extracts were cytosolic.

2.3.6 Phytoremediation

Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments. Fundamental and applied research have unequivocally demonstrated that selected plant species possess the metabolic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants (Mejare and Bulow, 2001).

Salt *et al*. (1996) described that there are three main techniques used to clean up sites with shallow, low to moderate levels of metal contamination. Phytoextraction, which is also called phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aboveground portions of the plants. Rhizofiltration refers to adsorption or absorption into the roots of contaminants that are in solution. Phytostabilization is to immobilize contaminants in the soil and ground water through absorption, and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (rhizosphere).

To enhance phytoremediation as a viable strategy, fast growing plants with high metal uptake ability and rapid biomass gain was tested with respect with chromium (Pulford *et al*. 2001). Recently, phytoextraction has emerged as a cost-effective, environment-friendly cleanup alternative (Blaylock and Huang, 1999). Particular attention was given to the role of phytochelators in making the heavy metals bio-available to the plant and their symbionts in enhancing the uptake of bio-available heavy metals (Chaney *et al*., 2000).

Many plant physiological properties have been reported to be of importance for phytoremediation such as low or high metal accumulation, low or high root-to-shoot translocation of metal, high biomass production and, often, high tolerance to the specific metal (Lasat *et al*., 1996).

Research has shown that if chromate was reduced to chromic by chemical or biological methods, the inertness and insolubility of chromic oxides in soil limited the formation of chromate and reduced environmental risk (James, 1996). Mycorrhizae and organic acids have been reported to play an important role in phytoremediation of chromium-contaminated soils by enhancing Cr uptake and restriction of translocation to shoot (Chen *et al*., 1994; Davies *et al*., 2001).

Nutrient culture studies revealed a marked enhancement in uptake and translocation of chelated ^{51}Cr in *Phaseolus vulgaris*. ^{51}Cr-DTPA was most effective followed by ^{51}Cr-EDTA and ^{51}Cr-EDDHA (Athalye *et al*., 1995). Chaney *et al*. (1997) reported that phytostabilization appears to have strong promise with respect to chromium.

Srivastava *et al.* (1999a) reported statistically significant increases in chromium accumulation from Cr(III)-treated maize plants in the presence of increasing concentrations of organic acid. Shahandeh and Hossner (2000b) have reported high increase in Cr uptake aided by organic acids. Srivastava *et al.* (1999b) reported that increasing concentrations of organic acids resulted in increased uptake of chromium, without affecting the distribution in plant parts. Source-to-plant transfer coefficients of chromium tended to increase with increasing concentrations of organic acids in wheat.

2.3.7 Amelioration of Cr toxicity and induction of tolerance

Literature survey until now shows that very few workers have reported ameliorative measures for Cr toxicity in crop plants. This is largely due the reason that most of the research has been focused on enhancing phytoaccumulation of Cr by plants and trees for its use in phytoremediation.

Impaired mineral nutrition due to chromium toxicity has been corrected by the application of mycorrhizal inoculation. Several studies have dealt with the possibility of alleviation of metal stress by mycorrhisation (Leyval *et al*., 1997; Jentschke and Goldbold, 2000). Khan (2001) reported the potential of mycorrhizae in protecting the host plant against the harmful effects of heavy metal and phytoremediation of Cr contamination in tannery effluent polluted soils. Trees species *Populus euroamericana*, *Acacia arabica* and *Dalbergia sisso* used in the study.

Karagiannidis and Zinoviadi (1998) studied the effect of the vesicular arbuscular mycorrhizal fungus (VAMF) *Glomus mosseae* on growth, yield and nutrient uptake of durum wheat and reported that VAMF enhanced yield in wheat and simultaneously decreased the Cr content in the plant. In a study on the effects of Cr on the uptake and distribution of micronutrients (Fe, Mn, Cu and Zn) in mycorrhizal soybean and maize in sand culture, Wang and Chao (1989) reported that the uptake of micronutrients by mycorrhizal crops with the addition of various heavy metals were less significantly different than in treatments without heavy metal addition. In general, mycorrhizal maize increased the uptake of Cu; mycorrhizal soybean plants increased the uptake of Fe, Mn, Cu, and Zn. Davies *et al*. (2001) reported that AM enhanced the ability of sunflower plants to tolerate Cr, similarly Davies *et al*. (2002) reported that AM had a positive effect on tissue mineral concentration, growth and gas exchange in Cr treated plants.

Iron uptake and transport is reported to be affected by chromium toxicity (Turner and Rust, 1971). Similarly, Cr strongly inhibited sulphate uptake (Skeffington *et al*., 1976; Shewry and Peterson, 1974). Glutathione and free amino acids are known to induce heavy metal tolerance by antioxidant action and metal chelator respectively (Rauser, 1999). Increased S-supply resulted in an overall increase in total S, SO_4 and GSH in leaves and tubers of potato. The concentrations of the total free amino acid pools in leaves and tubers show a 2- and 3-fold decrease respectively, with increasing S-supply (Hopkins *et al*., 2000). Hence, it is possible that sulphate and iron supplementation can counter Cr toxicity in crop plants.

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out primarily to understand the physiological, biochemical and molecular mechanisms underlying chromium tolerance and toxicity. An attempt was also made to induce tolerance by application of biological and chemical ameliorants. Organic acids and Vesicular Arbuscular Mycorrhizae (VAM) were tired to increase phyto accumulation of chromium in selected tree species. This research work was carried out in a series of experiments comprising of laboratory, glasshouse and field trails. A detailed account of the materials used and methodologies followed in different experiments in the present study are given below.

3.1. Experimental Materials

Seed materials of the crops and varieties thereof were obtained from the Department of Millets (*Sorghum bicolour* (L.) Moench), Department of Pulses (*Vigna radiata* (L.) Wilczeck), Department of Rice (*Oryza sativa* L.) and Department of Oil Seeds (*Helianthus annuus* L.), Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Three-month-old seedlings of trees species of *Tectona grandis, Leucaena luecocephala, Albizia amara,* and *Casuarina equisetifolia* were obtained from Department of Agroforestry, Forest College and Research Institute, Mettupalayam. Spores of VAM were obtained from Department of Microbiology, Centre for Soil and Crop Management Studies, Tamil Nadu Agricultural University, Coimbatore. Metallothionein 3 (MT3) gene cloned in plasmid vector pZL was obtained from Peter G. Goldsbrough, Department of Horticulture, Purdue University, West Lafayette, U.S.A.

3.2. Experiment Ia: Lab studies – screening of crops for chromium (Cr) tolerance

A solution culture study was conducted to screen the selected crops for tolerance and also to study the toxic effect of both speciation of Cr on the crops. An efficient screening procedure was employed according to Craig (1977). Seeds of all the crops were germinated in petri plates irrigated with half strength Hoagland's nutrient solution and added with different concentrations of chromium designated as treatments detailed below. Trivalent chromium was added as $Cr_2 (SO_4)_3.2H_2O$ and hexavalent chromium was added as $K_2Cr_2O_7$. Concentration of chromium was fixed according to (Hossner *et al*., 1998).

Table 3.1 Details of crops and varieties selected for study

Crop	Variety	Duration (days)
Green gram	CO-6	70
	CO-4	85
	CO-5	65-70
Sorghum	CO-26	105-110
	CO-27	65-70
	K-10	110-115
Sunflower	Modern	75
	CO-4	80-85
	CO-2	87
Rice	TRY-1	135-140
	ADT-37	105
	CO-43	135-140

Treatment details

T_1- Control

T_2 -Cr(III)- 100μM

T_3 -Cr(III)- 200μM

T_4 -Cr(VI)- 50 μM

T_5 -Cr(VI)- 100μM

T_6 -Cr(III)- 50μM + Cr(VI)- 50 μM

T_7-Cr(III)- 100μM + Cr(VI)- 100 μM

Replications: 3

Design: Completely Randomised Design (CRD)

After germination the seedlings were removed from petri dishes and placed in a beaker containing half strength Hoagland's solution (Hoagland and Arnon, 1938) with added treatment concentrations, as mentioned above. A fine plastic mesh was placed over the beakers. Roots of the seedlings were placed through the mesh so as to be completely immersed in the treatmental

solutions. All the beakers were kept in growth chamber under a light intensity of 430 μ M m^{-2} s^{-1}, 14 hours light duration and 8 hours of dark, 60 per cent relative humidity was maintained.

Composition of Hoagland's solution

2.5mM $CaNO_3$

0.1mM K_2HPO_4

75μM KCl

1mM Mg as $MgSO_4$

10μM Fe EDDDHA

10μM H_3BO_3

4μM Mn as $MnCl_2$. $4H_2O$

0.5μM Cu as $CuSO_4$.$5H_2O$

1μM Zn as $ZnSO_4$. $7H_2O$

0.2μM Mo as Na_2MoO_4. $2H_2O$

3.2.1 Germination and growth

3.2.1.1 Germination percentage

Emergence of 2 mm of radical from the seed was taken as the criteria for germination. (ISTA, 1999). Seed germination was recorded on 7^{th} day after sowing (DAS) for green gram, sunflower and sorghum and 14^{th} DAS for rice and the germination percentage was calculated using the formula

$$\text{Germination percentage} = \left(\frac{\text{Number of normal seedlings}}{\text{Number of seeds kept for germination}} \right) x100$$

3.2.1.2 Vigour index (VI)

Vigour index was taken on five seedlings in each crop and variety thereof at each concentration. Vigour index was calculated as follows

VI= Germination percentage x (Root length in cm + Shoot length in cm). (ISTA, 1999)

3.2.1.3 Promptness index (PI)

Promptness index under each treatment was computed as described by George (1967). PI is given by the formula PI = nd_2 (1.00) +nd_4 (0.75) +nd_6 (0.50) + nd_8 (0.25) Where nd_2, nd_4, nd_6 and nd_8 are the germination percentages at 2, 4, 6 and 8 days after soaking.

3.2.1.4 Germination stress index (GSI)

GSI was computed by using the formula described by Dhopte and Livera (1989) and expressed in percentage

$$GSI = \left(\frac{PI\,at\,T2\ to T7}{PI\,at\,T1} \right) x100$$

3.2.1.5 Root length

Root length was measured from the base of the shoot to the tip of the root by using graph paper line intersection method according to the procedure detailed by Tennant, (1975). Root length was expressed in cm.

3.2.2 Biochemical parameters

3.2.2.1 Root plasma membrane (PM) H^+ ATPase activity

The isolation of plasma membrane rich fraction of root segments and H^+ ATPase activity was carried out according to Hodges and Leonard (1974) and modified to suit chromium ion studies according to Zaccheo *et al*. (1982). The root segments were homogenized in a mortar and pestle at 4^0C in a homogenization buffer containing 250 mM sucrose, 2mM mercapto ethanol, 3mM Na_2-EDTAand 40mM Tris- HCl (pH 7.5). The ratio of tissue to buffer was 1g tissue 2ml $^{-1}$ buffer. A fraction enriched in PM was obtained by differential centrifugation and by subsequent centrifugation of the microsomal membrane pellet resuspended in 1ml of 18 per cent sucrose in a sucrose gradient formed by constructed gradient former. Membranous material banding at the 34-45 per cent interface was removed with a syringe. PM containing fraction was dialyzed at 4^0C against 10 per cent sucrose in Tris –HCl and repeated with no sucrose and kept overnight. Protein was determined by the method of Bradford (1976) with bovine serum albumin as standard. ATPase assay was performed taking 1ml reaction mixture, 40mM Tris-HCL, 1.5 mM Na_2-EDTA, 3.5 mM ATP and 50µg PM protein and 3mM $MgSO_4$ with 100mM KCL at a

concentration ratio of 80:20 mM. The reaction was terminated after 30 min incubation at 37^0C by mixing assay mixture with 150µl of 50 per cent trichloroacetic acid. The quantity of inorganic phosphate was determined Kondrashova *et al*. (1965). After centrifugation 500µl of the supernatant was added to 2.5 ml of absolute ethanol. This was then mixed with 400µl of 10 per cent ammonium molybdate prepared in 8N H_2SO_4. The absorbance at 390 nm was read immediately and the Pi released was calculated from a standard curve. The activity was expressed in µM P_i mg protein^{-1} h^{-1}.

3.2.2.2. Lipid peroxidation

The lipid peroxidation was determined by malondialdehyde (MDA) content produced by thiobarbituric acid (TBA) reaction at low pH as described by Behra *et al*. (1999). The pink chromogen was measured at 532 nm and 600 nm for the correction of blank. Lipid peroxidation was expressed as melondialdehyde content in µM g^{-1}fresh weight.

3.2.2.3 Root Fe (III) reductase EC 1.16.1.7

Root Fe (III) reductase was measured according to Alcantara *et al*. (1994). Root segments were washed thoroughly and pre-treated for 30 minutes in a standard nutrient solution without micronutrients at pH 6.0. Then they were transferred to 50ml of a Fe(III) reduction assay solution which consisted of standard nutrient solution without micronutrients, 10^{-4} M Fe(III)-EDTA and 3 x 10^{-4} M Ferrozine, pH 5.0. After 2 hours the absorbance at 562nm was measured and the root was cut and weighed. The activity was expressed in µM Fe^{2+} g^{-1}fresh weight h^{-1}.

3.2.2.4 Superoxide dismutase (SOD) EC 1.15.1.1

SOD activity was determined by using nitroblue tetrazolium (NBT) salt as described by Beau Champ and Fridovich (1971) and expressed as enzyme units mg^{-1} protein min^{-1}. The reagents used in the enzyme assay are as follows.

Reagents:

Sl. No	Stock	Working solution	Total volume
1.	Riboflavin 200 μM (100x) 3.8 mg/50ml	2 μM	30 μl
2.	Buffer 250 mM (5x) $KH_2 PO_4$ 3.402g/100ml K_2HPO_4 4.354g/100ml	50mM	600 μl
3.	Methionine 130 mM (10x) 0.9698 g/50ml	13MM	300 μl
4.	EDTA 10mM (100x) 0.3722 mg/5ml	0.1mM	30 μl
5.	NBT 7500 μM (100x) (4.08 mg/5ml)	75 μM	30 μl
6.	Enzyme extract in Phosphate buffer (pH 7.8)	-	50 μl
7.	Distilled water		
	i. For sample	-	1.96
	ii. Blank (NBT- enzyme extract)	2.04	-
	iii. Reference blank (Enzyme)	-	2.01

The absorbance was measured at 560nm

Calculations

Absorbance of reference blank = x

Absorbance of sample = y

$$\left(\frac{x - y}{x}\right) x100 \text{ w} = \text{Z per cent inhibition}$$

50 per cent inhibition = $z/50$ = A unit

i.e. 50 μl enzyme extract yielded A unit

1000 μl yields $\left(\frac{A}{50}\right) x1000$ = B unit

$$\frac{B}{\text{protein valve}} = \text{SOD assay valve (Enzyme units mg}^{-1}\text{ protein)}$$

3.2.2.5 Anthocyanin content

Anthocyanin content was estimated according to Hrazdina *et al*. (1977) and was expressed as μg g^{-1} fresh weight.

3.2.3 Molecular studies

3.2.3.1 Stress Protein profile

Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) analysis (Laemmli, 1970) was done to identify the proteins synthesised during chromium stress. A known quantity of leaf and root sample was homogenized in phosphate buffer (pH 7.0) in a pre chilled pestle and mortar and the homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was used for analysis.

Materials

Stock acrylamide solution	
Acrylamide	30g
Bisacrylamide	0.8g
Water	100 ml
Separating gel buffer (pH 8.8)	
1.875 m Tris - HCI	22.7
Water	100 ml
Stacking gel buffer (pH 6.8)	
0.6 M Tris - HCI	7.26 g
Water	100 ml
Polymerising agents	
APS	0.5g $20ml^{-1}$, freshly prepared
TEMED	Fresh from refrigerator
Electrode buffer (pH 8.2-8.4)	
0.05 M Tris	12g
0.192 M glycine	28.8g
0.1 per cent SDS	2g
Water	2L

Sample loading buffer (5 X conc.)

Tris HCI buffer (pH 6.8)	5ml
SDS	0.5g
Sucrose	5g
Mercaptoethanol	0.25 ml
Bromophenol blue (0.5per cent W/V)	1ml
Water	10ml
SDS 10 per cent solution (Stored at room temperature)	

Protein stain solution

Coomassie brilliant blue R 250	0.1g
Methanol	40ml
Acetic acid	10ml
Water	50ml
Destainer	as above without the dye

Procedure

Thoroughly cleaned glass plates and spacers were assembled properly and were clamped in an upright position on a gel casting unit with 2 per cent agar in the bottom to seal the chamber leak proof between glass plates. 10 and 15 per cent gels were tried to get better resolution.

Separating gel	**15per cent gel**	**10per cent gel**
Stock acrylamide	20ml	13.3ml
Tris-HCl (pH 8.8)	8 ml	8 ml
Water	11.4ml	18.1ml
Degassed for 3-5 min and then added		
APS (5per cent)	0.2ml	0.2ml
10per cent SDS	0.4ml	0.4ml
TEMED	20 µl	20 µl

The gel solution was poured in the chamber between glass plates carefully leaving 4 cm from the top and a layer of distilled water was added on top of the gel and allowed to polymerise for 30-60 min.

Stacking gel	**4 per cent**
Stock acrylamide	1.35 ml
Tris-HCI (pH 6.8)	1ml
Water	7.5ml
APS (5per cent)	50 µl
SDS (10per cent)	0.1ml
TEMED	10 µl

After removing water from the gel, stacking gel (4 per cent) was added; comb was placed and kept for polymerising (30-60 min).

After polymerization the comb was taken out and the gel plate was removed from the casting unit to the electrophoresis apparatus. Concentration of the protein sample was adjusted to 50-200 µg in a volume of 25-50 µl, by mixing with sample loading buffer, boiling was done for 2-3 minutes to ensure complete interaction between proteins and SDS. The sample was cooled and carefully injected in each well with a microsyringe. Then the apparatus was filled with electrode buffer in both tanks, cathode was connected at the top and anode at the bottom and the DC power pack was turned to a constant current of 15 mA initially till the dye front crossed the stacking gel. Then the current was increased to 30mA until the bromophenol blue reached bottom of the gel (about 3 h).
The gel was removed from the plates and immersed in staining solution overnight. After proper staining the gel was transferred to destainer with gentle shaking. The destainer was changed frequently till appropriate visibility of the bands on gel was observed.
The gel was photographed.

3.2.3.2 Glutathione Reductase (GR) Isozyme analysis

Glutathione reductase isozymes were separated according to the procedure of Anderson *et al*. (1990), 500 mg of root sample was homogenized in 1 ml phosphate buffer (pH 7.0) in a pre chilled pestle and mortar. The homogenate was centrifuged in a refrigerated centrifuge at 18,000 rpm for 15 min and the supernatant was used for analysis. Electrophoretic separation of isozyme was done with 7 per cent polyacrylamide gel without using SDS. Casting of the gel was done as described earlier, without using SDS anywhere. Loading of the samples was done in fridge and

chilled electrode buffer without SDS was used. The power supply was set to a constant 15mA current, 80 volts for 17 hours at 4^{o}C. Gels were stained in a solution of 0.25 M Tris, pH 7.8, containing 0.24 mM

3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide, 0.4 mM NADPH, 0.34 mM 2,6-dichlorophenolindophenol, and 3.6 mM oxidised glutathione (GSSG) in darkness for 1 h). Duplicate gels were also stained in the absence of GSSG to distinguish GR from other sources of 3-(4,5-dimethylthia- zol-2-yl)-2, 5-diphenyltetrazolium bromide reduction.

3.2.3.3. Metallothionein 3 (MT3) gene expression

The MT3 gene expression was studied by enzymatic amplification of RNA by Polymerase Chain Reaction (RT-PCR) as described by Butler and Roesijadi (2001). Crude RNA was extracted from stressed plant samples. An oligonucleotide primer was co precipitated with the RNA to maximize the efficiency of their annealing to each other. Following annealing, cDNA was synthesized using reverse transcriptase and enzymatic amplification of this cDNA was then performed by PCR. MT 3 gene (Fig. 3.1) was obtained from Peter B. Goldsbrough, the details of which are as follows

Gene	:	*Arabidopsis thaliana* Metallothionein like protein (MT3) gene
Vector	:	pZL
Resistance	:	Ampicillin
Restriction enzymes	:	SalI + NotI
Vector size	:	4.3 kb
Insert size	:	0.5 kb

3.2.3.3.1 RNA extraction by phenol/SDS method

Chemicals used were diethylpyrocarbonate (DEPC), liquid nitrogen, grinding buffer, phenol equilibrated with TLE solution, chloroform, 8 M and 2 M LiCl, 3 M sodium acetate and 100 per cent ethanol. Fresh root segments were cut and cells were lysed and protein was removed by phenol/SDS extraction. RNA was separated from DNA and other impurities by selective precipitation with LiCl. Mortar and pestle was cooled by pouring a little liquid nitrogen over it. 15 g frozen tissue was taken and homogenised in the mortar and pestle until tissue became a fine powder.

Liquid nitrogen was added to keep tissue frozen. It was immediately transferred to a 500-ml beaker containing 150 ml grinding buffer plus 50 ml TLE-equilibrated phenol. The mixture was homogenised by adding 50 ml chloroform. The mixture was centrifuged at 10,000 rpm at 4°C. Aqueous layer was taken without disturbing the interface and transferred to a clean 500-ml bottle. 50 ml TLE-equilibrated phenol was added to this aqueous layer, mixing was done by shaking, and then 50 ml chloroform was added. TLE-equilibrated phenol and chloroform were added to the freshly removed aqueous layer to reduce possibility of degradation of RNA.

Remaining aqueous layer together with interface from initial phenol extraction was removed and transferred to a 50-ml tube. This material was centrifuged at 20 min at 10,000 rpm at 4°C. All the aqueous layers were combined. The 500-ml bottle containing the combined aqueous layers to mix TLE-equilibrated phenol and chloroform with aqueous phase was vigorously shaken and centrifuged for 15 min at 10,000 rpm at 4°C. Reextraction was done three times to avoid interface. Selective precipitation of RNA was done by transferring the aqueous phase to a clean 250-ml bottle with 8 M LiCl (1/3 vol.) to bring solution to a final concentration of 2 M LiCl. The mixture was precipitated overnight at 4°C.

The precipitate was collected by centrifugation for 20 min at 10,000 rpm at 4°C. Pellet was rinsed with a few millilitres of 2 M LiCl. The pellet was resuspended in 5 ml water and transferred to a 15-ml tube with 8 M LiCl to bring concentration of LiCl to 2 M and the RNA was precipitated at 4°C for at least 2 hr. RNA was recovered by centrifugation for 20 min at 10,000 rpm at 4°C. Pellet was rinsed with 2 M LiCl. The pellet was resuspended in 2 ml water. 200 µl of 3 M sodium acetate and 5.5 ml 100 per cent ethanol was added and precipitated in ethanol for 30 min. The extracted RNA was stored ethanol at -20°C. RNA recovery was done by centrifugation for 15 min at 10,000 rpm at 4°C and resuspended in l ml water. 10 µl was diluted to 1ml. A260 and A280 was measured, 1 OD260 = 40 µg mL^{-1} RNA

3.2.3.3.2 Primer construction

The cDNA sequence of the MT3 gene was as follows

1 CAACAACAAA CAAGAACTCA AACACTTCAT AACTAAAACA TCCTTTAAAG CCTTTTCAAA

61 AACTCAATCA TGTCAAGCAA CTGCGGAAGC TGCGACTGTG CTGACAAGAC CCAGTGCGTA

121 AAGAAGGGAA CCAGCTACAC CTTCGACATC GTCGAGACTC AGGAGAGCTA CAAGGAGGCC

181 ATGATCATGG ACGTTGGTGC CGAGGAGAAC AACGCAAATT GCAAGTGCAA GTGCGGCTCC

241 TCTTGCAGCT GCGTCAACTG CACTTGCTGC CCCAACTAAT GAAGCTTCTT TAATCAAAAT

301 GTAATATGAA TAAAAGTTGA TGTGGGCTCA TCTATTGAGC TCATGTCTCT CTTATTACTA

361 CTCTCTAGTA TGGTGTGATG TAATGGGTTA TGACCCTTCT TTCCCTTCCC TATAAAACTA

421 AAGAAACTTG CAAGATAATT GAAAAGA

The above sequence was uploaded to the server of Gene Fisher online primer construction site (Query ID _1041256020_12071) with the following supplied parameters.

Maximum Primer size:		15 to18 base pairs
Primer GC Contents	:	65 to 45
Melting temperature	:	42 to 55 ^{0}C
PCR distance	:	300 to 500

The first of the top three primers returned was taken up for construction.

Primer sequence		
Forward primer	:	GTCAAGCAACTGCGGAA
Reverse primer	:	GAAGGGAAAGAAGGGTCA
Product length	:	319

3.2.3.3.3 PCR amplification of RNA

The following chemicals were used for PCR amplification Crude RNA, 25 µg mL^{-1}, cDNA primer in H_2O, 3 M sodium acetate pH 5.5, 100 per cent and 70 per cent ethanol, 400 mM Tris pH 8.3, 400 mM KCl, reverse transcriptase buffer, 10 mM Tris, 10 mM EDTA pH 7.5, phenol buffered with 10 mM Tris, 10 mM EDTA pH 7.5, 24:1 chloroform/isoamyl alcohol, ~150 µg mL^{-1} amplification primers in H_2O (~20µM each), 5 mM 4dNTP mix (5 mM each dNTP in H_2O), 10´ PCR amplification buffer, Taq DNA polymerase .

RNA and cDNA primer were coprecipitated by adding 2 µg crude RNA, 25 µg (3 pM) cDNA primer and H_2O to 90 µl. 10 µl of 3 M sodium acetate, pH 5.5, and 200 µl of 100 per cent ethanol was added mixed and incubated overnight -20°C. The mixture was centrifuged for 15 min at high speed at 4°C and supernatant was discarded. 200 µl of 70 per cent ethanol was added and mixed gently by inversion. High speed centrifuge was done for 5min at room temperature and supernatant was discarded. The pellet was dried in a desiccator.

12 µl H_2O, 4 µl 400 mM Tris pH 8.3, 4 µl 400 mM KCl. was added to the RNA pellet. The sample was centrifuged minimally to collect the condensate at the bottom of the tube and later it was incubated for 3 hr at 52°C. Reverse transcriptase buffer and 16U reverse transcriptase was added to the mixture and was incubated for 1 hr at 42°C. 150 µl of 10 mM TrisHCl/10 mM EDTA, pH 7.5 was added to this and mixed later. 200 µl buffered phenol was added and vortexed and centrifuged for 5 min at high speed at room temperature, and upper aqueous phase was saved. 24:1 chloroform/isoamyl alcohol was added and vortexed briefly and centrifuged again for 5 min at high speed at room temperature, and upper aqueous phase was saved.

To this mixture 20 µl 3 M sodium acetate pH 5.5 and 500 µl of 100 per cent ethanol was added, mixed and precipitated overnight at -20°C. Later the mixture was centrifuged for 15 min at high speed at 4°C and supernatant was discarded. The pellet was dried and resuspended in 40µl water. Amplifification of the cDNA by PCR was done by mixing 5 µl cDNA, 5 µl each amplification primer, 4 µl of 5 mM 4dNTP mix, 10 µl amplification buffer and 70.5 µl H2O. The mixture was heated for 2 min at 94°C and centrifuged minimally to collect condensate. 0.5 µl Taq DNA polymerase was added and overlaid with mineral oil. The amplification cycles were 39 cycles for 2 min at 55°C, 2 min at 72°C and 1 min at 94°C, Cycle for 2 min at 55°C and 7 min at 72°C.

The products were analysed by electrophoresis in agarose gels by choosing the gel matrix for 320 size of the amplification product.

3.2.4. Ultra structural studies in scanning electron microscope (SEM) (Charles, 1990)

Glass tubes were filled with the fixative solution containing 6ml of 25mM phosphate buffer pH 7.0 and 3mL of 25 per cent glutaraldehyde. The cut tissue was place in the tube with the solution; care was taken to place the tube sideways to avoid air contact with the tissue. The

sample was incubated at 4^0C overnight. One per cent osmiumtetraoxide in phosphate buffer was added to the tubes and incubated for several days till osmium turned black. Osmium was poured off and rinsed three times with 25mM phosphate buffer. The tissue was then put through a series of alcohol immersion for 15 to 30 minutes each at 30, 50, 65, 75, 89, 100 per cent concentration of alcohol. Soaking of tissue with alcohol at 100 per cent was repeated twice for 15-30 minutes. Finally the tissue was soaked in 100 per cent alcohol over night. Alcohol soaking was done again twice the next day after which the tissue was stored in 100 per cent alcohol.

At the time of scanning the tissue samples were dissected upto 1cm and were dried. Silver paste was added to the tissue and allowed to dry for several hours after which the samples were placed in the SEM sample holder using mount gripper and visualised.

3.2.5 Chromium content

Measurement of chromium content ($\mu g\ g^{-1}$) was made on individual plants. Plants were harvested and roots washed with running tap water. Roots and shoots were separated and oven dried for three days at 80^oC. Samples were then ground into fine powder using a grinding mill. The conditions used for digestion were modified from Cary and Olsen (1975). Five millilitres of concentrated HNO_3 was added to 0.25g of dried sample in a 50ml digestion tube and allowed to stand overnight at room temperature. The digestion tubes were placed in a heating block for one hour at 150^oC, tubes were then removed allowed to cool and 2ml of 30 per cent H_2O_2 was added. The tube contents were mixed by swirling, and then heated for 2 more hours at 150^oC. After cooling the solution was diluted to 50 mL total volume and the upper clear portion was used for chromium estimation. During dilution, NH_4Cl was added at 2 per cent and $CaCl_2$ was added at 0.5 per cent to each sample and standard to control interference caused by iron (Fe) and phosphorus (P) respectively during spectrophotometer analysis. Digested samples were analysed for Cr in atomic absorption spectrometer (Varion Spectra AA-220) with air- acetylene flame at 358nm with 0.2mm spectral slit width.

3.2.6 Index of Tolerance (IT)

Crop species dry biomass was used to provide an index for Cr tolerance. The IT was calculated as:

$$\text{Index of Tolerance} = \left(\frac{\text{Mean dry weight of seedlings} + \text{Cr}}{\text{Mean dry weight of seedlings} - \text{Cr}} \right) x100$$

A similar calculation was used by Baker *et al*. (1994) for heavy metal uptake, accumulation and tolerance for a number of plant species.

3.3 Experiment Ib: Wheat coleoptile bioassay of tannery effluent

A laboratory experiment was conducted to assess the toxicity of different percentages of tannery effluent by wheat coleoptile bioassay. Tannery effluent was fractionated into organic and inorganic fractions by ethyl ether solvent technique (USEPA, 1993). Five, 10, 15 and 20 percent raw untreated effluent was used for fractionation and wheat seedlings were germinated in petri plates. Four mm coleoptiles were cut uniformly from wheat germinated seedlings. Increase in length over time was used to assess the toxicity of tannery effluent. Based on the result the percentage was fixed for experiment II.

3.4 Experiment II

A glass house experiment was carried out in the Department of Crop Physiology to study in detail the physiological, biochemical and molecular basis of chromium toxicity and tolerance due to soil added and tannery effluent amended chromium. Biological and chemical ameliorants were tested for ability to mitigate toxicity and induce tolerance. One tolerant variety each of one tolerant and one moderately tolerant crop was taken up for the study based on the results of experiment Ia. Tannery effluent concentration was fixed based on the results of experiment Ib. The experiment was planed and setup according to (Agarwala and Sharma, 1976; Shahandeh and Hossner, 2000b). Experimental details are given below.

Crops: Sunflower var. CO 27

Sorghum var. CO 4

Treatments: 13

Treatment details

T_1 - Control

T_2 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil

T_3 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil

T_4 - 10% raw untreated tannery effluent irrigated every 10 days

T_5 - 15% raw untreated tannery effluent irrigated every 10 days

T_6 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil +VAM inoculation

T_7 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil +VAM inoculation

T_8 - 10% raw untreated tannery effluent irrigated every 10 days + VAM inoculation

T_9 - 15% raw untreated tannery effluent irrigated every 10 days + VAM inoculation

T_{10} - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{11} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil+ $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{12} - 10% Raw untreated tannery effluent irrigated every 10 days + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{13} - 15% Raw untreated tannery effluent irrigated every 10 days + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

Replications : Three

Design : CRD

The inoculum of *Glomus mossae* was applied at 2000 spores per pot (Davies *et al*., 2002) as a band at the bottom third of the container, then covered with soil and the roots of the sunflower and sorghum seedlings were allowed to grow into the band and colonize.

3.4.1 Experimental setup

Seeds of crops, Sorghum (CO 27), Sunflower (CO 4) to be tested for tolerance were collected from concerned departments. Pot mixture was prepared by mixing thoroughly two parts of soil and one part each of well-decomposed farmyard manure and sand and filled in pots of size 28 x 30 cm with 10 kg of soil in each pot. The inner side of the pot was coated with wax for preventing water leakage. Fertilizer was added in the soil at the following rate: Urea - 100 mg of N Kg^{-1}, DAP- 50 mg of P Kg^{-1}, KCl - 50 mg of K Kg^{-1}. The soil containing added chromium treatments were thoroughly mixed with chromium salts before filling into the pots. Normal plant protection measures were adopted through out the crop growth period.

3.4.2. Effluent collection and analysis.

To characterise a range of tannery effluent, replicates of effluent samples were collected from five individual sites adjacent to chrome tannery industries from effluents outlet points.

Composite sampling technique was used wherein three replicate samples were collected over a period of 18 hours with one collection every 6 hours in separate new one litre polyethylene containers which were rinsed with samples several times before filling. All the samples were stored at $4^{o}C$ until chemical analysis. The effluent samples were acidified with concentrated HNO_3 to pH of 3.0 to stabilise the original valence state of chromium. Effluent samples were analysed according to USEPA (1994) for total chromium, by Inductively Coupled Plasma Atomic Emission spectrometric (ICPAES) method using the instrument model 8440 plasmalab, Labtam, Australia with a monochromator wavelength range of 160nm to 800nm and a polychromator with 30 Channels. Hexavalent chromium was analysed by diphenyl carbazide (DPC) method according to USEPA (1993).

Trivalent Cr was taken as the difference between total Cr and Cr(VI). The volume of effluent sample taken was 45 ml and 1ml of DPC was added, the pH was acidified to 1.8 with H_2SO_4, turbidity was eliminated by subtracting the absorbance observed before DPC addition from the absorbance after DPC addition. Initial calibration was done to 1 mg l^{-1}. One preparation blank was used per batch of samples and a spike duplicate taken for every sample replicate. Sodium content was determined using flame photometer. Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), pH and electrical conductivity were measured using a combined electrode pH meter and an electrical conductivity bridge respectively. Collected effluent was pooled, mixed thoroughly and diluted according to treatment requirements and used for treatment imposition. All the other physio-chemical properties of the effluent was analysed as per USEPA (1993).

Table 3.2 Physico-chemical characteristics of tannery effluent

Parameter	Value ($mg\ L^{-1}$)
Colour	**Brown**
Odour	Foul
pH	8.83
EC ($dS\ m^{-1}$)	17.86
Carbonate	387.0
Bicarbonate	1673.0
Chloride	3679.0
Calcium	245.0
Magnesium	138.0
Sodium	3478.0
Potassium	87.0
Sulphate	526.8
Total solids	18670.0
T.D.S	12578.0
Organic carbon (per cent)	0.023
Total N	224.42
Parameter	Value ($mg\ L^{-1}$)
Ammoniacal N	103.7
Parameter	Value ($mg\ L^{-1}$)
Total P	0.5
C.O.D	3287.0
B.O.D	1650.0
Mn	0.10
Zn	0.5
Cu	0.11
Fe	1.09
Cr Total ($\mu g\ L^{-1}$)	24640.0

Cr(VI) (μg L^{-1})	2833.0
Cr(III) (μg L^{-1})	21807.0

3.4.3 Soil characteristics

The physical and chemical properties of the soil used for pot culture are given in Table 3.3.

Table 3. 3 Soil properties of the pot culture experiment

Particulars	Pot culture experiment
Physical properties	
Field capacity (per cent)	14.80
Bulk density (cm^3)	1.42
Permanent wilting point	7.50
PH	7.1
EC (m. mhos. cm^{-1})	0.2
Chemical composition (kg ha^{-1})	
Available nitrogen	241
Available phosphorus	16
Available potassium	421
Total Cr	Trace

3.4.4. Sampling for observation

Plant samples were drawn at 30, 50 and 80 DAS from sunflower and 25, 45, 65 DAS for sorghum from each treatment for recording various morphological, physiological, biochemical and anatomical characters. Yield parameters were recorded at harvest for sunflower and green fodder yield was recorded for sorghum at 65 DAS.

3.4.5 Morphological Observations

3.4.5.1 Root length

Root length was measured from the base of the shoot to the tip of the longest root and expressed in cm $plant^{-1}$.

3.4.5.2 Shoot length

Shoot length was measured from ground level to the growing tip expressed in cm plant^{-1}.

3.4.6 Growth attributes

3.4.6.1 Leaf Area

Leaf area was measured using a leaf area meter (LICOR, Model LI3000) and expressed in cm^2 plant^{-1}

3.4.6.2 Total Dry Matter Accumulation (TDMA)

The TDMA at each growth stages from each plant was recorded after oven drying at 85^{o}C for 72 hours. TDMA was expressed in g plant^{-1}

3.4.7 Leaf Gas exchange measurements

Gas exchange parameters *viz*., photosynthetic rate (μ mole CO_2 m^{-2} s^{-1}) and stomatal conductance (m mol m^{-2} s^{-1}) were recorded using portable CO_2 gas analyzer (model CL - CID Inc, USA). The readings were taken on clear sunny day when the PAR was more than 1000 μ M m^{-2} s^{-2}. Third fully opened single trifoliate leaves from the top were clamped to the leaf chamber and held perpendicular to the incident light and only the computed values were taken. The instrument was working in open type principle thus maintaining a constant CO_2 flux to the leaf chamber, which is at ambient concentration. RH was maintained at a steady state level equal to the ambient RH to simulate a condition very similar to that of the ambient air.

3.4.8 Photo physiological measurements

Chlorophyll Fluorescence was measured by Plant Efficiency Analyzer (PEA) (Hansatech, UK) following the method advocated by Smillie (1983). Measurements were made on intact leaves which were dark adapted for 20 minutes prior to measurement .
The key fluorescence parameters *viz*., the fluorescence raise from the ground state value (Fo), its maximum value (Fm) and variable fluorescence (Fv) and the ratio of Fv/Fm and Fv/Fo are automatically calculated using the instrument.

$$\frac{Fv}{Fm} = \frac{\text{Ratio of variable fluorescence}}{\text{Maximum fluorescence}}$$

At the beginning of each experiment the background fluorescence from a dark-adapted leaf is measured when the LED light was on (Fo). Next maximum fluorescence yield of the leaf was measured which was the maximum fluorescence signal achieved when all chlorophyll molecules were saturated with light, and all electron acceptors were fully reduced (Fm). It was measured after applying a brief (0.8 second) saturating pulse of actinic light to the leaf after a period of dark equilibration. Quantum flux during the saturating pulse was in excess of 5000 µmol quanta m^{-2} s^{-1}. Fv/Fo was also calculated as follows.

$$\frac{Fv}{Fo} = \frac{\text{Ratio of variable fluorescence}}{\text{Ground state fluorescence}}$$

3.4.9 Biochemical metabolites and enzymatic parameters

3.4.9.1 Chlorophyll content

The chlorophyll content was estimated in fully expanded in the main branch at the specified stages (Arnon, 1949), and expressed in mg g^{-1} fresh weight.

3.4.9.2 Nitrate reductase activity (EC 1.6.6.1)

Nitrate reductase (NR) activity in all three stages. NR activity was estimated by using the method described by Hageman and Hucklesby (1971) and the enzyme activity was expressed in µ moles NO_2 produced g^{-1} h^{-1} fresh weight.

3.4.9.3 Catalase (EC 1.11.3.6)

Catalase activity was determined following the method of Luck (1974). One gram of the sample was extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of the extract was added to the experimental cuvette containing 3 ml H_2O_2 - PO_4 buffer. The time taken for change in absorbance at 240 nm was recorded for calculating the enzyme activity and expressed as enzyme units g^{-1} min^{-1} tissue. All the operations were carried out at 0-5^oC.

3.4.9.4 SOD (EC 1.15.1.1)

The method described in section 3.2.2.4 was used.

3.4.9.5. Ascorbate-glutathione pathway

3.4.9.5.1 Ascorbate peroxidase (APX) (EC 1.11.1.11)

The enzyme activity was measured according to Gerbling *et al*. (1984) following the oxidation of ascorbate to dehydraoascorbate spectrophotometrically. Reaction mixture contained, in a final volume of 1ml, 100μM HEPES-KOH buffer at pH 7.0, 2μM EDTA, 1ml of crude sample extract, and 2μM ascorbate and 1μM H_2O_2 for reading at 298 nm and 20μM ascorbate and 10μM H_2O_2 for reading at 310nm. The reaction was started by the addition of H_2O_2 and the enzyme activity was calculated from the observed rates of absorbance decrease and the milli molar extinction co efficient for ascorbate which is 0.80 and 0.055 mM^{-1} cm^{-1} at 298 and 310 respectively. The reaction was done at 25^oC. One unit of enzyme activity was that which catalysed the oxidation of 1μM ascorbate min^{-1}. Activity was expressed in mU g^{-1} fresh weight.

3.4.9.5.2 Dehydroascorbate reductase (DHAR) (EC 1.8.5.1)

DHAR was measured similar to MDHAR by measuring the reduction of dehydroascorbate at 265nm according to Hussain and Asada (1984). The activity was expressed in mU g^{-1} fresh weight.

3.4.9.5.3 Monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4)

Frozen root tissue was homogenized in ice cold 0.1 M Tris HCL buffer at pH 7.8, containing 1mM EDTA,1mM Dithiotreitol and 4 percent insoluble polyvinylpyrolidone (5ml buffer g^{-1} fresh weight). The homogenate was squeezed through a cheese cloth and centrifuged for 10 minutes at 20000g at 4^oC. The enzyme activity was measured in the supernatant at 25^oC as described by Foyer *et al*. (1989). MDHAR was assayed spectrophotometrically by following the decrease in absorbance at 340nm due to NADPH oxidation using an absorbance coefficient of 6.2mM^{-1} cm^{-1}. Monodehyroascorbate formed by ascorbate oxidase was used as standard. The enzyme activity was expressed in mU g^{-1} fresh weight.

3.4.9.5.4 Glutathione reductase (GR) (EC 1.6.4.2)

The method based on the increase in absorbance at 412 nm when 5.5'- dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by reduced glutathione (GSH) was used according to Smith *et al*. (1988). The reaction mixture contained 1ml of 0.2 M potassium phosphate buffer at pH 7.5 containing 1mM EDTA, 0.5ml 3mM DTNB in 0.01 M phosphate buffer, 0.25ml water, 0.1ml of 2mM NADPH, 0.05ml of GR ($1Uml^{-1}$) and 0.1 ml of 20 mM GSSG. The components

of the reaction mixture were added in the order stated in 4.5ml cuvette and the reaction was initiated by the addition of GSSG. The temperature was maintained at 24^{o}C. The increase in absorbance at 412 nm was monitored. The activity of the enzyme was expressed in unit activity which is the amount of enzyme which reduces 1μM of GSSG in 1hout at 24^{o}C.

3.4.9.5.5 Ascorbic acid

Ascorbic acid content was determined by volumetric method as proposed by Law *et al*. (1983). One gram of root was homogenised with four per cent oxalic acid and centrifuged at 5000 rpm for 10 minutes. The supernatant was made upto 25 ml. To 5ml of the aliquot, 10 ml of 4 per cent oxalic acid was added and titrated against 2,6- dichlorophenol indophenol dye. 5 ml of working standard containing 100 μg ml^{-1} of ascorbic acid was also titrated for standard reference value. The amount of ascorbic acid was expressed as μg g^{-1} fresh weight.

3.4.9.5.6 Total acid soluble thiols (TAST)

Non protein thiols was estimated according to De Vos *et al*. (1992) Roots were separated washed and dried, non protein thiols were extracted by grinding 20 mg of dry weight in 2ml of 5 per cent sulfosalicylic acid and 6.3mM diethylenetriaminepentaacitic acid (DTPA) at pH < 1 at 0^{o}C with quartz sand in a mortar. The homogenate was centrifuged at 10,000g for 10 minutes at 4^{o}C. The clear supernatants were collected and immediately used for determination of thiols. The level of total acid soluble thiols (TAST) was determined with Ellman's reagent. Three hundred micro litters of supernatant was mixed with 630 μl of 0.5 M K_2HPO_4 and 25 μl of 10mM 5.5'-dithiobis (2-nitrobenzoic acid)(DTNB) pH7. The absorbance was read 412 nm after 2 min. Values were corrected for the absorbance of supernatant and DTNB. TAST was expressed as μM g^{-1} dry weight

3.4.9.5.7 Reduced glutathione (GSH), oxidised glutathione (GSSG) and total glutathione

GSH and GSSG were assayed by the enzymatic GSSG recycling method advocated by Bergmeyer *et al*. (1974) with modifications according to Theodorus *et al*. (1981). One ml of 0.001 M EDTA at pH 7.0 was added to 1ml of crude extract of sample containing 100μl of 0.5 nM glutathione, 50 μl NADPH, 20μl DTNB, 20μl GR. The contents were mixed and the linear increase in absorbance was read at 412nm. A blank assay was run separately without glutathione.

A standard calibration was run, a standard graph was charted out and the amount of total glutathione was calculated from the graph. The amount of GSSG in the sample was determined by taking 1ml of sample with 1ml of 0.1 M potassium phosphate buffer to which 10µl of EDTA was added along with 10µl NADPH.

The reaction was monitored by stoichometric conversion of NADPH spectrophotometrically at baseline level of NADPH absorbance at 340mn. Standard calibration was done using 100µl GSSG instead of sample. The final concentration of GR amounted to 0.5 unit ml^{-1} in the total glutathione assay (GSH+ GSSG), and 1unit ml^{-1} in the assay of GSSG. GSH content was calculated by subtracting GSSG content from total glutathione. Both GSH and GSSG were expressed in µM g^{-1} dry weight.

3.4.9.5.8 Phytochelatin Sulph Hydryl (PC-SH)

The difference between TAST and GSH was taken as the level of phytochelatin SH as described by De Vos *et al*. (1992) and expressed in µM g^{-1} dry weight.

3.4.9.6 Ethylene content of roots

Ethylene content was measured according to Tani *et al*. (1997). 1 g roots from all treatments and control plants were cut and transferred to 25 ml vials containing ambient air and sealed with serum caps. Accumulated ethylene including ethylene produced in response to the wound was determined by withdrawing 2 ml gas samples using hypodermic syringe and injected into Gas Chromatography (GC) instrument.
This reading was compared with standard sample for retention time, height of peak and area covered by the peak and the ethylene concentration was computed using the formula below:

Standard amount of ethylene (E) in µmol = $\left(\frac{0.0446 \text{x Z } \mu l}{\text{Peak height in mm}}\right) x$ attenuation

Amount of ethylene evolved = E ×peak height of the sample in mm ×attenuation

3.4.9.7 Total free amino acids

Total free amino acids content was estimated from the ethanol extract of leaves by the reaction with ninhydrin solution (Shah *et al*., 1997) and expressed in µg g^{-1} fresh weight.

3.4.10 Molecular studies

3.4.10.1 Stress protein

The method described in section 3.2.3.1 was used.

3.4.10.2 GR isozyme profile

The method described in section 3.2.3.2 was used.

3.4.11 Nutrient Uptake

3.4.11.1 Total Nitrogen

Total plant nitrogen content was estimated by micokjeldhal method according to AOAC (1990) and expressed in percentage on dry weight basis

3.4.11.2 Total Phosphorus

Total Phosphorus was determined spectrophotometrically by the molybdo-vanadate method according to Chapman and Pratt (1961) and expressed in percentage dry weight basis.

3.4.11.3 Total Potassium

Potassium uptake was estimated according to Chapman and Pratt (1961) in triple acid extract using flame photometer type-21 and expressed in percentage dry weight basis.

3.4.11.4 Total Sulphur

Sulphur content was estimated from triacid extract using spectrophotometer according to Johnson and Ulrich (1959). Sulphur content was calculated by referring to a standard graph and expressed in per cent.

3.4.11.5 Iron

The micronutrients iron was determined by using Atomic absorption spectrophotometer (Varion Tectron 120) and expressed in ppm.

3.4.11.6 Boron content

The content of boron in leaves was estimated by the method proposed by Banuleos *et al*. (1992) using azomethine –H and expressed in $\mu g\ g^{-1}$ of sample on dry weight basis

3.4.11.7 Chromium content

The procedure followed for determination of chromium content was as described in section 3.2.5.

3.4.12 Yield and yield components for Sunflower

The data on the following yield and yield components were recorded employing standard procedures.

3.4.12.1 Number of seeds head^{-1}

Number of filled and unfilled seeds from the tagged heads was recorded.

3.4.12.2 Number of filled seeds head^{-1}

Number of filled seeds from the tagged heads was recorded.

3.4.12.3 Filled seed percentage

Percentage of filled seeds head^{-1} was worked out by dividing the number of filled seeds per head by the total number of seeds head^{-1}.

3.4.12.4 100 seed weight

One hundred filled seeds were counted and their weight was recorded and expressed in g.

3.4.12.5 Seed yield

Seed yield from each tagged heads was recorded at 14 per cent moisture level and expressed as g plant^{-1}.

3.4.12.6 Fodder yield of Sorghum

The data on the fodder yield were recorded employing standard procedures. Green fodder yield was recorded on the 65th day after sowing

3.5 Experiment III

A glass house experiment was carried out in the Department of Crop Physiology to study the effect of soil added chromium and tannery effluent on the growth and phytoaccumulation of selected tree species. Organic acid amendments were tested for their ability to enhance chromium phytoaccumulation. The experiment was planned and set up according to Pulford *et al*. (2001). Experimental details are given below.

Tree Species

1) *Albizia amara*
2) *Casuarina equisetifolia*

3) *Tectona grandis*
4) *Leucaena luecocephala*

Treatments details:

T_1 - Control

T_2 - 10 per cent raw untreated tannery effluent irrigated every 20 days

T_3 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil

T_4 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil

T_5 - 10 per cent raw untreated tannery effluent irrigated every 20 days + VAM

T_6 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + VAM

T_7 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil +VAM

T_8 - 10 per cent raw untreated tannery effluent irrigated every 20 days + 20mM citric acid

T_9 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + 20mM citric acid

T_{10} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil + 20mM citric acid

T_{11} - 10 per cent raw untreated tannery effluent irrigated every 20 days + 25mM oxalic acid

T_{12} - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + 25mM oxalic acid

T_{13} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil + 25mM oxalic acid

Replications : Three

Design : CRD

3.5.1 Experimental setup

Three month old seedlings of the afore mentioned tree species were collected from Department of Agroforestry, Forest College and Research Institute, Mettupalayam. Pot mixture was prepared by mixing thoroughly two parts of soil and one part each of well-decomposed farmyard manure and sand and filled in polyethylene bags of 300 gauge of size 20 x 30 cm with 5 kg of soil in each bag. Fertilizer was added in the soil at the following rate: Urea - 100 mg of N Kg^{-1}, DAP- 50 mg of P Kg^{-1}, KCl - 50 mg of K Kg^{-1}. The soil containing added chromium treatments were thoroughly mixed with chromium salts before filling into the pots. Normal plant protection measures were adopted through out the seedling growth period

3.5.2 Sampling for observation

Plant samples were drawn at five months after the initiation of the experiment from each treatment for recording growth and chromium uptake pattern.

3.5.3 Soil characteristics

The soil characteristics were the same as mentioned in Table 3.2

3.5.4 Observation recorded

3.5.4.1 Collar diameter

Growth in terms of collar diameter (CD) was recorded using a measuring tape and expressed in cm.

3.5.4.2 Chromium content

The procedure followed for determination of chromium content was as described in section 3.2.5.

3.5.4.3 Accumulation factor (ACF)

The accumulatory ability of the tree species of soil added Cr was estimated according to (Baker *et al*., 1994). ACF is given by the formula:

$$\text{ACF} = \frac{\text{Cr content in tissue}}{\text{Total Cr in soil}}$$

3.5.4.4 Bio magnification ratio (BMR)

The biomagnifications ratio for Cr(VI) was calculated according to Baker *et al* (1994). BMR is given by the formula:

$$\text{BMR} = \frac{\text{Cr content in tissue}}{\text{Cr(VI) in soil}}$$

3.6 Experiment IV

Field evaluation of CO 27 fodder sorghum variety was done using chemical amelioration technique. The field trial was conducted in the field of Mr. Gajendra Babu, Jayapuram village, Tirupattur Taluk, Vellore District, Tamil Nadu. Seeds of CO-27 fodder sorghum were sown in 2.5 cent plots with treatments designated as follows

T_1 - Control (recommended package of practice)

T_2 - 10 per cent raw untreated effluent irrigation

T_3 - 10 per cent raw untreated effluent irrigation +VAM

T_4 - 15 per cent raw untreated effluent irrigation

T_5 - 15 per cent raw untreated effluent irrigation +VAM

Design : Randomised Block Design (RBD)

Replication : four

3.6.1 Observations recorded

3.6.1.1 Leaf Area

The procedure followed for determination of chromium content was as described in section 3.4.6.1.

3.6.1.2 Chromium content

The procedure followed for determination of chromium content was as described in section 3.2.5.

3.6.1.3 Nitrogen

The procedure followed for determination of was as described in section 3.4.11.1.

3.6.1.4 Phosphorus

The procedure followed for determination of was as described in section 3.4.11.2.

3.6.1.5 Potassium

The procedure followed for determination of was as described in section 3.4.11.3.

3.6.1.6 Green fodder yield

Green fodder yield was measured as described in section 3.4.12.6.

3.7 Statistical analysis

The data was analysed statistically using a general linear model (Wilkinson *et al*., 1996) for analysis of variance in the respective designs. Critical difference (CD) was used to compare treatments.

CHAPTER IV

EXPERIMENTAL RESULTS

The present study was carried out with the aim of elucidating the toxicity of Cr in crops and screening crops and varieties thereof exhibiting tolerance to Cr. Physiological, biochemical and molecular aspects of Cr toxicity and tolerance was studied in detail. In addition, biological and chemical ameliorants to counter Cr toxicity were tested. Cr accumulation pattern of selected tree species were also studied. A series of experiments were conducted in the laboratory, glass house and farmers' field. The results obtained from each experiment was analysed statistically and is presented hereunder with appropriate tables and suitable graphs.

Experiment I: Laboratory studies – screening of crops for Cr tolerance

Treatment details

T_1 - Control

T_2 - Cr(III)- 100μM

T_3 - Cr(III)- 200μM

T_4 - Cr(VI)- 50 μM

T_5 - Cr(VI)- 100μM

T_6 - Cr(III)- 50μM + Cr(VI)- 50 μM

T_7 - Cr(III)- 100μM + Cr(VI)- 100 μM

4.1 Germination and Growth

4.1.2 Germination percentage (percentage) (Table 4.1)

Germination per cent decreased significantly in all treatments in comparison to control. T_7 showed the lowest germination percentage in all the crops. Green gram recorded the lowest among the crops (4.8) and sorghum recorded the highest (7.64) at the highest concentration of Cr(III) and Cr(VI). T_3 and T_4 were not significantly different from each other in all the crops and varieties. There was no significant difference between varieties in all treatments in green gram and rice. Sorghum CO-27 and sunflower CO-4 varied significantly in comparison to other varieties in T_7 and T_5 respectively. Treatments T_5 and T_6 were on par with each other in all the crops studied. There was no interaction between varieties.

4.1.3 Vigour Index (VI) (Table 4. 2) (Fig. 4.1)

There was a progressive decline in VI in all the crops studied as the concentration of Cr increased in the nutrient media. Lowest VI was seen in T_7 in green gram variety CO-6 (56) and the highest VI was observed in T_2 in sorghum variety CO-27 (889). There was no significant difference between T_3 and T_4; and T_5 and T_6. Varieties did not vary significantly in green gram and rice, whereas in sorghum and sunflower, varieties CO-4 and CO-27 respectively were significantly different from the rest of the varieties in T_2-T_6. Mean VI of 96.67 and 94.33 was observed in sorghum and sunflower as compared to 66 and 60.67 in rice and green gram respectively.

4.1.4 Promptness Index (PI) (Table 4. 3)

Significant variations in PI between T_5 and T_6 were not observed in all the crops. All the treatments were significantly lower than control with T_7 exhibiting least values (20.76 in sorghum and 10.17 in green gram). There was no significant difference between varieties in green gram and rice. Sorghum CO-27 exhibited significantly higher

PI (25.87) in T_7 than the rest of varieties. Similarly CO-4 sunflower was significantly higher than the rest of the varieties. There was no interaction between varieties and treatments. Highest PI of 178.51 was observed in sorghum CO-27 in T_2. The least PI was recorded by green gram CO-5 (144.48) in the same treatment. T_3 and T_4 did not vary significantly among themselves, although both recorded significantly lower values than control in the all the crops.

4.1.5 Germination stress Index (GSI) (Table 4.4)

GSI values of the treatments followed a concentration dependant decline in all the crops and varieties thereof. There was a three fold reduction in the GSI in comparison to control as an effect of Cr concentration the medium. The mean GSI values reduced from 236.88 to 42.92 in green gram, 246.12 to 42.82 in rice, 247.72 to 51.25 in sunflower and 245.98 to 50.90 in sorghum. The highest GSI at the mixed speciation concentration (T_7) was observed in sunflower var.CO-4 (10.51). This was followed by sorghum var. CO-27 in the same treatment. There was no significant interaction between varities and treatments.

4.1.6 Root length (cm plant^{-1}) (Table 4.5) (Fig. 4.2)

Root length decreased from 7.17 to 4.23, 7.47 to 4.10, 7.57 to 5.07 and 10.27 to 6.23 in green gram, rice, sunflower and sorghum respectively as the Cr concentration increased. T_6 recorded higher root length than T_5 in all the crops although they were not significantly different. Maximum reduction in root length was observed in T_7 and T_5 in comparison with control in all the crops. Sunflower variety CO-4 recorded significantly higher root length in T_7 (5.60) than Modern and CO-2 (4.80). Root length of rice variety TRY-1(7.3) was significantly lower in T_2 in comparison with ADT 36 (7.6).

A clear progressive decline in root length in response to Cr concentration and speciation was seen in all the crops. T_6 and T_4 were on par with each other in all the crops. Sunflower variety CO-4 and sorghum variety CO-27 were significantly different in their response to all the Cr concentration in comparison to other varieties respectively. Green gram and rice recorded 50.19 and 54.10 per cent reduction in root length over control in T_5 as against 32.60 and 31.49 in sunflower and sorghum respectively.

4.2 Biochemical traits

4.2.1 Anthocyanin content (μg g^{-1}fresh weight) (Table 4.6)

Anthocyanin content ranged from a highest of 0.0055 in sunflower variety CO-4 in T_4 to a minimum of 0.0009 in rice variety CO-43 and ADT-36 in T_7. In general T_4 recorded the maximum anthocyanin content in all crops. There was a significant increase in the anthocyanin content over control in T_2, T_3 and T_4 after which it declined in T_5 and T_6 to reach a minimum value in T_7 in all the crops. Significant varietal variations was observed in

sunflower variety CO-4 and sorghum variety CO-27 under combination treatments of Cr(III) and Cr(VI). Green gram and rice did not exhibit significant varietal difference in T_6 and T_7. No significant increase in anthocyanin content was observed in T_2 and T_3 when compared with each other.

4.2.2 Lipid peroxidation (μM g^{-1}fresh weight) (Table 4. 7)

Lipid peroxidation increased as the concentration of Cr increased irrespective of speciation of Cr. Increases was significant over control and between each treatment from T_2-T_5 whereas in T_6 and T_7 the increase was not significant between each other. Green gram variety CO-6 recorded the maximum value of 11.25 in T_7 as compared to 6.82 in control.

Significant varietal differences were observed in sunflower CO-4 and sorghum CO-27 with decrease in lipid peroxidation over other varieties in T_4, T_5 and T_6. There was no effect of interaction between treatments and varieties. Maximum increase in comparison with control was observed in rice and green gram (4.43) as against 3.93 and 3.89 in sorghum and sunflower respectively.

4.2.3 Superoxide Dismutase (SOD) (units mg^{-1} protein $hour^{-1}$) (Table 4.8)

SOD activity increased from T_2 to T_3 and took a declining trend from T_5 to T_7 in all the crops. Higher increase in SOD activity was observed in sunflower and sorghum in comparison to rice and green gram. There was no significant difference observed between treatments T_5 and T_6 whereas a steep decline was seen in T_7. All the treatments were significantly different from control. Lowest mean decrease in SOD activity (28.57, 17.31, 48.89 and 44.41) was observed in T_7 in green gram, rice, sunflower and sorghum respectively. Significant difference was observed between varieties in sorghum and sunflower. Interaction between varieties and treatments was absent. Significant difference between T_2 and T_3 was not present although both the treatments showed increased activity over control.

4.2.4 Plasma membrane H^+ ATPase (μM P_i mg $protein^{-1}$ h^{-1}) (Table 4. 9)

Plasma membrane H^+ ATPase activity reduced progressively over control as the concentration of Cr increased in the nutrient medium irrespective of speciation. Mean control values ranging from 17.23 to 17.53 were observed in green gram, sunflower and sorghum whereas higher control mean value of 19.63 was recorded in rice. T_2 and T_3 did not show any significant difference in plasma membrane H^+ ATPase activity in comparison with control although there was a general decline in these treatments. Significant decrease in activity was seen in T_4-T_7 over control. T_6 and T_5 were not significantly different between them.

Lowest activity was seen in T_7 at the highest concentration of Cr(VI) in medium. There was no significant difference observed between varieties in any crop in T_2 and T_3, whereas in T_6 and T_7 sorghum variety CO-27 and sunflower variety CO-4 exhibited significant increase in activity in comparison to other varieties. There was no interaction effect. Rice and green gram recorded 61.46 and 57.03 reduction in activity over control in T_7 in comparison with 46.44 and 45.65 in sorghum and sunflower respectively.

4.2.5 Root Fe (III) reductase (μM Fe(II) g^{-1}fr.wt h^{-1}) (Table 4.10)

Root Fe (III) reductase in control was 1.73 and 1.40 in sunflower and green gram respectively. All the varieties in both the crops recorded a general decline in activity from T_2 to T_5. T_6 recorded a slight increase in activity over T_5 and was significantly different. Treatments T_4 and T_6 did not vary significantly although both were significantly higher than control. Least root Fe (III) reductase activity of 0.65 in green gram variety CO-4 and 0.80 in sunflower variety CO-2 was observed in T_7. There was no significant interaction between varieties and treatments. Sunflower variety CO-4 exhibited significant variation over other sunflower varieties in treatments T_2 to T_7.

4.3 Molecular studies

4.3.1 Stress protein

4.3.1.1 Protein profile of Cr(III) 100 µM treated sunflower and sorghum varieties (Plate 7)

Sunflower var. CO-4 in lane 4 distinctly developed stress protein of 19 kDa Similarly a 23 kDa protein in the same variety was seen as evidenced by a band much darker than the rest of the sunflower varieties. Similarly sorghum var. CO-27 exhibited a stress protein of 45 kDa represented by a dark band as against the other two sorghum varieties K-10 and CO-26.

4.3.1.2 Protein profile of green gram var. CO-5 and rice var. CO-43 treated with Cr(III) and Cr(VI) (Plate 8)

Cr(VI) 50µM exhibited distinct dark bands with the presence of proteins in the range of 43-75 kDa indicating that Cr(VI) induced these bands in green gram var. CO-5. Very distinct 17-20 kDa proteins were formed due to the addition of both the species of Cr in the same variety. Unlike green gram, rice var. CO-43 exhibited strong stress protein production induced only by high concentration of both Cr(III) and Cr(VI) as seen in the last lane, a 50 kDa protein was distinctly seen in the band.

4.3.1.3 Root and leaf protein profile of Cr treated sunflower var. CO-4 (Plate 9)

Root protein profile showed that there were distinct proteins formed due to Cr(III) 200 µM, of molecular weight ~28, ~32 and ~40 kDa in sunflower var. CO-4 whereas the other Cr treatments did not exhibit any specific variation in comparison with control.
The leaf protein profile was distinctly different from root protein profile exhibiting rich presence of proteins at the full range of 14 – 83 kDa observed. Unlike roots, leaves exhibited a diverse range of stress proteins due to Cr(III) 100µM with very thick bands formed at low molecular weight range indicating the presence of stress proteins of ~14 – 40 kDa. Cr(VI) treated sunflower leaves exhibited bands of ~ 80 kDa molecular weight in sunflower var. CO-4.

4.3.2 GR isozyme pattern due to Cr(VI) stress in roots of sunflower, sorghum,

green gram and rice (Plate 10)

Three distinct isozymes were seen in all the crops due the treatment of Cr(VI) 100µM The isozyme bands were dark and distinct in the treated plants indicating that Cr stress induced the production of different isoforms of

the enzyme. Very distinct dark bands were observed in sunflower var. CO-4. Sorghum var. CO-27 showed more than three discernible bands for GR isozyme as did rice var. CO-43 due to Cr(VI) stress.

4.3.3. MT gene expression (Plate 14)

The RT-PCR product in sorghum var. CO 27 was distinct and corresponded to the band of MT3 gene for both the speciation of Cr. The bands formed by sorghum var. K 10 were lighter in comparison to CO 27 indicating low copy number of the MT RNA in K 10. Both the speciation of Cr induced the expression of MT3 in sorghum.

4.4 Cr content (μg g^{-1} DW) (Table 4.11) (Fig. 4.3)

Treatments T_2 and T_3 showed a progressive increase in Cr content in all the crops. T_4 showed significantly lesser Cr content in comparison with T_2 and T_3. There was significant difference in Cr content among all the treatments. Highest Cr content was observed in rice var. ADT-37 in T_7 (342.0) as against the lowest observed in sunflower var. CO-4 (282.8). All the crops showed significantly higher Cr content in treatment T_5 when compared to T_2. Lowest mean values of 118.58, 123.92, 111.33 and 119.13 in green gram, rice, sunflower and sorghum respectively were observed in T_4. Cr content in all the crops in T_6 and T_4 was significantly lower than T_5. There was no interaction between varieties and treatments. Sunflower var. CO-4 exhibited significantly reduced Cr content (268.1) in comparison with CO-2 (308.0) in T_3 and T_5. Sorghum var. CO-27 recorded significantly lower Cr content (297.0) in comparison with K-10, which recorded 329.3 in T_7. There was no significant difference between T_2 and T_5 in Cr content in all crops.

4.5 Ultra structure (Plate 13)

The scanning electron microscope photographs of sorghum root showed indications of clogging of vascular tissue treated with 300μM Cr(VI) for 7 days in nutrient culture.

Experiment Ib

4.6 Wheat coleoptile bioassay of tannery effluent (mm) (Table 4.12)

Organic fraction of the tannery effluent showed lesser toxicity in terms of coleoptile elongation in comparison with the inorganic fractions as an indication of lesser inhibition of endogenous auxin present in the coleoptiles. Elongation of wheat coleoptiles was the least in fifteen percent tannery effluent (4.01 at 5hrs to 4.43 at 30 hrs) and there was no elongation in 20 percent effluent after 5 hrs indicating the toxic effect of the effluent. In the case of inorganic fraction of the effluent the elongation was nil at 15 per cent concentration as well as 20 per cent concentration which is indicative of the presence of toxic concentration of metals and salts in the inorganic fraction. Minimal increase in the coleoptiles was seen in 5 (4.23) and 10 (4.21) per cent concentrations after 10 hrs.

Experiment II: Pot culture studies

Treatment details

T_1 - Control

T_2 - Cr (III) as $Cr_2(SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil

T_3 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil

T_4 - 10% raw untreated tannery effluent irrigated every 10 days

T_5 - 15% raw untreated tannery effluent irrigated every 10 days

T_6 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil +VAM inoculation

T_7 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil +VAM inoculation

T_8 - 10% raw untreated tannery effluent irrigated every 10 days + VAM inoculation

T_9 - 15% raw untreated tannery effluent irrigated every 10 days + VAM inoculation

T_{10} - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{11} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil+ $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{12} - 10% Raw untreated tannery effluent irrigated every 10 days + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

T_{13} - 15% Raw untreated tannery effluent irrigated every 10 days + $FeSo_4$ 0.5% spray at 25 and 45 DAS in sunflower and 15 and 40 DAS in sorghum

4.7 Morphological traits and growth

4.7.1 Plant height (cm $plant^{-1}$) (Table 4. 13)

Plant height increased progressively stage wise in sunflower and sorghum but the rate of increase was markedly reduced in stage III in comparison with stage II. Control treatment recorded the highest plant height of 139.5 and 156.5 in sunflower and sorghum respectively. Least height (107.0 and 112.5) was seen T_3 in sunflower and sorghum respectively at stage III. T_2 and T_3 were significantly different from control at all stages. T_3 recorded significantly less plant height in comparison with T_2 in both the crops in all stages of growth. T_6 recoded significantly higher values in comparison with T_2 as did T_8 in comparison with T_4 in both the crops at all the three stages of crop growth.

No significant differences were observed between T_7 and T_3, T_{10} and T_2 and T_{12} and T_4 at any stage although these treatments recorded increase in height. $FeSO_4$ sprays increased plant height but were not significant in the ameliorative treatments. A difference of 10.5 and 10.0 was seen under VAM treatment over 10 per cent effluent in sorghum and sunflower respectively as against 7.9 and 7.3 over Cr(III) at stage III.

4.7.2 Root length (cm $plant^{-1}$) (Table 4.14)

Least root length at stage III in sunflower was 9.20 in T_3 and was 15.0 in T_5 in sorghum although T_3 recorded least root length (4.71 and 8.12) in both the crops at stage I. Treatments T_2 and T_3 were significantly different from control at all stages of growth in both the crops. There was significant decrease in root length in T_3 in comparison with T_2. Rest of the treatments did not show any significant difference in stage I.

The effect VAM inoculation was evident in T_6 and T_4 where significant increase in root length over T_2 and T_8 at stage II and stage III respectively was observed . Similarly VAM was instrumental in increasing root length in T_9 which was significantly different from T_5 at stage III whereas it did not show any significance in the preceding

growth stages in spite of increase in root length. A difference of 2.81 between T_6 and T_2 was observed in sorghum as against 1.71 in the case of sunflower.

4.7.3 Leaf area (cm^2 $plant^{-1}$) (Table 4.15)

There was a steady increase in leaf area in sunflower as the age of the crop advanced; however, the rate of increase in leaf area was markedly reduced in the final stage. The maximum leaf area attained was 1436.0 and 1143.0 in sunflower and sorghum respectively. There was a decline in leaf area in sorghum at stage III in all the treatments. Effect of Cr(VI) was pronounced as evidenced by decline in leaf area significantly in comparison with both control and Cr(III) in both the crops. Lowest was observed in T_3 (946.00 and 923.00) followed by 15 per cent effluent treatment (964.92 and 941.46) at stage II in sunflower and sorghum respectively.

The ameliorative effect of VAM was evident as it increased leaf area 56.25 and 50.10 in Cr(III) treated plants. VAM treatment was significantly effective in 10 per cent effluent and Cr(III) treated plants but the increase in leaf area was lesser. VAM treatments were not significant in sorghum at stage I in Cr(VI) treatments whereas in Cr(III) treatments there was significant increase in leaf area. $FeSO_4$ treatments showed increase in leaf area but where not significant at any stage.

4.7.4 TDMA (g $plant^{-1}$) (Table 4.16)

Dry matter accumulation increased progressively in all the stages of the crop growth in both the crops in control from 6.29 to 63.46 in sunflower and 4.64 to 72.34 in sorghum. Effect of Cr(VI) was very evident as it decreased to 48.20 in sunflower and 44.53 in sorghum. T_2 was significantly less than control and significantly more that T_3 in all stages of crop growth in both the crops. T_5 recorded low dry matter (19.97 and 23.84) in stage II as against 28.44 and 32.32 in control in sunflower and sorghum respectively.

VAM as an ameliorant in Cr(III) and 10 per cent effluent showed significant increase in total dry matter in all stages of growth in both the crops. VAN as an ameliorant in Cr(VI) showed significant increase in TDMA only in sunflower at the reproductive stage. $FeSO_4$ as an ameliorant brought about an increase of 0.51 and 0.52 in sunflower and sorghum at stage III respectively. Cr(VI) caused a reduction of 24.14 and 18.93 in sunflower and sorghum in comparison with control.

4.8 Physiological processes

4.8.1 Photosynthetic rate (μ M CO_2 m^{-2} s^{-1}) (Table 4.17) (Fig. 4.4)

Photosynthetic rate increased from 22.30 and 23.56 in stage I to 29.90 and 32.24 in stage II and latter reduced to 26.40 and 27.10 in stage III in sunflower and sorghum respectively. Least photosynthetic rate of 17.59 was observed as consequence of Cr(VI) addition in sunflower at stage III. Cr(III) treatment reduced photosynthetic rate significantly as compared to control in all stages on the other hand it was significantly higher than Cr(VI) by 3.81 and 3.35; 3.09 and 4.74 in stage I and stage III in sunflower and sorghum respectively. An increase of 1.00 and 0.99 in sunflower and sorghum respectively was brought about by VAM inoculation at stage I.

The least effective was $FeSO_4$ as an ameliorant as it brought about an increase of only 0.31 and 0.17 in sunflower and sorghum at stage I. The highest significant increment of 1.32 was observed in T_6 in comparison with T_2. All the $FeSO_4$ treatments were instrumental in increasing the photosynthetic rate but the increase was not statistically significant.

4.8.2 Stomatal conductance (Table 4.18) (m mol m^{-2} s^{-1})

Stomatal conductance decreased steadily as the stage of the crops advanced in both the crops. Sorghum recorded higher stomatal conductance than sunflower at all stages. Highest value of 1.02 was observed in control at stage I in sorghum. The effect of Cr speciation was markedly seen as decline in stomatal conductance by 0.17 -0.09 in stage I and 0.15-0.09 in the stage II in both the crops. Cr(VI) was instrumental in decreasing stomatal conductance significantly more than Cr(III) in both the crops.

A significant increase in stomatal conductance was observed as a result of VAM inoculation as an ameliorant in Cr(III) and 10 per cent tannery effluent wherein VAM was able to significantly increase stomatal conductance by 0.028 and 0.032 in sunflower and sorghum respectively at stage III. VAM as an ameliorant increased stomatal conductance as did $FeSO_4$ in other treatments but the increases were not significantly different.

4.8.3 Chlorophyll fluorescence

4.8.3.1 fv/fm (Table 4.19)

The control plants exhibited a normal ratio of fv/fm expected of a healthy plant, although there was a general decline at senescence (0.81-0.75 in sunflower and 0.82-0.78 in sorghum). fv/fo reduced drastically due to Cr(VI) which was significant. The effect of Cr(VI) was significant in comparison with Cr(III) in both the crops and at all stages.
T_5 was on par with T_3 in sunflower and sorghum, which was the effect of effluent irrigation in the crop. VAM amelioration to Cr(III) and 10 per cent effluent was significant in alleviating the effect of Cr as evidenced by higher fv/fm ratio. At the final stage of crop growth Cr(VI) reduced fv/fm ratio to the least 0.51 and 0.56 in sunflower and sorghum respectively indicating its deleterious effect on the photosystem of the plants.

Ameliorants of VAM and $FeSO_4$ increased the fv/fm ratio slightly in Cr(VI) treated crops at all stages but the increase was not significant. The highest significant difference over control of 0.25 and 0.24 was observed in Cr(VI) treated plants in sunflower at stage I and stage III respectively.

4.8.3.2 fv/fo (Table 4.20)

There was a marginal decline in the fv/fo ratio in both the crops as the phenology of the crops advanced. The crops exhibited a range of 4.62 to 4.41 (sunflower) and 4.48 to 4.38 (sorghum), which is typical of a non stressed plant. The reduction in the fv/fm ratio due to senescence was evident in both the crops. A lowest value of 3.83 as against 4.62 in control was recorded in Cr(VI) treatment in sunflower at stage I. Cr(III) treatment exhibited a significantly higher value of 4.25 and 3.88 in sunflower and sorghum in comparison with Cr(VI).

T_5 recorded significantly reduced ratios in comparison with T_2 at all stages in both the crops and both the treatments were significantly lesser that control values. T_8 exhibited an increase of 0.084 and 0.095 over T_4 at stage III in sunflower and sorghum respectively, which was significant. VAM amelioration over Cr(III) also was instrumental in increasing fv/fo ratios by 0.169 to 0.154 through out the growth stages of the crop in both the crops. The highest nonsignificant increase in fv/fo was observed in T_7 over T_3.

4.9 Biochemical metabolite and enzymes

4.9.1 Chlorophyll *a* (mg g^{-1} fresh weight) (Table 4.21) (Fig. 4.5)

Chlorophyll *a* content increased as the growth stage of the crops advanced till stage II after which there was a decline in chlorophyll *a* content in both the crops at stage III. The decline resulted in chlorophyll *a* content was less than the amount present in stage I as an evident effect of senescence in leaves. Control recorded a maximum chlorophyll *a* content of 1.34 and 1.43 in sunflower and sorghum respectively. The least values in control were 1.12 and 1.06 at stage III in both the crops.

The least value for Chlorophyll *a* was 0.75 and 0.74 in sunflower and sorghum irrespective of the stage of growth. The addition of Cr(VI) and Cr(III) decreased significantly than control. The treatments T_2 and T_3 were significantly different from each other. T_6 recorded a significant increase in chlorophyll *a* content than T_2 as did T_8 and T_4. The inoculation of VAM as an ameliorant in Cr(VI) treated plants did increase chlorophyll contents but were not significant. A similar trend was seen in $FeSO_4$ treatment as well.

4.9.2 Chlorophyll *b* (mg g^{-1} fresh weight) (Table 4.22)

In comparison to chlorophyll *a*, chlorophyll *b* values were lesser, but similar trend associated with the growth of the crops was seen in control and treatments. However sunflower recorded increase in chlorophyll *b* contents in stage III in comparison to stage I. A maximum of 0.95 was seen in sunflower at stage II. In stage I chlorophyll content reduced to 0.67 in T_3 from 0.76 in control in sunflower.

There was no significant reduction of chlorophyll *b* observed in T_2 in comparison with control although there was a reduction of 0.04. Similarly T_2 and T_3 were not significantly different from each other indicating that speciation of Cr did not affect chlorophyll *b* contents. None of the ameliorative measures was effective in significantly increasing chlorophyll *b* contents in both the crops in at all stages of growth.

4.9.3 Chlorophyll *a*/*b* ratio (Table 4.23)

A distinct pattern of reduction in the chlorophyll *a*/*b* ratio was observed in both the crops as the growth stage advanced. The ratio reduced from 1.51 and 1.58 in sunflower in stage I to 1.33 and 1.39 in the same crop at stage III. The effect of 10 per cent effluent was least on chlorophyll *a*/*b* ratio, which exhibited a decrease of only 0.01 from control. Least ratio was observed in VAM inoculated in Cr(VI) plants however it was not significant.

Similar to chlorophyll *b* none of the ameliorative treatments were able to cause a significantly different effect on plants at any stage in both the crops. $FeSO_4$ treatments on the other hand reduced the chlorophyll *a*/*b* ratio significantly when applied to Cr(VI) treated plants.

4.9.4 Nitrate reductase (NR) (μ moles NO_2 g^{-1} h^{-1} fresh weight) (Table 4.24) (Fig. 4.6)

NR activity exhibited an increasing trend as the growth of crop advanced in both the crops and reduced at the final stage of the crop. NR activity was higher in sorghum than in sunflower through the stages of crop growth. The effect of senescence was evident in both the crops as it reduced the NR activity. There was a decrease from 10.12 in stage II to 6.27 at stage III in sorghum and from 6.98 at stage II to 5.12 at stage III in sunflower.

Cr(VI) treatment to plants effected a significant decrease in NR activity in comparison to control and similarly Cr(III) too had a significant decrease in NR activity as compared to control. Tannery effluent irrigation at 10 and 15 per cent also caused a significant decrease in NR activity compared to control. The difference between Cr(III) and Cr(VI) was not significant in both the crops at all stages of growth.

The effect of VAM inoculation was seen as it increased the NR activity when used as an ameliorant in Cr(III) and 10 per cent effluent irrigation at stage I of plant growth in both the crops. The highest increase was seen in Cr(III) treated plants with VAM inoculation (0.326) and it was 0.177 in 10 per cent tannery effluent irrigated plants at stage I in sunflower. The same significant effect of VAM was absent in both the crops at the final stage of observation although the NR increased from the respective stressed plants. $FeSO_4$ used an ameliorant did not effect any significant increase in the NR activity at any stage of the crop growth in both the crops.

4.9.5 Catalase (units g^{-1} min^{-1} fresh weight) (Table 4.25)

Catalase activity increased from stage I to stage II and latter it reduced at stage III in both the crops (3.32 to 4.10 at stage II and reduced to 3.10 at stage III in sunflower, and 4.68 to 5.34 at stage II and reduced to 4.21 at stage III in sorghum). The activity of the enzyme was lesser at stage III than at stage I indicating the senescence of the leaves. There was a general increase in the enzyme activity due to the effect of Cr and tannery effluent irrigation.

The highest increase was seen Cr(VI) (4.15 and 5.35) in sunflower and sorghum respectively at stage II. The influence of VAM and $FeSO_4$ was evident in the plants resulting in the increase of the catalase activity at all stages of growth of both the crops; however the increase was not significantly different. The increase in catalase activity due to Cr (VI), Cr(III) and tannery effluent irrigation was not significant indicating that catalase does not play a significant role in the antioxidant response of the plants under heavy metal stress.

4.9.6 SOD (units mg^{-1} protein h^{-1}) (Table 4.26)

SOD activity decreased with age of the crop in both the crops indicating the effect of senescence on the enzyme activity. The activity in sunflower reduced from 78.30 in stage I to 46.42 in stage III. Similarly in sorghum the SOD activity reduced form 61.40 in stage I to 38.31 in the stage III. The effect of Cr addition was evident by the increase in the activity of the

enzyme in both the forms of soil added Cr. Cr(III) and Cr(VI) treatments exhibited significantly higher activity than control in both the crops.

The difference between the two-speciation forms of Cr was not significantly different. The effect of VAM as an ameliorant did not show significant difference with the respective Cr treatments. Whereas the $FeSO_4$ treatment was effective in increasing the SOD activity significantly as evidenced by an increase of 5.810 in T_{13} in comparison to T_5 and an increase of 5.69 in T_{10} in comparison to T_2 in the case of sunflower in stage I.

4.9.7 Ascorbate-glutathione pathway

4.9.7.1 Ascorbate peroxidase (APX) (mU g^{-1} fresh weight) (Table 4. 27)

APX activity in fresh weight basis was higher in sunflower than in sorghum through out the stages of plant growth (3248.4 in sunflower and 2987.9 in sorghum at stage III). There was a high increase in Cr(VI) treated plants in both crops. A high increase of 191.43 in Cr(VI) and 141.29 in Cr(III) treated plants were seen at stage I in sunflower. On the other hand in sorghum the increase was lower (49.09 and 22.74 in Cr(VI) and Cr(III) respectively). The increase in both the crops at all stages was significant.

The increase in activity reduced as the growth stage of the crops advanced. The effect of VAM increased the activity but the increase was not significant. Whereas the effect of $FeSO_4$ as an ameliorant was evident by high significant increase in activity. The highest increase caused by $FeSO_4$ was seen in T_{13} in comparison to T_5 (64.81) at stage I in sunflower. In sorghum at stage III the same treatments showed a significant increase of 40.02 in sunflower. However, $FeSO_4$ treatments did not bring about the same significant increase in sorghum as seen in sunflower. The response of sorghum was different from sunflower.

4.9.7.2 Monodehydroascorbate reductase (MDHAR) (mU g^{-1} fresh weight) (Table 4.28)

In general MDHAR was lesser in sorghum than sunflower at all the stages of crop growth. In sunflower MDHAR ranged from 283.5 in stage I to 256 in stage II and it reduced further to 243.0 at stage III. In the case of sorghum the values followed a similar trend although it was lower than the values observed in sunflower; 247.0 at stage I to 204 .0 at stage II to a low of 184 at stage III. There was a general increase in MDHAR activity due the application of Cr and tannery effluent.

The highest increase was seen in T_5 (300.52) in the case of sunflower as against Cr(VI) (250.53) in the case of sorghum at stage I. Similar to other antioxidant enzymes assayed MDHAR increased at a lower rate in sorghum than in sunflower at all stages of growth. The increase in MDHAR activity at stage III in both the crops was less in comparison with the preceding stage of growth (243.0 in control to 251.0 in Cr(VI) in sunflower and 184 in control to 186.22 in Cr(VI) in sorghum). The increase in MDHAR activity due to heavy metal stress was significant in stage I over control. T_3 (298.71) recorded significantly higher activity compared to T_2 (294.73) in sunflower at stage I. Cr(III) did not increase MDHAR activity significantly in comparison to control in sorghum at stage I, whereas Cr(VI) showed significantly higher values over control.

Cr(III) and Cr(VI) treatments were on par with each other. A similar trend was observed in all the stages of growth the case of sorghum unlike sunflower wherein significant increases in activity was noted. The difference between the Cr speciation was not significant at final stages of growth in sorghum. The inoculation of VAM did not bring about significant changes in the activities of MDHAR at any stage in both the crops. However the $FeSO_4$ treatments were instrumental in increasing the activity of the enzyme significantly in all the stressed plants.

4.9.7.3 Dehydroascorbate reductase (DHAR) (mU g^{-1} fresh weight) (Table 4.29)

DHAR activity in terms of plant fresh weight was lesser than the activity observed in MDHA in both the crops at all stages of growth. The range of activity in control was 240.06 and 232.03 at stage I and 221.02 and 220.02 at stage II to a final value of 203.01 and 207.03 in sunflower and sorghum respectively. In the first two stages of observation sunflower exhibited higher values of DHAR activity than sorghum but in the final stage sorghum recorded slightly higher values than sunflower.

The increase in DHAR activity due to the addition of Cr speciation and tannery effluent was not significantly different at any stage of the crop in both the crops. Similarly the increase in enzyme activity brought by both biological and chemical ameliorants did not show any significance in spite of slight increase in activity.

4.9.7.4 Glutathione reductase (GR) (mU g^{-1} fresh weight) (Table 4.30)

GR activity consistently reduced as the crop stage advanced to reach a low of 293.0 at stage III from 356.0 at stage I in sunflower and 304.0 at stage III from 339.0 at stage I in sorghum. The activity of GR was higher than sunflower at stage III than sorghum although it was lesser in other preceding stages in comparisons to sunflower. The addition of both forms Cr increased the activity of the enzyme as did the addition of tannery effluents significantly.

T_2 was significantly different from T_3 as was T_5 and T_4. The addition of VAM was instrumental in increasing the GR activity by 3.33 (T_6-T_2), 3.75 (T_7-T_3) however the increase was not significantly different at stage I. The increase brought about by VAM inoculation was less compared to the increase at earlier stages of crop growth. $FeSO_4$ treatments were instrumental in effecting an increased activity of GR in sunflower plants at stage I significantly.

Similar increases were seen in stage II whereas there was no significant increase at the final stage of the crop. In the case of sorghum $FeSO_4$ treatments did not influence the enzyme activity significantly as was seen in sunflower. The Cr treatments significantly exhibited higher GR activities at stage III of the crop in both the crops.

4.9.7.5 Ascorbic acid (AA) (μg g^{-1} fresh weight) (Table 4.31)

Ascorbic acid contents were much lower in sorghum as compared to sunflower (425 and 403 at stage III in sunflower and sorghum respectively). There was a high increase in the AA content due to heavy metal stress in sunflower than sorghum although both were significantly different from control.

There was no significant difference between Cr(III) and Cr(VI) whereas there was a significant increase in 15 per cent effluent irrigations compared to 10 per cent effluent irrigation at stage I in sunflower. The difference between Cr(III) and Cr(VI) was significant in sorghum at stage II (412.87 in T_2 and 416.72 in T_3).

Similar to other thiol pathway enzymes the metabolite production of ascorbate was significantly increased by $FeSO_4$ treatments under all the forms of Cr stress. The highest increase in AA content was seen in T_{13} in comparison with T_5 (8.409) in sunflower at stage I. The increase brought by $FeSO_4$ as an ameliorant was not significant in the case of sorghum as it was in sunflower

4.9.7.6 Total acid soluble thiols (TAST) (μM g^{-1} dry weight) (Table 4.32)

TAST showed definite pattern of decrease in relation to stages in both the crops and there was wide variation of TAST content in both the crops. The TAST content ranged from 0.09 and 0.43 in sunflower in stage I thereafter it reduced to 0.03 and 0.33 in stage II and at the final stage it recorded minimum value of 0.02 and 0.15 in sunflower and sorghum respectively. Sorghum exhibited increased TAST in comparison to sunflower at all stages. The increase in TAST was very high in sunflower in all the Cr treatments (0.09 to 5.02 at stage I; 0.03 to 4.99 in stage II and 0.02 to 4.11 at stage III).

In contrast, the increase in sorghum was not as high as that observed in sunflower (0.43 to 0.64 in stage I; 0.15 to 0.81 at stage III). At stage II the effect of Cr treatments were instrumental in decreasing the TAST content in sorghum significantly. There was no significant difference observed between the two speciation levels of Cr. Fe SO_4 treatment were effective in reducing the TAST in all the Cr and tannery effluent treated plants. The decrease brought about by FeSO4 spray was significantly different in the case of Cr(VI) treated plants. VAM as an ameliorant did not cause any significant effect in both the crops at any stage.

4.9.7.7 Total Glutathione (μM g^{-1} dry weight) (Table 4.33)

The content of total glutathione decreased from stage I to stage III in both the crops. Sorghum recorded more total glutathione than sunflower at all stages of growth. At stage I sunflower and sorghum recorded 3.57 and 4.38 total glutathione respectively. The rate of decrease was almost the same in both the crops in control as the crop phase advanced.

The effect of Cr addition and the addition of tannery effluent was evident by a marked increase in the total glutathione contents in both the crops at all stages observed. Cr(III) addition was instrumental in increasing total glutathione significantly more than control as was the addition of Cr(VI). However, Cr(III) and Cr(VI) treatments were not significantly different from each other. At stage III the content glutathione, content did not show a significant increase over control in T_2 and T_3.

The effect of $FeSO_4$ as an ameliorant was effective in increasing the total glutathione content in all the treatments, with the highest increase observed in T_{12} as compared to T_4 at the stage III in sorghum (0.53). In sunflower too there was a corresponding increase as observed in sorghum. VAM did not show any significant effect on total glutathione in all the

treatments when used as an ameliorant. There was a slight decrease in total glutathione content due to VAM inoculation but it was not significantly different.

4.9.7.8 Reduced glutathione (GSH) (μM g^{-1} dry weight) (Table 4.34) (Fig. 4.7)

There was general decrease in reduced glutathione content as the crop stage advanced in both the crops; the rate of decrease however was less from stage II to stage III. In sunflower, the reduced glutathione content reduced from 3.21 to 3.18 from stage II to stage III and 4.10 to 4.03 in sorghum. The Cr treatments and tannery effluent treatments increased the reduced glutathione content in both the crops.

There was no significant difference between T_2 and T_3 at all stages of the crop in both the crops. Similar to total glutathione $FeSO_4$ treatments increased reduced glutathione contents at all stages of crops growth. The highest increase was seen in stage III of the crop 0.420 and 5.18 in sunflower and sorghum. The increase in reduced glutathione was not significantly different from control in T_2 and T_3 at the final stage of observation in both the crops.

4.9.7.9 Oxidised glutathione (GSSG) (μM g^{-1} dry weight) (Table 4.35)

GSSG content was much lesser than GSH in both the crops at all stages indicating that the conversion of GSSG to GSH was an active process in the crops. Similar to other thiols sorghum recorded more GSSG that sunflower at all stages (0.16, 0.14 and 0.13 in sunflower compared to 0.18, 0.16 and 0.14 in sorghum at stage I, stage II and stage III respectively). VAM as an ameliorant did not alter GSSG levels in sunflower at stage I, however there was an increase in VAM inoculated plants at stage II and stage III in both the crops, which was not significantly different from the two forms of Cr or tannery effluent treatment.

Unlike total glutathione and reduced glutathione $FeSO_4$ treatment in Cr(III), Cr(VI) and tannery effluent irrigation did not increase GSSG contents significantly, although there was a slight increase in the content itself. The highest increase in GSSG brought about by $FeSO_4$ spray was in T_{10} in comparison to T_2.

4.9.7.10 Phytochelatin Sulph Hydryl (PC-SH) (μM g^{-1} dry weight) (Table 4.36) (Fig. 4.8)

The increase in PC-SH due to Cr stress was much higher in sunflower than in sorghum at all stages of crop growth in control. There was steep increase in PC-SH in Cr treated plants as well as tannery effluent treated plants. The highest increase was seen in Cr(VI) treatment which was 8.78 as against 3.50 (T_1) in sunflower at stage I and 5.26 as against 4.63 (T_1) in sorghum at the same stage. The increase in PC-SH contents because of Cr addition was more in sunflower than in sorghum (4.26 in sunflower and 0.65 in sorghum at stage III).

$FeSO_4$ treatments brought about a significant increase in PC-SH over the respective non sprayed plants in both the crops. VAM treatments were instrumental in increasing PC-SH contents in both the crops at all stages but the increase was not significantly different from the respective Cr and tannery treatments. The increase brought about by $FeSO_4$ treatments at the final stage of observation was not significantly different unlike the previous two stages wherein the difference was significant.

4.9.8 Ethylene (Table 4.37) (μM g^{-1})

Ethylene content in roots of both crops increased as the crop growth stage advanced. The increase was 1.71 in stage I to 2.16 in stage II to a highest value of 2.26 at stage III in sunflower. In the case of sorghum, the content of

ethylene was more in comparison to sunflower. The increase was from 1.93 in stage I to 2.16 in stage II and a highest value of 2.63 at the final stage of crops growth in control plants. The effect of metal stress and tannery effluent increased the ethylene contents in the plants significantly.

Cr(III) increased the ethylene content to 2.46 from 2.26 in control in sunflower at stage III and 2.81 from 2.63 in the same stage in sorghum, the increase was significant. Similarly Cr(VI), 10 per cent tannery effluent and 15 per cent tannery effluent also increased the ethylene content significantly in comparison with control. The difference between T_2 and T_3 was not significant in both the crops at any stage. None of the ameliorants had any significant effect on the ethylene content of the roots at any stage in both the crops.

4.9.9 Free amino acids (μg g^{-1} fresh weight) (Table 4.38)

Free amino acids increased in stage II as compared with stage I in both the crops after which there was a decline in the content of free amino acids as an indication of plant senescence. There was a distinct and significant increase in the free amino acid content due to Cr treatment and effluent treatments at all stage of crop growth in both the crops. The increase was highest in Cr(VI) and 15 per cent tannery effluent treated plants (262.69 and 267.32) in comparison with Cr(III) and 10 per cent tannery effluent irrigation at final stage. The comparative increase in these treatments was to a lesser degree in the stage I and stage III.

The inoculation of VAM did not bring about a significant increase in the free amino acids when used as an ameliorant. On the other hand, $FeSO_4$ spray increased the free amino acid content significantly when used as an ameliorant. The highest increase in free amino acids brought about by $FeSO_4$ spray was in Cr(VI) treated plant (from 262.69 to 283.74). The increase in free amino acids brought by Fe SO_4 was significant in T_{11} at all sages of crop growth in both the crops.

4.10 Molecular studies

4.10.1 Stress protein: Leaf protein profile of Cr(III) and Cr(VI) treated sorghum var. CO-27 and sunflower var. CO-4 (Plate 11)

Cr(VI) induced a distinct band of ~ 80 Kda in sunflower var.CO-4 similarly, there were 48 and 75 kDa proteins seen in both Cr(III) and Cr(VI) in comparison with none seen in control in the same variety. In the case of sorghum var. CO-27 both the Cr speciation induced a protein of approximately 78 kDa, which was absent in control. There was a distinct variation in response to the speciation of Cr by both the crops. The bands representing lower molecular weight proteins were thick in all the treatments indicating that lower molecular weight were formed specifically due to Cr addition.

4.10.2 GR isozyme pattern: Leaf GR isozyme pattern of Cr(VI) and tannery effluent treated sorghum and sunflower varieties (Plate 12)

GR isozyme bands were thicker and distinct in both the crops due to both Cr in the soil and tannery effluent addition. A clear pattern of the presence of an extra band was seen in both the crops in Cr(VI) treatment as against tannery effluent treatment indicating the role of glutathione metabolism in the stress response due to Cr(VI).

4.11 Mineral nutrition

4.11.1 Nitrogen, Phosphorus and Potassium (percentage DW) (Table 4.39)

Total N content of the crops sunflower and sorghum was 1.12 and 1.18 respectively at stage III in control conditions. The least N was in T_3 (1.01 and 1.02 in sunflower and sorghum respectively) followed by Cr(III) treatment which exhibited 1.08 in sunflower and 1.12 in sorghum. The effect of effluent treatments was not as detrimental as the effect of soil added Cr as 10 per cent and 15 percent effluents treatments showed value of N (1.12) which was on par with control.

Similarly, there was an increase in the N content (1.19 and 1.19) in both the effluent treatments ameliorated with VAM. The increase in N content due to the inoculation of VAM was significant in all the treatments. On the other hand $FeSO_4$ treatments did not increase the N content in Cr(III) and Cr(VI) treated plants significantly. This trend was different in sorghum wherein T_7 was not significantly different form T_3 although the treatment increased the N content by 0.041.

Total P content was 0.072 and 0.079 in sunflower and sorghum respectively. T_3 recorded the least P (0.053 and 0.052) followed by Cr(III) treatment. Both speciation levels of Cr were significantly different from each other as they were from control. T_4 and T_5 gave slightly lower values than control and were on par with control values in sunflower whereas the same treatment increased P content in sorghum but still were not significantly different. Highest increase in P of 0.005 and 0.006 was observed in T_8 over T_4 in sunflower and sorghum respectively. VAM as an ameliorants was able to significantly increase the P content over their respective Cr or effluent levels in sunflower, which was similar in n sorghum wherein T_6 showed significantly higher P in comparison with T_2 as, did T_7 and T_8 over T_3 and T_4 respectively.

Potassium content was higher in sorghum (0.79) than in sunflower (0.74) at stage III in control conditions. The effect of Cr(VI) was evident in both the crops as it reduced the K content to 0.62 in sunflower and 0.63 in sorghum which was statistically significant. The next lowest K content was seen in Cr(III) treated plants which reduced

K content by 0.04 and 0.05 in sunflower and sorghum respectively. The treatments T_3 and T_2 were significantly different from each other. T_8 and T_9 increased K content significantly over T_4 and T_5 respectively. VAM as ameliorant increased K content in all treatments but was not significant.

4.11.2 Iron (percentage DW), sulphur (ppm) and boron ($\mu g\ g^{-1}$ DM) (Table 4. 40)

Iron content was high in sunflower (119.5) in comparison with sorghum (113.2) at stage III of the crop. Iron content was reduced significantly by T_2, and T_3 in both the crops, T_2 and T_3 were significantly different from each other with Cr(VI) reducing iron content more than Cr(III). Both 10 per cent effluent and 15 per cent effluent reduced iron content significantly than control as an evident effect of tannery effluent. Unlike other elements VAM treatment did not significantly increase iron content as an ameliorative effect. VAM treatments increased iron content by a range of 1.09 to 3.23 in all the Cr speciation treatments however the increase was not significant. $FeSO_4$ as an ameliorative measure was effective in increasing Fe content in all the Cr treatments. T_{10} significantly increased

iron by 8.51 in sunflower and 9.23 in sorghum over T_2. However, the highest increase in Fe content (11.86 and 12.46 in sunflower and sorghum respectively) was seen in T_{11} in comparison with T_2.

Sulphur content was 0.09 and 0.06 in sunflower and sorghum respectively. S content decreased significantly to 0.085 and 0.057 in T_2 as against 0.079 and 0.051 in T_3 in sunflower and sorghum respectively in comparison with control. S content was reduced in tannery effluent treatments and was significantly less than control, with 15 per cent effluent showing the maximum decrease (from 0.09 in controls to 0.082 in T_5). S followed a similar trend exhibited by iron content when used as an ameliorant. Highest significant increase in S content was seen in T_{11} by 0.016 over T_3 in sunflower. Whereas the S content increased only by 0.008 in the same treatments in sorghum. In general $FeSO_4$ treatments increased S when used as an ameliorant.

Boron content was 6.47 in sunflower and 6.89 in sorghum. There was no significant variation in boron content induced by the application of ameliorants. Cr(III) reduced B content significantly over control in both the crops as did Cr(VI), however T_2 and T_3 did not vary significantly with each other.

4.11.3 Cr content (μg g^{-1} DW) (Table 4.41) (Fig. 4.9)

Twenty to thirty fold increases in Cr accumulation in roots was seen in all the treatments irrespective of speciation and amelioration application when compared to shoots. Cr content in shoots was below detection limit, very low or in trace amounts in control in both the crops at stage III. Highest accumulation of Cr (228.9) in roots was seen in T_7 as an evident effect of VAM inoculation in sunflower whereas T_6 showed the highest Cr accumulation (231.8) in sorghum. Lowest accumulation of Cr was seen in 10 per cent effluent treatment (124.58 and 141.90 in sunflower and sorghum respectively).

There was high significant difference between T_2 and T_3 in both the crops with respect to Cr content in roots as well as shoots in both sunflower and sorghum. In general VAM increased uptake of Cr in all the treatments and was significantly higher in T_6 over T_2 and T_7 over T_3 in both the crops in roots. Least increase in Cr accumulation by ameliorants was seen in $FeSO_4$ treatment over Cr(VI) in sunflower and T_{10} recorded lesser Cr content than T_2 in the same crop although there was no statistical significance.

Shoot Cr content ranged from low of 4.76 and 2.79 in 10 per cent effluent to a high of 7.10 and 4.17 in Cr(III) treatments in sunflower and sorghum respectively. There was no significant difference in shoot Cr accumulation among the ameliorative treatments. In general keeping in view the amount of Cr applied to soil, Cr(VI) induced enhanced uptake of total Cr than Cr(III) in both the crops in root as well as shoots. T_9 increased Cr accumulation significantly in comparison with T_5 in sorghum whereas it was not significant in the case of sunflower.

The root to shoot ratio of Cr was lower in sunflower (26.4 to 30.57) than in sorghum, which exhibited higher values of 47.9 to 51.5 indicating, that lower amount of Cr was translocated to leaves of sorghum in

comparison to sunflower. The lowest root shoot ratio of Cr content was observed in T_{10} (25.84 and 51.02 in sunflower and sorghum respectively). There was no significant change in root shoot ratio of Cr accumulation pattern induced by ameliorants although there was a numerical decrease in ratios.
Cr content was lower in VAM treated shoots in comparison with pure Cr treated plants.

4.11.3 Cr uptake (μg plant^{-1}) (Table 4.42)

Total uptake in roots was higher in sorghum (Cr(III)-3792.46, Cr(VI)-3442.31) than sunflower (Cr(III)-3493.21, Cr(VI)-3117.61). Whereas in the above ground parts sunflower recorded higher values (Cr(III)-262.69, Cr(VI)-203.95) than sorghum (Cr(III)-162.70, Cr(VI)-132.51). VAM treatments in both the crops showed high uptake in roots, with T_6 recording 3899.80 in roots and 276.90 in shoots and T_7 recording 3563.18 in roots and 166.30 in shoots of sunflower. Similar results were seen in sorghum wherein T_6 recorded 4598.34in roots and 161.15 in shoots and T_7 recorded 3640.09 in roots and 137.13 in shoots. Least uptake was in seen $FeSO_4$ treated stressed plants mainly due to reduced dry of the plant. Tannery effluent recorded significantly reduced uptake in both the crops in roots and shoots.

4.12 Yield and yield components. (Table 4.43)

Sunflower

4.12.1 Number of seeds head^{-1}

The number of seeds per head was 543 in control. There was a significant reduction in seed per head due to Cr treatments. Cr(III) treated plants recorded 432 and Cr(VI) treated plants recorded 392 seeds per head. Tannery effluent treatment at 10 per cent showed higher seeds yield in comparison to both the soil added Cr treatments (442). Whereas, 15 per cent effluent did not show any significant difference in comparison with Cr treatments, although it was significantly lesser than the seed yield obtained in control. VAM as an ameliorant was instrumental in increasing the yield of 10 per cent tannery effluent treated plants and Cr(III) significantly (442 in T_4 and 463 in T_8; 432 in T_2 and 452 in T_6). $FeSO_4$ as an ameliorant did not have any significant effect on the seeds per head in sunflower.

4.12.2 Filled seeds head $^{-1}$

The control treatment recorded the highest file seeds per head (461.55). There was a significant reduction in filled seeds per head due to Cr speciation, with Cr(VI) treated plants recording the lowest filled seeds percentage (239).

Both the concentrations of tannery effluent showed significantly higher seed filling in comparison with the soil added Cr. VAM as an ameliorant was instrumental in increasing the filled seed per head in both soil added Cr as well as tannery effluent treatments. This indicated the effectiveness of VAM under both the stresses. On the other hand, $FeSO_4$ as an ameliorant did not have any significant effect on the seed filling of sunflower plants. The maximum increase due to VAM was seen in T_8 (344) in comparison with T_4 (304).

4.12.3 Percentage filled seeds and 100 seed weight (g)

Seed filling percentage was 0.85 in the case of control. This parameter was reduced significantly due to soil added Cr and tannery effluent in sunflower. The least recorded percentage was seen in Cr(VI) followed by Cr(III). Similar to filled seeds per head the seed filing percentage was significantly increased in VAM treated plants under stress. There was no significant effect of $FeSO_4$ treatments on the seed filling percentage when used as an ameliorant. 100 seeds weight was the highest in control (4.23) and there was significant decrease in the 100 seed weight due to Cr treatments and due to tannery effluent treatments. The ameliorant VAM as well as $FeSO_4$ did not influence 100 seed weight in the stress plants significantly.

4.12.4 Seed yield (g plant $^{-1}$)

Seed yield of sunflower plants was affected significantly by Cr speciation added to the soil as well as the irrigation of 10 and 15 per cent tannery effluent. The control yield was 19.52 and it reduced to 11.71 and 9.92 by Cr(III) and Cr(VI) respectively. The difference between the seed yield of Cr(III) and Cr(VI) was significantly different. The difference between Cr(III), 10 and 15 per cent tannery effluent treated plants was not significantly different. The use of VAM as an ameliorant was instrumental in increasing the seed yield per plant calculated on the basis of filled seeds per head. This indicated the effectiveness of VAM as an ameliorant in the case of stress caused by Cr speciation present in the tannery effluent

4.12.5 Green fodder yield (sorghum) (g plant $^{-1}$)

Green fodder yield was the highest in control (109.45). There was a significant reduction in green fodder yield due to Cr speciation. The lowest Green fodder yield was seen in Cr(VI) (88.96). VAM as an ameliorant increased fodder yield significantly in Cr(III) treated plants and this was not the case in Cr(VI0 treated plants wherein there was no significant difference in green fodder yield. FeSO4 used an ameliorant in Cr and tannery effluent stressed plants did not have any significant effect on green fodder yield.

Experiment III: Phytoaccumulation growth of Cr treated trees species

Tree Species

1) *Albizia amara*
2) *Casuarina equisetifolia*
3) *Tectona grandis*
4) *Leuceana luecocephala*

Treatments details:

T_1 - Control

T_2 - 10% raw untreated tannery effluent irrigated every 20 days

T_3 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil

T_4 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil

T_5 - 10% raw untreated tannery effluent irrigated every 20 days + VAM

T_6 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + VAM

T_7 - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil + VAM

T_8 - 10% raw untreated tannery effluent irrigated every 20 days + 20mM citric acid

T_9 - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + 20mM citric acid

T_{10} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil+20mM Citric acid

T_{11} - 10% raw untreated tannery effluent irrigated every 20 days + 25mM oxalic acid

T_{12} - Cr (III) as $Cr_2 (SO_4)_3.2H_2O$ @ 250mg kg^{-1} of soil + 25mM oxalic acid

T_{13} - Cr (VI) as $K_2Cr_2O_7$ @ 100mg kg^{-1} of soil+25mM oxalic acid

4.13 Growth as collar diameter (CD) increment (cm) (Table 4.44)

Among the tree species highest CD increase was seen in *C. equisetifolia* indicating that it is a fast growing tree, this was followed by *L. leucocephala* and *A.amara*. The least growth was observed in *T. grandis* indicating that it is a slow growing species. Among the treatments Cr(VI) affected the CD increments significantly in comparison to control in all the tree species (1.38 in T_3 and 1.65 in T_1).

A similar trend was seen in all the other tree species. VAM inoculation was instrumental in increasing the CD in Cr treated trees although it was not significant in Cr(III) whereas, significance was observed in Cr(VI) indicating that VAM has the potential to mitigate the stress caused by Cr(VI). The decrease in CD caused by Cr(III) was not significantly different from Cr(VI), although both were significantly different from control. This trend was not seen in *A. amara* where in VAM did not bring about significant increase in Cr treatments. Organic acids did not significantly affect growth in terms of CD when compared in both the Cr speciation treatment.

4.14 Cr content (µg g^{-1}) (Table 4. 45)

The general pattern of Cr accumulation was highest in *A. amara* followed by *T. grandis*, C. *equisetifolia* and the least accumulation was seen in *L. leucocephala*. Among the Cr treatments the Cr(III) treated trees accumulated significantly more Cr in tissue in comparison to Cr (VI) although the amount of Cr added to the soil was low indicating that the mobility of Cr(III) was high. Cr(III) treated trees exhibited a Cr content of 476, 524, 532 and 623 in the roots of *L. leucocephala*, *C. equisetifolia*, *T. grandis* and *A. amara* respectively.

In general roots accumulated more Cr than shoots in all the trees species at all the concentrations of Cr. There was an approximately 10 fold increase in root Cr content in the roots in comparison with shoot Cr content in all the trees at all concentration of Cr and all sources of Cr. The organic acids namely citric and oxalic acid significantly increased the Cr content in the tissues of roots in all the species under both speciation of Cr. A similar trend was seen in the case shoots of the Cr(III) treated tree species.

The highest increase brought by 20mM citric acid addition was seen in T_8 in comparison with T_2 in the case of *A. amara* followed by *C. equisetifolia*, *T. grandis* and the least was seen in *L. leucocephala* in the same treatments in roots of the respective tree species. In Cr(VI) tree species the above trend was not evident as the increase in Cr

content in these tree species due to organic acid addition was not significant although there was a numerical increase. Unlike organic acids, VAM treatment did not bring about a significant increase in the Cr contents of the entire tree species studied although there was a slight increase in the Cr content of all the trees.

4.15 Accumulation factor (Table 4. 46) (Fig. 4.10)

ACF was calculated in soil added Cr treatments. Cr (VI) had a general a high accumulation factor due to its faster and higher mobility in the soil. With regards, to the plant parts analysed it was seen that the roots had a higher accumulation factor due to higher retention of Cr by the roots in comparison with shoots. *L. leucocephala* had ACF of 1.90 in roots as against 0.18 in shoots in pure Cr(III) treatment. In Cr(VI) the same tree species had an ACF of 3.44 in roots and 0.35 in shoots ACF was higher in the case of Cr(VI) in all the tree species. VAM increased the ACF values significantly in both the Cr speciation in all the trees species.

There was a significant increase of ACF due to VAM inoculation in *A.amara* in the Cr(VI)(4.52 in control and 6.03 in VAM). Similarly higher ACF was observed due to addition of both organic acids. The increase brought by organic acids was higher than VAM. The highest increase in ACF was seen in citric acid treatment. Followed by oxalic acid treatment in all the tree species. The increase brought by organic acids was consistent in both root and shoots of all the tree species. The highest accumulation factor was observed in *A.amara* followed by *T. grandis*, *C. equisetifolia* and the least ACF was seen in *L. leucocephala* irrespective of the species of Cr added to the soil.

4.16 Bio magnification ratio (BMR) (Table 4.47)

BMF values were calculated in Cr(VI) treated tree species. General increase in BMF was seen due to VAM and organic acids as compared to pure Cr treatments. The BMF was much higher in roots when compared to shoots which almost 10-12 times higher (15.95 and 1.52 in *A.amara*). *A.amara* exhibited the highest BMF values in organic acid treated tree seedlings which were 17.0 and 1.68 in citric acid treatment in roots and shoots respectively and a little lesser (16.02 and 2.64) in roots and shoots respectively in oxalic acid treatments. The BMF values also followed the same trend seen in AMF values indicating that *A.amara* was a good remover of Cr from the soil.

Experiment IV

Treatment particulars

T_1 - Control

T_2 - 10 per cent effluent irrigation

T_3 - 10 per cent effluent irrigation +VAM

T_4 - 15 per cent effluent irrigation

T_5 - 20 per cent effluent irrigation +VAM

4.17 Leaf area (cm^2 $plant^{-1}$) (Table 4.48)

Leaf area was 2835 in control plot as against a reduction to 2640 due to effluent irrigation at 10 per cent concentration and 2743 in the case of 15 per cent effluent irrigation. The leaf area increased significantly due to VAM treatment in both tannery effluent treatments

4.18 Chromium content (μg g^{-1}) and macronutrients (percentage DM) (Table 4.48)

Cr content was trace in untreated control as against 4.56 and 5.78 in effluent irrigated plots. VAM increased the Cr content in the effluent treated plots although the increase was not significantly different. Nitrogen content was 1.21 in control plot and there was no significant difference between treatments in the N content of the plants. VAM inoculation increased the N content in the effluent irrigated plants although the increase was not significantly different from control.

Unlike N there was a significant increase in P content due to inoculation of VAM in effluent treated plots indicating the potential of VAM in mobilising nutrients to the plant. The increase was from 0.075 to 0.082 in the case of 10 per cent effluent irrigation and 0.072 to 0.081 in the case of 15 per cent effluent irrigated plots. The potassium content in the crops was reduced due to effluent irrigation but was not significant and VAM did not have any significant effect in the case of both the effluent concentration used.

4.19 Green fodder yield (tons ha^{-1}) (Table 4.48)

There was a significant difference in the green fodder yield due to effluent irrigation at higher concentration. The green fodder yield reduced by 3.86, 4.65 in 10 and 15 percent tannery effluent irrigation and was significantly different. The effect of VAM as an ameliorant was instrumental in increasing the green fodder yield significantly in comparison with 10 per cent effluent treatment whereas it was not significantly higher in the case of 15 per cent tannery effluent although there was an increase in the green fodder yield.

CHAPTER V
DISCUSSION

Chromium toxicity in the present study reduced growth, vigour, photosynthesis, respiration, water relations and reproduction, caused changes in certain organelles and disrupted membrane structure and function. Majority of Cr present in natural form is in the trivalent state, which is less toxic; hence it is very rare to observe Cr toxicity in nature. It has been known that Cr in very low concentration produces certain stimulatory effects in plants (Bonet *et al*., 1991). It has been proposed that the positive effects may be due to the minimal substitution of Cr for molybdenum, but this effect has been in general very difficult to explain (Huffman and Allaway, 1973a).

Plant responses to metals are dose dependent. For essential metals, these responses cover the phases from deficiency through to sufficiency. For non-essential metals, only the tolerance and toxicity phases occur. Solution culture is frequently used to determine plant sensitivities to metal toxicity. The main advantage that solution culture has over soil culture is that, the composition of the growing medium can be defined, manipulated and measured with a high level of precision, thus reducing confounding variables and giving quantitative answers.

As research has shown that precipitated metals are not plant-available, and that complexed metals are generally not as available as the free metal, hence the results obtained in the solution culture study can be decisively taken as a true effect of Cr on plants. Soil culture experiments are more precise in providing results closer to the natural effect of Cr in view of the fact that the bioavailability of Cr is greatly affected by the soil properties. Compounds with potential for chelating Cr(III) or reducing Cr(VI) may be present in soils amended with tannery effluent due to the presence of organic matter in it. Thus, potential toxicity of Cr speciation added to soils may be affected by the interactions between oxidation-reduction and organic complexation. Plants cannot usually access the total pool of a metal present in the growth substrate. Instead, the fraction of the metal, which plants can absorb, is known as the available or bio available fraction.

Metals present in a soil can be divided into a number of fractions including; the soluble metal in the soil solution, metal-precipitates, metal absorbed to clays, hydrous oxides and organic matter, and metals within the matrix of soil minerals (Reichmann, 2002).

These different fractions are all in dynamic equilibrium with each other. However, while the soluble metal in the soil solution is directly available for plant uptake other soil metal pools are less available (del Castilhos *et al*., 1993). Metal tolerant plants obtained from the results of solution culture experiments offer an excellent opportunity to analyse the impact of complex metal mixtures on the performance of plants during a full life cycle.

An understanding of the dynamic mechanism of Cr speciation and its physiological, biochemical and molecular mechanism of action in causing toxic effects have been gained

from the present study. The use of biological and chemical ameliorants in this study has given interesting results. The possibility of phytoremediation of Cr contaminated soils has been effectively addressed in the present study with worthy results.

The results obtained from all the experiments conducted in the present study are discussed hereunder in detail with an attempt to throw light into the physiological, biochemical and molecular mechanism of chromium toxicity and tolerance in higher vascular plants.

5.1 Cr speciation effects

Cr has a complex chemistry and hence the detailed mechanism of toxicity of Cr is yet to be clearly explained in higher vascular plants. There is evidence that no conversion occurs for Cr species in the nutrient solution before absorption by plant roots. McGrath (1982) measured the valence of Cr remaining in solution that originally contained either Cr(III) or Cr(VI) after culturing oat plants for 4 weeks. The results showed that both Cr ions remained unchanged in the nutrient solution for the duration of the experiment.

This contradiction can be basically attributed to the differences in bioavailability of Cr(III) and Cr(VI) at various pHs, amounts of the two forms of the element, and concentrations of other ions in the root substrate in culture techniques. The results obtained in all the morphological, physiological and biochemical parameters in the solution culture experiment showed that Cr(VI) and Cr(VI) + Cr(III) combination was more toxic than Cr (III) to all the varieties of all the crops, although it was a concentration based response which is in accordance with Zayed *et al*. (1998). Similar results were confirmed in soil culture experiment, which is in corroboration with Davies *et al*. (2002).

The reason for this differential toxicity of Cr speciation can be attributed to the fact that as a soluble anion, Cr(VI) readily penetrates cell membranes and is toxic as an oxidising agent which is evident by the high redox potential of the Cr(VI) with a range of 1.33 and 1.38 eV. In contrast, Cr(III) is soluble at biological pHs only when organically complexed (Kotas and Stasicka, 2000).

Thus Cr(III) can be taken up by plants and induce toxic symptoms only when present in high concentration. In addition to this Cr(III) is kinetically inert to ligand substitution and therefore can form substitution inert metalloproteins complexes *in vivo* (Kendrick *et al*., 1992).Thus its role in causing toxic symptoms is greatly reduced.

5.2 Effect of Cr on plants

5.2.1 Germination

In general, all the parameters recorded with respect to germination the inhibition of was more pronounced in crop varieties grown at the higher concentrations of chromium. Cr(III) was less toxic than Cr(VI) in terms of

germination percentage, vigour index, promptness index and germination stress index. Similar results have been reported by Rout (2000) and Jetly and Srivastava (1995). This might be attributed to depressive effect of Cr(VI) on the activity of amylases and subsequent transport of sugars to the embryo axes.

Protease activity, on the other hand, increases with the chromium treatment, which could also contribute to the reduction in seed germination of Cr treated seeds (Zeid, 2001). Green gram was the least tolerant crop, which is in accordance with the results obtained by Subramani *et al*. (1998). Under high concentrations of Cr speciation combination, the reduction in germination percentage and in seedling vigour could be due to the direct contact of seedlings roots with Cr in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium (Bishnoi *et al*., 1993).

5.2.2 Morphology and growth

The root growth development of seedlings are important stages of whole plant growth, it is also the most sensitive stage of plants to changes in their surrounding environment. Root growth was seriously affected by Cr concentrations in solution culture and was confirmed in the soil culture experiment. This is in accordance with Davies *et al*. (2002) and Suseela *et al*. (2002). General response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division, inhibition of root elongation or due to the extension of cell cycle in the roots (Chen *et al*., 2003).

Roots do not grow as a unit, they grow to a large extent independently of each other (Burstrom and Svensson, 1972). As Cr could be present at an irregular distribution pattern within the different root tissues, there may be different modes of injury and toxicity effects in different root tissues. Cr(VI) was more toxic to root growth than was Cr(III) possibly due to the fact that Cr(VI) or Cr(III)+Cr(VI) were able to penetrate the cell membrane and cause extensive biochemical imbalance and apart from this, it could have caused membrane disruption by lipid peroxidation as evidenced by MDA production.

The root growth inhibition was much reduced in pot experiment by soil added Cr and tannery effluent as compared to solution culture experiment. This could be due to chelation, adsorption or immobilization of soil added Cr and also may be due to the presence of nutrients and organic substances in the effluent.

An important arbuscular mycorrhizal genus is *Glomus mossea*, which colonizes a variety of host species (Marschner, 1995), including sunflower and sorghum (Chandrashekara *et al*., 1995; Medeiros, 1994). VAM was effective in mitigating root growth inhibition in inoculated plants in the present study as also reported by Davies *et al*. (2001) and Clark (2002). This could be due to its ability to accumulate high amounts of Cr and protect the roots from injury (Galli *et al*., 1994). There was a decrease in shoot growth and was brought about by both the species of Cr. Similar results have been reported by (Samantaray, 2002). Cr stress induced, reduced root growth had a direct impact on the shoot growth of the stressed plants.

The reduction in plant height was mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above ground parts of the plant. In addition to this, the Cr transport to the ariel part of the plant contributed to the reduction in plant height. The Cr reaching the shoots could have had a direct impact on the metabolism of shoots, which could have manifested in decreased height.

The deleterious effect of Cr on the growth of all the tree species was evident from the reduction in collar diameter of the seedlings. The reduction in collar diameter could have been due to Cr induced toxicity to the outer cells of the tree barks affecting the secondary thickening processes of the trees. The possibility of reduced root growth and resultant impaired water and nutrient status of the trees could have caused decrease in collar diameter increments. The results obtained are in confirmation with Pulford *et al*., (2001).

Leaf area was reduced by both the speciation of Cr and to as lesser extent by tannery effluent, which is in accordance with Barceló *et al*. (1986). The process of cell division is completed early in leaf development and final leaf area is achieved during the subsequent cell expansion phase, whereas the final leaf size is determined by cell division occurring throughout development (Ferris *et al*., 2002). Cr binding to the cell walls could have reduced the cell expansion, resulting in reduced leaf area. Another possible explanation could be that interference of Cr in the processes associated with the biosynthesis of cell wall components, which could lead to changes in cell wall structure and ultimately bring about a reduction in leaf area (Barceló *et al*., 1986).

All crops species tend to invest most of the energy in leaf expansion in order to convert maximum solar energy to dry matter. Total dry matter accumulation is directly related to the efficiency of the leaf in intercepting and utilising the available solar energy. The decrease in leaf area brought about by both the species of Cr as well as tannery effluent caused a decrease in the assimilatory surface. This in turn caused a reduction in solar energy conversion resulting in reduced dry matter and biomass.

Apart from this, the photosynthetic apparatus was damaged by the presence of Cr in the leaf. A combination of both reduction in the leaf area and reduced photosynthesis caused a reduction in the dry matter in the stressed plants. The presence of organic substance and nutrients in tannery effluent could have been the reason for the reduced effect of Cr on the above parameters in comparison with pure Cr addition in soil. This is in accordance with the report of Singh (2001).

Mycorrhizae increase nutrient and water uptake, alleviate cultural and environmental stresses, and enhance disease resistance and plant health (Bethlenfalvay and Linderman, 1992). The effect of VAM was pronounced in mitigating the deleterious effects of Cr and tannery effluent, Diaz *et al*. (1996) have reported similar results. The possible mechanism for this could be that, higher mobilization of essential nutrients by VAM to the leaf tissue, which was successful in partially alliveiating the damaging effect of Cr on leaf area expansion, and photosynthesis of the plants thus bringing about an increase in the dry matter than the corresponding soil added Cr and tannery effluent treated plants.

A possible explanation for the overall growth reduction brought about by Cr stress could be due to increased cross linking of pectins in the middle lamellae causing increased cell adhesion, which would be a

significant source of resistance to growth (Mukhopadhyay and Aery, 2000). Cr induced increase in peroxidase was seen in the present study this might enhance phenolic cross-linking, which could inhibit cell wall expansion (Biggs and Fray, 1987).

5.3 Water relations and photo physiology

5.3.1 Stomatal conductance

Stomatal conductance is a numerical measure of the maximum rate of passage of either water vapour or carbon dioxide through the stomata. Stomatal conductance plays an important role in the plant-atmosphere water exchange and hence it is a key parameter in many ecological models (Chen *et al*., 1999). Diffusion of CO_2 into the mesophyll of leaves and water vapour from the leaves to the atmosphere is mainly driven by the stomatal aperture, which is controlled by a complex system of plant physiological processes. Stomatal conductance decreased steadily as the stage the of the crops advanced in both the crops, this phenomenon can attributed to the operation of senescence mechanism in the crops.

A picture of stomatal conductance not only throws light into the water relations of the plant but also the CO_2 flux into the plant which is related to dry matter production. Water relations in terms of stomatal conductance exhibited a steady decline due to soil added Cr and to a lesser extent due to raw untreated tannery effluent. The reduced stomatal conductance was due to impaired water relations caused by soil added Cr and tannery effluent amended Cr. Impaired spatial distribution and reduced root surface of Cr stressed plants lowered the capacity of these plants to explore the soil surface for water. This could be the reason for the decrease in the stomatal conductance of the plants (Ferris *et al*., 2002).

The significantly higher toxic effect of Cr(VI) in declining the stomatal conductance could be due to the high oxidative potential of Cr(VI) which in turn may have been instrumental in damaging the cells and membrane of stomatal guard cells. VAM was effective in partially alleviating the decline in stomatal conductance caused by soil added Cr and effluent irrigation. Davies *et al*. (2002) have given similar reports with soil added Cr in sunflower plants.

The effect of VAM in partially alleviating the decline in stomatal conductance could be attributed to the positive effect on root growth of the plants. Increase in water uptake more than the uninoculated Cr treated plants could be due to better root system brought about by VAM (Entry *et al*., 2002).

5.3.2 Photosynthesis

Photosynthesis is one of the most important physiological processes affected in stressful condition. The deleterious effect of Cr stress on photosynthesis directly affected the partitioning of dry matter to all the parts of the plant, which in turn affected leaf area expansion, and total dry matter accumulation at all stages of crop growth. The reduction in photosynthetic rate as the age of the crops advanced was mainly due to lesser leaf area and senescence. There was a marked reduction in photosynthetic rate of Cr stressed plants at all stages of crop growth. This result is in accordance with Vajpayee *et al*. (1999).

The inhibition of photosynthesis by Cr in soil added form and tannery effluent amended form could be due to disorganization of the chloroplast ultrastucture and inhibition of electron transport processes as it has been shown by Bishnoi *et al*. (1993a) that there can be a diversion of electrons from the electron donating side of PS I to Cr(VI).

It is also possible that Cr present in the leaf tissue can affect the activities of enzymes of the Calvin cycle thus causing a decline in photosynthesis. It was seen in this study, that phosphorus content was reduced significantly by Cr stress. It is known that P and Cr are competitors for surface sites (James, 1996). The reduction in P can have regulatory effects of inorganic phosphate (Pi) on photosynthetic metabolism in chloroplasts. It is possible that low Pi acts by inhibiting RuBP regeneration. Thus reducing the photosynthetic carbon fixation. Mn is an imperative metal for water splitting process, interaction between Mn and Cr is known (James and Bartlett, 1983). This could possibly result in the interference with the process of water splitting in the water – water cycle resulting in reduced CO_2 fixation (Asada, 2000).

This explanation also could be the reason for VAM induced increase in photosynthesis, as increased mobilization of P by VAM in the roots and subsequent transport to the leaves of the plants was seen in the present study. Similar results have been reported by Rice *et al*. (2002). VAM association significantly altered the water status of host plants as a result the CO_2 fixation of the VAM inoculated stressed plants increased which was evident by the increased photosynthetic rates in these plants.

5.3.3 Chlorophyll fluorescence

Each quantum of light absorbed by a chlorophyll molecule raises an electron from the ground state to an excited state. Upon de-excitation from a chlorophyll *a* molecule from excited state 1 to ground state, a small proportion (3-5% *in vivo*) of the excitation energy is dissipated as red fluorescence. The indicator function of chlorophyll fluorescence arises from the fact that fluorescence emission is complementary to alternative pathways of de-excitation, which are primarily photochemistry and heat dissipation. Generally, fluorescence yield is highest when photochemistry and heat dissipation are lowest (Krause and Weis, 1991).

Therefore, changes in the fluorescence yield reflect changes in photochemical efficiency and heat dissipation. Upon the application of a saturating flash, fluorescence raises from the ground state value (Fo) to its maximum value (Fm). In this condition, Q_A, the first electron acceptor of PSII, is fully reduced.

This allows the determination of the maximum quantum efficiency of photosystem II (PSII) primary photochemistry, given by Fv/Fm. In healthy leaves, this value is always close to 0.8, independently of the plant species studied (Krupa *et al*., 1992). Typically, lower values were observed in Cr stressed plants irrespective of the method of application in both the crops at all the stages observed. The results observed in all the fluorescence parameters are in accordance with Dan *et al*. (2000).

This reduction in the ratio could be due to the fact that, a proportion of PSII reaction centre was damaged, a phenomenon called photo inhibition, often observed in plants under heavy metal stress conditions. Since the parameters maximal fluorescence (Fm), variable fluorescence (Fv) and Fv/Fm ratio characterize the functional state of PSII in dark adapted leaves (Briantis *et al*., 1986). The Fv/Fm ratio is considered to be a measure of PSII effectiveness in the primary photochemical reactions (Buttler, 1977). The observed reduction in the Fv/Fm ratio clearly indicated that Cr(VI) and to a lesser extant Cr(III) caused ineffectiveness of the PSII thereby by causing a

reduction in the primary photochemical reactions. This also explains the reduced photosynthetic rate observed in the stressed leaves.

The effect of Cr stress was evident because the electrons produced by the photochemical process were not necessarily used for carbon fixation as evidenced by low photosynthetic rate of the Cr stressed plants. Due to the known oxidative potential of Cr (VI) it is possible that alternative sinks for electrons could have been enhanced by reduction of molecular oxygen (part of Mehler reaction) which in part explains the oxidative stress brought about by Cr(VI). The results obtained in the present study are in accordance with Peixoto *et al*. (2002).

The obtained data on the decrease of PSII effectiveness of Cr-treated plants are in confirmation with Vassilev *et al*. (1995). This could be partly explained by Cr induced disorders in chloroplast ultra structure (Vassilev *et al*., 1997). In the swollen and highly disorganized thylakoid, the architecture of photosynthetic units and membrane bound electron transport processes could be disturbed due to the presence of Cr *per se* or due to singlet O* evolution by Cr(VI).

There are evidences that leaf structural status may also affect the primary fluorescence parameters when the Fv/Fm ratio shows drastic change (Araus and Hogan 1994). Hence, the ameliorative effect of VAM in the case of Cr stressed plants could be due to its effectiveness in keeping the structural status of the leaf healthy by the supply of adequate nutrients to the growing leaf because of its nutrient mobilizing ability in the rhizosphere. The ratio of variable fluorescence to unquenchable portion of fluorescence (Fv/Fo) is an indicator of the size and the number of active reaction centres.
This parameter was also reduced by 17 per cent due to the addition of Cr(VI) speciation in soil added form and effluent form. This ratio depicts the emission of excited antenna chlorophyll *a* before excitation migrates to the reaction centre and it is independent of photochemical events. Cr(VI) induce changes in this ratio indicated that there was a partial damage in the energy transfer from antenna pigments to reaction centres. The results obtained are in conformation with Dan *et al*. (2000).

Desmet *et al*. (1975) had reported that Cr(VI) could function as a Hill reagent. Thus, it is possible that Cr(VI) could inhibit the PSI by diversion of electrons from the electron donating side of PSI to Cr(VI) instead of sending the electrons to the auto-oxidizable methyl viologen. Similar results of photosystem inhibition by Cr were reported by several workers (Jana *et al*., 1987; Bishnoi *et al*., 1993a). The overall effect of Cr ions on photosynthesis and excitation energy transfer could also be due to Cr(VI) induced abnormalities in the chloroplast ultra structure like a poorly developed lamellar system with widely spaced thylakoid and fewer grana.

5.4 Ultra structure

Scanning electron micrographs revealed that the plants treated with high concentration of Cr(VI) (500 μM) for 4 days has distinct changes in the micro anatomical level. The observed closed vascular bundles as an effect of Cr(VI) could have been due to disturbances in the translocation of water and solutes and direct physical interference of the transport process by Cr ions. The results obtained are in agreement with the reports of (Susheela *et al*., 2002;

Setia and Bala, 1994). Insufficient supply of nutrients and other metabolites could also have been the reason for changes in the vascular bundles observed in Cr(VI) treated plants.

5.5 Chromium uptake, transport and accumulation. (Fig.5.1)

The content and uptake of Cr in the plant tissue was directly related to the concentration in the medium in both the solution culture and soil culture experiments. Cr(III) was more readily taken up by the plants in solution culture as compared to the soil added Cr speciation. The observed difference in the uptake of Cr(III) and Cr(VI) in soil and solution culture experiments was mainly due to the solubility and ready availability of Cr (III) in solution as against the complexation and possible chelation and immobilization in the case of soil culture experiment.

However, it was noted that the content of Cr in Cr(III) treated plants was less at higher concentration compared to the content of Cr in Cr(VI) treated plants at lower concentration. This is explained by the fact that Cr(III) is known to penetrate plant cells only very slowly by diffusion and the low toxicity of Cr(III) is attributed to this specific reason. This finding is supported by many studies, which have also demonstrated that Cr(VI) is better absorbed by the plants than Cr(III) in the case of solution culture experiments (Barcelo *et al*.,1993; Zayed *et al*.,1998) and in soil culture experiments with added tannery amendments (Grubinger, 1994; Mishra, 1995).

The possibility of modification of the Cr form after it is supplied in either Cr(III) or Cr(VI) cannot be ruled out in all the experiments. This can occur because of action of root exudates with the capacity to reduced or oxidise either form of Cr in solution culture and in the form of organic complexes or soil micro flora or fauna with the capacity to participate in the redox reactions of Cr in soil. Hence when attempting to answer the central question it has to taken into account that bulk soil Cr may differ from rhizosphere Cr and plant tissue Cr because root activity and micro organism could influence the oxidation of state of Cr and the formation of Cr(III) organic complexes (Bartlett, 1988). Cr(VI), which is potentially toxic, is taken up by the plants by active mechanisms involving molecular transporters and carriers, thus the reason for its higher uptake could be that it is taken up with the essential elements with similar molecular structure like Fe and S.

Pot culture experiments indicated that sunflower absorbed more Cr in both forms than sorghum in both the above and below ground plant parts irrespective of the mode of supply of Cr. This finding in is confirmation with the finding of Cary *et al*. (1977) who found that in general dicots absorbed more Cr and transported more Cr to the shoots than did monocots. The reasons for this could be that these two groups of crops exhibit contrasting rooting pattern, different transpiration rates and a high degree of complex differences in the metabolism.

The high degree of tolerance by sorghum (Table 5.1) can be attributed to the hardy nature of the plant and due to the compartmentation of Cr in the roots largely rendering it non toxic to the above ground parts. In the case of sunflower, the tolerance was mainly due to the action of various defence mechanisms in averting excessive oxidative stress. Both this mechanism was not actively observed in green gram and rice. The results obtained are in confirmation with the report of Mei *et al*. (2002).

There was a nearly ten times higher concentration of Cr applied as both forms in roots as that present in shoots. This trend was observed in both crops and trees although sorghum tended to hold more Cr in roots than sunflower. Cr is known to predominantly accumulate in roots (Cary *et al*., 1977). The reason of the high accumulation in roots of the plants could be because Cr is immobilised in the vacuoles of the root cells to render it non-toxic, which may be a natural toxicity response of the plant. Since both Cr(VI) and Cr(III) must cross the endodermis via symplast, the Cr(VI) in cells is probably readily reduced to Cr(III) and the Cr(III) is retained in the root cortex cells under low concentration of Cr(VI) which in part explains the lower toxicity at this concentration. The presence of Cr(III) forms inside the plant tissue was reported by Zayed *et al*.(1998) in study with X ray atomic absorption studies.

According to the chemosmotic hypothesis (Mitchel, 1985), it is envisioned that selective solute accumulation can proceed *via* secondary transport process where the "uphill" movement of solutes is linked to the pH gradient and membrane potential components of the proton electrochemical gradient established by the H^+ ATPase.

This mode of operation could be one of the basis for the selective accumulation of solute by plants and this is central to the important process of Cr accumulation by plant roots.

The low concentration of Cr in the stems as compared to roots suggests that Cr is poorly translocated in phloem of the plant tissue (Barcelo *et al*., 1993). Least increase in Cr accumulation by ameliorants was seen in $FeSO_4$ treatment over Cr(VI) in sunflower. This could be because Fe and S sufficient plants accumulate less Cr as reported by several workers (Cary *et al*., 1977; Bartlett, 1988; Bonnet *et al*., 1991). Another important reason for the lack of transport of Cr from roots to shoots could be because the plants lack any specific mechanism of transport of Cr, as it is a toxic and nonessential element in itself. A slight decrease in the Cr content of shoots of VAM inoculated plants was seen in the present study as compared to uninoculated plants. These results are in confirmation with the results of Weissenhorn *et al*. (1995). The reason for this could be that more of Cr was bound to the roots and the fungal hyphae thus reducing upward transport of the metal to arial parts.

The accumulation factor and magnification factor for Cr(VI) values derived in the present study in tree species revealed the differing capacity of the four tree species studied. The results are in confirmation with the reports of Khan (2001). This could be because the uptake pattern of Cr from soil depends on the tree species and that within the tree species the concentration largely differs between different parts of the trees (Pulford *et al*., 2001). In general it is possible that the restriction of Cr to roots is more in non-accumulator species.

There was a pronounced enhancement of Cr uptake in tree species treated with low molecular weight organic acids. This result is similar to the report of Shahandeh and Hossner (2000) and Srivastava *et al.* (1999). The reason for this could be that the organic acids modified soil properties, increased the labile fraction of Cr and mobilised Cr for easy uptake by roots (Wu *et al*., 2003). This technology can be effectively used for phytoremediation of Cr contaminated sites with special reference to tannery industrial areas.

5.6 Mineral nutrition

The nutrition of all the major elements (N,P and K) was decreased by the addition of Cr in the soil added as salt or by the irrigation of tannery effluent. This result is in confirmation with Moral *et al*. (1995) and Davies *et al*. (2002). The reduction in nitrogen, potassium, phosphorus and other elements could be due to the reduced root growth and impaired penetration of the roots into the soil due to Cr toxicity, which in turn caused a decrease uptake of major nutrients. Phosphate (H_2PO_4) inhibits Cr(VI) adsorption in equilibrated soil suspensions (James and Bartlett, 1983). Thus, P and Cr are competitive species for surface sites, which could be the reason for the lesser levels of P in Cr(VI) treated plants. One of the reasons for the decreased uptake of nutrients in Cr stressed plants was because of the inhibition of the activity of plasma membrane H^+ ATPase, which was observed in the solution culture experiment in the present study.

Similarly Fe and S are known also to compete with Cr for transport carrier binding (Wallace *et al*., 1976). Dual uptake mechanism has been reported for S, P and K (Shewry and Peterson 1974). The reduction in the uptake of the elements S and Fe could be mainly due to the chemical similarity of these ions in solution. It is also known that the mechanism of Cr(VI) transport is an active mechanism involving anion carriers, which may be specific to other essential anions. Hence, the competitive binding to these sites by Cr(VI) could have reduced the uptake of S and Fe (Kraemer *et al*., 2002). The uptake of Fe could have been reduced by direct interference with root Fe(III) reductase enzyme present in the root transfer cells thus inhibiting reduction of Fe(III) to Fe(II) which is the form that plants absorb as observed in the present study.

VAM was instrumental in increasing all the mineral elements in the Cr stressed plants. Similar results were reported by Liao *et al*. (2003). Thus the use of VAM to colonize a Cr-stressed host plants had obvious advantages. Compared to uninoculated plants VAM plants were able to increase uptake of less soluble metals, help alleviate nutritional stress, including P stress, and enhance nutrient use efficiency. The increased amount of Fe and S in $FeSO_4$ treated plants were mainly due to the foliar application of these elements. The results obtained are consistent with that of Davies *et al*. (2001) and Weissenhorn *et al*. (1995).

5.7 Biochemical traits

5.7.1 Pigments

Chlorophyll *a, b* and the ratio of the two chlorophylls reduced in response to soil added Cr and due to Cr present in the tannery effluent. The above observation in the present study is in accordance with (Chaterjee and Chaterjee, 2000; Samantaray *et al*., 2001). The chlorophyll reductions due to the application of Cr(VI) was more pronounced than due to Cr(III) or tannery effluent irrigation which is in accordance with the finding of Bera *et al*. (1999). The reasons for this could be the damaging effect of Cr on the enzymes involved in chlorophyll biosynthesis.

The antenna complex is comprised of the central part and the peripheral part in the photosynthetic apparatus. The components of the central part and the peripheral part change in response to Cr stressful environments (AnZhi *et al*., 2000). As opposed to chlorophyll *a*, which is found in every part of photosystems, chlorophyll *b* is only found in the peripheral part of the antenna complex in a proportional amount to the size of the peripheral part.

The size of the peripheral part is given by chlorophyll *a* to *b* ratio. The decrease in the ratio brought about by Cr indicated that Cr toxicity reduced size of the peripheral part. This was clearly seen in the results obtained in the fluorescence kinetics of the Cr affected leaves. The decrease in the Chlorophyll *b* due to Cr could have brought about the destabilization and degradation of the proteins of the peripheral part, which was evident by the reduction of photosynthetic rate in the Cr stressed plants.

There was an increase in the anthocyanin content in response to stress caused by Cr in the solution culture experiment. Anthocyanin is an important pigment in the plant system and has specific role in the photosynthetic processes. In the present study root, anthocyanin content was quantified, as it is known to enhance tolerance to heavy metals by posing a barrier to the passage of metals into the roots. It was found that Cr treatments increased the anthocyanin content in roots and it was more in sorghum and sunflower varieties, which can be taken as an index of tolerance.

The increase observed in the content of anthocyanin could be due to activation of the enzymes involved in the anthocyanin biosynthetic pathway by Cr ions. A number of regulatory genes mediate transcriptional activation of the anthocyanin biosynthesis pathway genes (Selinger and Chandler, 1999). To activate the genes of the anthocyanin pathway, a protein from each class must be expressed. The Bronze2 (Bz2) gene in maize encodes a glutathione S-transferases that performs the last genetically defined step in anthocyanin biosynthesis namely the tagging of cyanidin-3-glucoside with glutathione, allowing for transport to the vacuole *via* a tonoplast Mg-ATP-requiring GS-X pump (Holton and Cornish, 1995).

Heavy metal ions are known to increase the activity of glutathione S-transferase (Marrs and Walbot, 1997). Hence, it is possible that this could be the reason for increase in anthocyanin synthesis in plants grown with added Cr. However the increase in anthocyanin content decreased under higher concentrations of Cr(VI) in the present study. The reason for this could be that the toxic oxidative effect of Cr(VI) impaired the function of the various enzymes in the anthocyanin pathway.

5.7.2 Lipid peroxidation and plasma membrane H^+ ATPase

The peroxidation of lipids is an oxidative degradation of polyunsaturated fatty acids and is a basic membrane damage process. In the present study, Cr ions in solution of both speciations damaged the membranes and the damage was concentration dependant. The increase in lipid peroxidation in chromium treated roots might be due the production of free radicals by chromium mediated reaction. As a direct effect free radicals contributed to the destabilization of the membrane. The effect of Cr(VI) was more pronounced because of the higher oxidation potential of the species. The results are in accordance with Toppi *et al*. (2002) who observed small osmiophilic droplets in close vicinity to the membranes as result of membrane disruption, in Cr stressed plants.

The comparatively lesser membrane damage in the roots of sorghum could be taken as an index to identify tolerance to Cr. The damage of the membrane resulted in fluidity, elasticity of the membrane, which may have been the reason for various other toxic effects, observed in the stressed plants. The plasma membrane H^+ ATPase

plays a significant role in all life functions of the plant cells. It catalyses the reaction ATP + H_2O + H^+(in) = ADP + phosphate + H^+(out). It mediates the ATP dependant H^+ extrusion to the cell exterior and sets up the driving force for solute transport in terms of an inwardly directed proton electrochemical gradient at the plasma membrane (Briskin and Hanson, 1992). In the present study, the root cell plasma membrane H^+ATPase activity was reduced in the Cr treated seedlings of all the crops.

The inhibition of ATPase activity could be due to the fluidity and disruption of the membrane because of free radical formation. The results are in corroboration with Zaccheo *et al*. (1985). The decrease in the activity of ATPase could also be due to generation of free radicals by Cr(VI), since it is known that free radicals regulate the ATPase activity (Demidchik *et al*., 2003).

The decrease in ATPase activity caused a decrease in H^+ proton extrusion. This in turn could have caused a decrease in the transport activities of the root plasma membrane thus reducing the uptake of most nutrient elements (Kraemer *et al*., 2002). It is also possible that Cr interfered with the mechanism controlling the intracellular pH, this possibility is supported by the fact that Cr could be reduced in the cells thereby utilizing the protons (Zaccheo *et al*., 1985).

5.7.3 Root Fe(III) reductase

Fe uptake by dicots and non-graminaceous plants involves the active participation of the plasma membrane bound Fe(III) reductase since there is no production of phytosiderophores as in the case of graminaceous plants (Hell and Stephan, 2003). The enzyme catalyses the reaction 2 Fe(II) + NAD^+ = 2 Fe(III) + NADH + H^+ In the present study the activity of root Fe(III) reductase was measured in the roots of dicot plants (green gram and sunflower). The results showed that root Fe(III) reductase activity reduced as the concentration of Cr in the nutrient medium increased. The rate of decrease in the activity reduced at higher concentrations.

These results are in agreement with the results obtained by Barton *et al*. (2000) who observed drastic reduction in the activity of root Fe(III) reductase due to Cr, and other heavy metals in the nutrient medium. The reasons for the reduction in the enzyme activity could be due to blocking of the differentiation of the root transfer cells where the root Fe(III) reductase is located, thus preventing the enzyme action.

Cr could also directly inhibit the function of root Fe(III) reductase by competing for active sites due to the similar valence dynamics shown by these two elements. In addition to the above reasons, it was seen that Cr caused lipid peroxidation and membrane damage thus disturbing the sub cellular localisation of the enzyme and preventing its action. The possibility of Cr interference with the induction, function and localisation of root Fe(III) reductase was seen from the present study. The results of this study indicated that root plasma membrane ATPase activity was reduced causing a corresponding reduction in proton extrusion, this could have increased in the pH in the rhizosphere, thereby decreasing the solubility of Fe(III) and thus restricting the Fe uptake, as evidenced in the pot culture study. The results observed are in accordance with the report of Han *et al*. (2002).

5.7.4 Ethylene production

In the present study, it was seen that Cr stress in both the soil applied and tannery effluent applied form increased ethylene production. The results are in confirmation with the findings of (Poschenrieder *et al*., 1993) who reported that Cr ions supplied to plants either with the nutrient solution or to discs with the incubation medium increased the conversion of exogenous ACC to ethylene.

The induction of the enzymes of ethylene synthesis namely ACC synthase could be the reason for increased production of ethylene. The loss of membrane integrity due to free radical damage and lipid peroxidation under Cr stress could have triggered the activity of ethylene forming enzymes thus increasing ethylene production. The induction of ethylene production under Cr stress could possibly be a stress signal transduction mechanism from the roots to shoots to counter the stress by various strategies (Vess *et al*., 2002; Vangronsveld *et al*., 1993).

5.7.5 Nitrate reductase

The enzyme catalyses the reaction nitrite + NAD^+ + H_2O = nitrate + NADH + H^+. The activity of the enzyme was reduced at all the stages due to the addition of Cr in the soil medium as a salt and due to the presence of Cr in the tannery effluent. The results obtained are in corroboration with the reports of (Panda and Patra, 2000; Vajpayee *et al*., 2000). It is possible that, firstly due to the reduced substrate availability the enzyme activity was reduced as it was seen that N content in the leaves were reduced.

Secondly, the reduction in activity may be due to the interference of Cr present in the tissue with the catalytic activity of the enzyme. The affinity of metals to sulphydryll groups involved in the catalytic action or structural integrity of enzymes is said to be one of the main mechanisms of enzyme inhibition (Karataglis *et al*., 1991).

The increase in activity seen in the VAM inoculated plants may be due to the fact that in general N content was increased in the VAM inoculated plants. Similarly, the increase in the $FeSO_4$ treated plants over the corresponding stressed plants may be due the fact that S increased the production of thiols particularly PC-SH, as evidenced by the results of the present study. Kneer and Zenk (1992) have reported that phytochelatins protect nitrate reductase from heavy metal poisoning. They have reported that free phytochelatin peptides reactivated metal-poisoned nitrate reductase *in vitro* up to 1000-fold in heavy metal treated plants.

5.7.6 Anti-oxidant enzymes, antioxidants and phytochelatins (Fig.5.2)

Almost all of the toxic effects of Cr species can be traced to its ability to cause oxidative damage. In contrast to O_2, these partially reduced or activated derivatives of oxygen (O_2^1, O_2^-, H_2O_2, and $HO^·$) are highly reactive and toxic, and can lead to the oxidative destruction of cells Hall, 2002). The enzymes responsible for scavenging of these radicals conferred tolerance from the destructive action of Cr to the plants. The increase in the activity of these enzymes in general can be taken as an index for

tolerance. The pattern of induction of these enzymes by Cr stress was unique and differed with different enzymes depending on the substrate of the enzyme.

5.7.6.1 Superoxide dismutase (SOD)

SOD in plants exists as a family of enzymes localized at different cellular location with a variety of wide range of metal co-factors *viz.*, Cu, Mn, Zn etc. The enzyme catalysis the reaction $2\ O_2{}^{*-} + 2\ H^+ = O_2 + H_2O_2$. The induction of intracellular free radicals by Cr(III) and Cr(VI) was effectively countered by this enzyme as evidenced by the higher activity seen

in the solution culture experiment and latter confirmed in the soil culture studies. The results obtained were in confirmation with Dixit *et al*. (2002).

The increase in the SOD activity observed might have been in direct response to the generation of O_2^- by Cr induced blockage of the electron transport chain in the mitochondria. The higher increase noticed due Cr(VI) indicated that Cr(VI) addition generates more singlet oxygen than Cr(III). The decrease in the activity of the enzyme as the concentration of the external Cr increased might be because of the inhibitory effect of Cr ions on the SOD itself (Dixit *et al*., 2002).

5.7.6.2 Catalase

Catalase is the next logical step in the quenching of singlet oxygen derived dangers as it catalysis the reaction $2\ H_2O_2 = O_2 + 2\ H_2O$. The increase in catalase activity was seen in the present study, which is in corroboration with the report of Samantaray *et al*. (2001). The increase in the activity of the enzyme was less as compared to the increase in SOD. Firstly, the increase in the activity may have been due to the production of H_2O_2 by SOD, which is the substrate of catalase. Secondly, the lesser increase in comparison with SOD may be because catalase had extremely high maximum catalytic rates but low substrate affinities, since the reaction requires the simultaneous access of two H_2O_2 molecules to the active site (Willekens *et al*., 1995).

5.7.6.3 Ascorbate glutathione pathway enzymes

The ascorbate glutathione pathway involves the following enzymes: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) dehydroascorbate reductase (DHAR) and glutathione reductase (GR).

The enzyme APX similar to catalase acts on H_2O_2 catalysing the reaction L-ascorbate + H_2O_2 = monodehydroascorbate + $2\ H_2O$. The enzyme unlike catalase requires ascorbate as a reductant. The activity of this enzyme increased significantly as an effect of Cr stress on crop plants. Similar increase has been observed in studies with other heavy metals (Gupta *et al*., 1999a).

The increase in the activity of this enzyme was higher than that of catalase, this could be because APX are found throughout the cell (Jimenez *et al*., 1997) and which have a much higher affinity for H_2O_2 than CAT. The monodehydroascorbate produced by peroxidase reaction is further catalysed to oxidised glutathione and ascorbate is

replenished by the action of the enzyme (2 glutathione + monodehydroascorbate = oxidized glutathione + ascorbate).

The activity of this enzyme increased in comparison with control due to the presence of Cr. This phenomenon can partly be explained by the fact the presence of substrate for this enzyme namely MDHA in higher quantity and also the requirement by the tissue to produce ascorbate which is the product of the reaction and which in itself is an active antioxidant in conditions of metal stress (Weckx and Clijsters, 1996).

The activity of DHAR was not significantly affected by the presence of Cr stress although there was an increase in the activity. This result is in confirmation with the findings of Gupta *et al*., (1999) who obtained similarly result under Cu stressed plants. The reason for this could be the fast use of MDHA as a substrate by MDHAR and subsequent reduced formation of the substrate DHA for the activity of this enzyme.

It is known that MDHA is a radical with a short lifetime and that, if not rapidly reduced, disproportionates to ascorbate and dehydroascorbate (Noctor and Foyer, 1998). In this study it was seen that MDHA was rapidly reduced by MDHAR, thus leaving not enough substrate for the action of DHA, which was evidenced by low activity of the enzyme DHAR.

The final step in the removal of Cr induced singlet oxygen and subsequent rendering of H_2O_2 non toxic is performed by glutathione reductase (GR) which is an important enzyme in the glutathione pathway as it is involved in the inter conversion of reduced and oxidised glutathione. The reaction catalysed by the enzyme is 2 glutathione + $NADP^+$ = glutathione disulfide + NADPH + H^+. The activity of this enzyme was increased as a result of Cr stress.

The reason for this is that the whole glutathione pathway was under operation as a stress response and countering mechanism. Glutathione was used in the plants to synthesis phytochelatins as observed in the present study. Consequently, this would have caused a decrease in the glutathione pool (De Vos *et al*., 1992). Hence, the increase in the activity of GR would have been necessary to keep the cellular glutathione levels high since glutathione is an effective antioxidant in itself and can efficiently counter the oxidative stress caused by Cr ions in the plant tissue.

5.7.6.4 Ascorbate glutathione pathway metabolites

The flux in the metabolite content was seen as a response to the stress induced by the presence of Cr ions in the plant root tissue. Ascorbate content was high in the stressed plants. This result is in confirmation with the results obtained by Gupta *et al*. (1999). The increase in ascorbate could be due to the enzymatic generation of ascorbate by the action of both the enzymes DHAR and MDHAR in tandem. It was clear, from the present study, that ascorbate acted, as a major primary antioxidant reacting directly with hydroxyl radical in superoxide, and singlet oxygen by being a substrate for APX.

Total glutathione, reduced glutathione and oxidised glutathione content of the stressed plants were increased in the present study, which is corroboration with the results obtained by Gupta *et al*. (1999). Although, the

increase in GSSG was not high in comparison with reduced glutathione. The enhanced levels of the components of glutathione could have been mainly due to the increased activity of GR as well as the direct increased biosynthesis of glutathione in the stressed cells.

The increase in singlet oxygen due to the presence of Cr could have contributed to the higher observed levels of glutathione. Studies have shown that glutathione accumulates in response to increased reactive oxygen generation (May and Leaver, 1993; Shi *et al*., 1994). The reduced content of GSSG in comparison with the GSH could have been solely due to the enhanced activity of the enzyme GR and reduced activity of DHAR which together are responsible for the inter conversion of reduced and oxidised glutathione.

The deduced content of PC-SH from thiols was very high in sunflower plants in comparison to sorghum; the reason for this could be that the content of Cr in the sorghum leaves was low in comparison to sunflower. This observed phenomenon can be made use of to hypothesize that sorghum is tolerant by excluding foliar transport of Cr in contrast to a glutathione metabolism mediated tolerance or detoxification mechanism in sunflower. The increase in the levels of PC-SH has also been reported by De Vos *et al*. (1992) although the authors reported that glutathione pool depleted in tandem with the increase in PC-SH synthesis. Hence the tolerance observed in sorghum can be attributed to exclusion as against accumulation observed in sunflower.

In the present study, the glutathione levels increased which as discussed earlier can be attributed to increased biosynthesis of glutathione and that the increased total glutathione was in itself an effective stress protectant. The cellular mechanism of protection from the harmful effects of Cr by PC-SH was evident in the present study, which has also been reported by Toppi *et al*. (2002) and Rea *et al*. (2002). Although PCs clearly can have an important role in metal detoxification, alternative primary roles of PCs in plant physiology have also been proposed. These have included roles in essential metal ion homeostasis and in Fe or sulphur metabolism (Toppi and Gabbrielli 1999; Toppi *et al*., 2002)

Sulphur nutrition to the plants by means of foliar sprays was effective in enhancing the overall glutathione metabolism including the enzyme activity and metabolites. Lopez *et al*. (2002) and Rennengerg *et al*. (1988) have reported similar results. The reason for this could be that increase in sulphur content had a direct influence on the sulphur containing amino acids and which contributed to the biosynthesis and metabolic regulation of all the constituents of the glutathione pathway.

Thus in the present study the contribution of foliar sprays of $FeSO_4$ to the antioxidant stress response was by way of sulphur mediated increase in the glutathione ascorbic acid pathway. Free amino acids were also seen to increase in response to the stress caused by Cr present in the soil in salt form and tannery effluent form. This is in confirmation with the report by Kramer *et al*. (1996) that free amino acids increase multi fold in response to heavy metal stress. The reason for this could be a natural toxicity countering mechanism by plants, as free amino acids could play a role in detoxification and tolerance by acting as potential ligands for Cr (Toppi *et al*., 2002).

5.8 Molecular aspects

5.8.1 Glutathione reductase isozymes

In the present study, different isoforms of GR was observed in response to stress caused by Cr in solution culture and this was confirmed in soil culture experiments. Foyer *et al*. (1991) have reported the induction of several isoforms of GR under stressful conditions. The increase in the number of isoforms could in part explain the increased activity of GR observed in the present study.

The induction of these isoforms could be due to increase in the synthesis of mRNA coding for the enzyme. The increased translation of the enzyme specific mRNA could have been induced by special transcription factors synthesised in response to Cr stress as seen by the production of stress proteins in the present study. The induction of multiple forms of these enzymes could be to counter the stress metabolically by increased activity of the enzyme as this enzyme plays a key role in the quenching of reactive oxygen species. The increase in the isoforms could also have been due to differential expression of distinct GR genes or a post translational modification of one or more of the GR subunits (Anderson *et al*., 1990).

5.8.2 Stress proteins

Stress proteins were detected in response to Cr stress in solution culture experiments and were confirmed in the soil culture experiments. The proteins formed were from 19 – 43 kDa range in general and was induced by high Cr concentrations. Samantaray *et al*. (2001) reported similar results. These proteins were gene products and may have been induced as a response to counter the toxicity caused by Cr at molecular level. This could be a signal transduction mechanism by which transcription may be modulated in response to Cr toxicity (Hall, 2002).

These proteins may act as molecular chaperones in normal protein folding and assembly and may function in the protection and repair of proteins and enzyme under stress. These proteins could also contribute to a more resistant membrane or actually help in the repair of the damaged plasma membrane.

The possibility that these stress proteins may act as transcription enhancing factors for the synthesis of specific stress related enzymes also cannot be ruled out. Hence, the presence of these proteins can be taken as an index of tolerance of the particular crop or species (Hall, 2002). In this respect, it was seen that sunflower and sorghum were in part tolerant to Cr stress by the production of these stress proteins.

5.8.3 Metallothionein (MTs) gene expression

Metallothioneins are one of the two types of cysteine rich metal binding peptides present in higher plants. These are gene encoded polypeptides unlike phytochelatins, which are metabolically synthesised from glutathione. MTs are of various types and have been classified as MT1, MT2, MT3 and MT4 based on the number and alignment of cysteine residues (Cobett and Goldsbrough, 2002). In the present study, it was found by RT-PCR analysis that mRNA encoding for MT3 like proteins were present in the sorghum var. CO-27 and its expression was seen under Cr stress.

Similar results have been shown by (Matsumura *et al*., 1999) wherein the authors reported transcripts of two MT3 genes accounted for an additional 1.25 per cent of the mRNAs in rice. In plants, there is a lack of information concerning the metals likely to bind to these polypeptides. There is a possibility that MTs could confer tolerance to plants against metal stress by binding heavy metal ions and rendering them non-toxic. A clear role for MTs is yet to be established although they certainly are thought to play a role in metal metabolism. MTs may have a function as antioxidant and a role in plasma membrane repair is another possibility (Salt *et al*., 1998).

5.9 Yield and yield components

Seed yield per head and per plant, filled seeds per head and seed filling percentage were reduced in sunflower under Cr(III) and Cr(VI) stresses and 10 per cent tannery effluent irrigation showed higher yields in comparison with soil added Cr. The reduction in yield in soil added Cr treatments was a direct effect of reduced photosynthesis, impaired water relations and poor nutritional status of the plants. The decrease in photosynthesis directly contributed to low partitioning of assimilate to the economic part of the plants resulting in poor seed yield.

The yield reduction was mainly due to less number of seeds per head and low filling percentage because it was seen that the 100 seed weight was not affected by Cr stress. The results are in confirmation with the findings of Sharma and Sharma (1993) and Sharma and Mehrotra (1993). The green fodder yield reduction in CO 27 in both pot culture and field experiments was due to reduced leaf area which was a direct consequence of soil added Cr. The slightly higher yields in sunflower and sorghum irrigated with tannery effluent was because of the lesser amount of Cr in the tissue and also due to the presence of minimal amount of nutrient mainly N in the effluent as against the pure toxic effect of soil added Cr.

The VAM inoculated plants showed higher yield in Cr(III) treated plants and 10 per cent effluent treated plants. The higher yields brought about by VAM inoculation under stress conditions was further confirmed in field experiment in sorghum var. CO 27. The higher yield due to VAM inoculation could be because of the ability of VAM to mobilise nutrients and counter act Cr induced stress by improving the water relations and by having a positive effect on the photosynthesis of the stressed plants. VAM had a positive effect on the overall tolerance of Cr stressed plants. Davies *et al*. (2002) and Karagiannidis *et al.* (1998) have reported similar results. VAM is an effective heavy metal stress protectant and stimulator of defence system in plants (Schutzendubel and Polle, 2002). VAM induced increase in antioxidant enzymes could also have contributed to the yield increase observed in the present study.

5.10 Cr content and its effects on whole plant physiology (Table 5.2)

The correlations coefficients worked out on the basis of Cr content in tissue {Soil/ medium source of Cr(III) and Cr(VI)} and various physiological and biochemical parameters discussed above showed that apart from the antioxidant enzymes all other plant physiological and biochemical parameter were negatively correlated with Cr. In general, Cr(VI) form added to the soil or nutrient medium exerted more toxic effect evidenced by significantly negative correlation with root growth, yield, photosynthesis and Fe content. The positive

correlation with antioxidant enzymes illustrated the detoxification mechanism employed by the plants to counteract toxicity.

Table 5.2 Pearson's co-relations between Cr content (applied speciation), growth, physiological, and biochemical parameters

Cr speciation	Growth characteristics			
	Plan Height	Root Growth	LA	Yield
Cr(III)	-0.465783	-0.643589*	-0.473245	-0.587345*
Cr(VI)	-0.497832	-0.709342**	-0.508935	-0.698743**
	Leaf Physiology			
	Photosynthesis	Stomatal conductance	Fv/Fm	Fv/Fo
Cr(III)	-0.494573*	-0.392758	-0.487564*	-0.467292*
Cr(VI)	-0.657839**	-0.456783*	-0.685463*	-0.574392**
	Nutrient content			
	N	P	K	Fe
Cr(III)	-0.346574	-0.395674	-0.403823	-0.473920*
Cr(VI)	-0.410329*	-0.421939*	-0.449673	-0.520395**
	Enzymes and Chlorophyll			
	Nitrate reductase	Fe(III) reductase	PM H^+ATPase	Total Chlorophyll
Cr(III)	-0.324955	-0.327594	-0.385738	-0.379584
Cr(VI)	-0.458739	-0.403928*	-0.495839*	-0.395839
	Anti oxidant enzymes			
	SOD	CAT	APX	GR
Cr(III)	0.574953*	0.293935	0.539293*	0.523945*
Cr(VI)	0.603943*	0.294822	0.573949*	0.594945*
	Metabolites			
	Total Glutathione	Ascorbate	Free amino acids	PC-SH
Cr(III)	0.415894	0.435739	0.274853	0.393945
Cr(VI)	0.483829*	0.494832*	0.285938	0.410394

* Significant at 0.05

** Significant at 0.01

Table 5.1 Tolerance index of selected crops to Cr speciation concentration levels in nutrient medium in experiment Ia

Tolerance Index (TI)	T_2	T_3	T_4	T_5	T_6	T_7
Green gram	54.77	48.00	34.46	15.69	15.38	12.31
Rice	68.58	65.91	48.46	27.93	32.85	33.26
Sunflower	72.54	68.57	62.70	41.59	38.10	43.49
Sorghum	71.03	68.97	61.72	40.34	40.00	48.28

CHAPTER VI
SUMMARY AND CONCLUSION

A series of studies were carried out in laboratory, glass house and farmers field to understand and elucidate the mechanism of tolerance and toxicity to Cr in nutrient medium, soil added Cr and Cr from tannery effluent. VAM as a biological ameliorant and $FeSO_4$ spray as a chemical ameliorant was tried in soil added Cr and Cr from tannery effluent treatments. A cereal (rice), a pulse (green gram), an oilseed (sunflower) and a coarse cereal (sorghum) were taken as experimental materials. *Tectona grandis, Leucaena luecocephala, Albizia amara*, and *Casuarina equisetifolia* were evaluated for their phytoaccumulatory potential of soil added and tannery effluents added Cr. In addition, biological amendment in the form of VAM and chemical amendment in the form of citric and oxalic acid was tried to enhance Cr phytoaccumulation.
The results obtained are summarised below:

Both the speciation of Cr reduced germination percentage, vigour index, germination stress index and promptness index. Cr(VI) affected the above parameters more than Cr(III). Combination of both the speciation was most deleterious to the all the above parameters. Root length decreased in a concentration dependant manner and the most deleterious was the combination of speciation. Green gram and rice were more affected by Cr in the medium than sunflower and sorghum. Anthocyanin content increased with the initial increase in concentration of Cr in the medium and was more in sorghum and sunflower and particularly in varieties CO 27 and CO 4 respectively. This parameter can be taken as an index for selecting tolerant plants.

Lipid peroxidation in terms of malondialdehyde formation was seen in the presence of Cr in the medium by all the crops and varieties studied. This indicated that Cr had high oxidising ability and thus caused membrane damage in the cells. Sorghum and sunflower were tolerant in comparison to the other crops. Plasma membrane ATPase activity and Fe (III) reductase activity reduced and as an effect of Cr in the nutrient medium. SOD was induced and its activity increased as the concentration of the Cr the nutrient medium increased but later at higher concentration and mixed speciation there was reduction in the activity of the enzyme. Sunflower var. CO 4 distinctly developed stress protein of 19 kDa. Cr(VI) 50µM exhibited distinct dark bands with the presence of proteins in the range of 43-75 kDa indicating that Cr(VI) induced these bands in green gram var. CO-5. Very distinct 17-20Kda proteins were formed due to the addition of both the species of Cr in the same variety. Root protein profile showed that there were distinct proteins formed due to Cr(III) 200 µM of molecular weight ~28, ~32 and ~40 in sunflower var. CO-4 whereas the other Cr treatments did not exhibit any specific variation in comparison with control. Three distinct isozymes were seen in all the crops due the treatment of Cr(VI) 100µM. MT 3 gene expression was seen in CO 27 sorghum as a response to Cr(VI)100 µM exposure for 10 days in nutrient medium.

There was a progressive concentration dependant increase in Cr content in the seedlings. Highest Cr content was observed in rice var. ADT-37 (342.0µg g^{-1}against the lowest observed in sunflower var. CO-4 (282.8µg

g^{-1}) at the high concentration treatment. On the basis of the above parameters sorghum and sunflower were found to be tolerant and the varieties CO 27 and CO4 receptivity performed better than other varieties.

In the pot studies with soil added Cr, tannery effluent added Cr and ameliorants plant height, root length, leaf area, TDMA was reduced in the Cr(VI) treated plants. Cr(VI) was more toxic in terms of the above parameters than Cr(III). Least photosynthetic rate of 17.599 μ mole CO_2 m^{-2} s^{-1} was observed as consequence of Cr(VI) addition in sunflower at maturity phase. An increase of 1.00 μ mole CO_2 m^{-2} s^{-1} and 0.99μ mole CO_2 m^{-2} s^{-1} in sunflower and sorghum respectively was brought about by VAM inoculation at vegetative stages. The least effective was $FeSO_4$ as an ameliorant. Stomatal conductance decreased steadily as the stage of the crops advanced in both the crops.

Chlorophyll fluorescence decreased in the Cr treated plants and indicated the damage to both the photosystem PSI and PSII. There was decrease in the excitation energy transfer due to presence of Cr ions. The overall efficiency of the photochemical reaction was reduced as evidenced by decline in the fluorescence parameters observed. There was a consequent decline in the chlorophyll *a* chlorophyll *b* and *a*/*b* ratio as response to Cr treatment. Nitrate reductase was also reduced due to Cr addition. VAM effectively alleviated the stress caused by Cr. The antioxidative enzyme SOD increased in response to Cr addition but a similar increase was not seen in the case of catalase activity. The predominant antioxidative quenching of free radical was by the ascorbate- glutathione pathway as evidenced by the differential activity and contents of the enzymes (APX, MDHAR, DHAR and GR) and metabolites of the pathway. Glutathione increased in response to stress. Phytochelatin S-H was deduced to be present in response to Cr addition which may plant a role in the detoxification of Cr inside the tissue. Sunflower synthesised more PC-SH than sorghum. $FeSO_4$ as an ameliorant increased the thiol pathway enzymes and metabolites.

There was increase in free amino acids and ascorbic acid indicating the possible role of these metabolites in detoxification of tissue Cr. Ethylene increase brought about by Cr was also seen suggesting a role for ethylene in signal transduction of root perceived stress to the above ground parts of the plants. Nutrients NPK and micronutrients S, Fe reduced due to the presence of Cr. B content was not influenced by stress. Cr content was 10 times more in roots than in shoots. Sorghum accumulated lesser Cr than sunflower. There was a significant reduction in seed per head due to Cr treatments. Cr(III) treated plants recorded 432 seeds per head and Cr(VI) treated plants recorded 392 seeds per head. There was a significant decrease in yield due to Cr addition.

$FeSO_4$ was effective in increasing the glutathione metabolic pathway enzymes whereas it was not effective in increasing yield significantly in the stressed treatments. VAM was effective as an ameliorant to 10 per cent tannery effluent irrigated plots in the case of fodder sorghum CO 27. *Albizia amara* was found to accumulate more Cr than the other species studied in terms of bio magnification ratio and accumulation factor. Organic acids, oxalic and citric acid were effective in increasing the uptake of Cr from soil added and tannery effluent added form.

The results of the present studies indicates that Cr(VI) is more toxic than Cr(III) to plant growth and development. Ten per cent and 15 per cent tannery effluent were also detrimental to plant growth, development and yield. VAM effectively ameliorated the deleterious effect of soil added Cr(III) and 10 per cent tannery effluent by increased restriction of Cr in the roots. The mechanism of tolerance in sorghum was by restriction of translocation of Cr to the above ground parts as against antioxidant mediated detoxification mechanism seen in sunflower. Sorghum variety CO 27 inoculated with VAM can be effectively cultivated in the tannery effluent affected lands. Organic acids increased the uptake of Cr in tree seedlings and *Albizia amara* was found be an effective accumulator of Cr and it can be used as a potential phtoremediator species in view of the fact that the Cr accumulated by the tree will not get into the food chain since the tree is mainly used as a furniture wood source.

Table 4.13 Effect of different sources of Cr and ameliorants on plant height (cm plant^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	29.45	36.45	135.00	138.50	139.50	156.50
T_2	22.26	28.54	115.00	122.50	123.00	132.50
T_3	17.32	22.23	95.50	103.50	107.00	112.50
T_4	23.45	30.40	124.00	126.00	125.60	132.00
T_5	18.43	24.34	101.00	107.40	111.60	117.40
T_6	23.60	34.25	121.90	147.00	130.38	140.45
T_7	17.67	22.67	97.41	105.57	109.14	114.75
T_8	25.33	32.83	133.92	136.08	135.65	142.56
T_9	19.54	25.80	107.06	113.84	118.30	124.44
T_{10}	22.71	29.11	117.30	124.95	125.46	135.15
T_{11}	17.67	22.67	97.41	105.57	109.14	114.75
T_{12}	23.92	31.01	126.48	128.52	128.11	134.64
T_{13}	18.80	24.83	103.02	109.55	113.83	119.75
CD(0.05)	1.31	2.41	6.86	9.83	7.28	7.91

Table 4.14 Effect of different sources of Cr and ameliorants on root length (cm plant^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	8.24	12.23	15.56	19.76	20.13	27.54
T_2	5.89	9.64	10.12	13.87	12.22	20.13
T_3	4.71	8.12	8.56	10.43	9.20	15.32
T_4	5.86	9.43	10.00	13.13	12.43	21.05
T_5	4.78	8.32	8.34	11.00	9.98	15.00
T_6	6.95	10.99	11.54	15.81	13.93	22.95
T_7	5.13	8.85	9.33	11.37	10.03	16.70
T_8	6.86	11.03	11.70	15.36	14.54	24.63
T_9	5.37	9.35	9.37	12.36	11.22	16.86
T_{10}	6.01	9.83	10.32	14.15	12.46	20.53
T_{11}	4.80	8.28	8.73	10.64	9.38	15.63
T_{12}	5.98	9.62	10.20	13.39	12.68	21.47
T_{13}	4.88	8.49	8.51	11.22	10.18	15.30
CD(0.05)	1.13	1.34	1.43	1.56	1.37	1.67

Table 4.15 Effect of different sources of Cr and ameliorants on leaf area (cm^2 $plant^{-1}$) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	762.00	386.00	1374.00	1276.50	1436.00	1143.00
T_2	686.87	323.50	1121.00	1056.00	1125.00	1002.00
T_3	600.50	289.00	946.00	923.00	956.76	802.00
T_4	707.48	333.21	1154.63	1087.68	1158.75	1032.06
T_5	612.51	294.78	964.92	941.46	975.90	818.04
T_6	721.21	339.68	1177.05	1108.80	1181.25	1052.10
T_7	618.52	297.67	974.38	950.69	985.46	826.06
T_8	728.70	343.20	1189.27	1120.31	1193.51	1063.02
T_9	618.64	297.73	974.57	950.87	985.65	826.22
T_{10}	693.05	326.41	1131.09	1065.50	1135.13	1011.02
T_{11}	605.90	291.60	954.51	931.31	965.37	809.22
T_{12}	713.84	336.20	1165.02	1097.47	1169.18	1041.35
T_{13}	618.02	297.43	973.60	949.93	984.68	825.40
CD(0.05)	20.13	15.98	32.56	31.34	34.71	34.51

Table 4.16 Effect of different sources of Cr and ameliorants on TDMA (g plant^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	6.29	4.64	28.44	32.32	63.46	72.34
T_2	5.67	3.89	23.20	26.74	55.64	56.68
T_3	4.95	3.47	19.58	23.37	44.53	48.20
T_4	5.84	4.00	23.90	27.54	57.30	58.38
T_5	5.05	3.54	19.97	23.84	45.42	49.16
T_6	5.95	4.08	24.36	28.08	58.42	59.51
T_7	5.10	3.58	20.17	24.07	45.87	49.65
T_8	6.01	4.12	24.61	28.37	59.02	60.13
T_9	5.10	3.58	20.17	24.08	45.88	49.66
T_{10}	5.72	3.92	23.41	26.98	56.14	57.19
T_{11}	5.00	3.50	19.75	23.58	44.93	48.63
T_{12}	5.89	4.04	24.11	27.79	57.82	58.90
T_{13}	5.10	3.57	20.15	24.06	45.83	49.61
CD(0.05)	0.17	0.12	0.63	0.81	1.67	1.43

Table 4.17 Effect of different sources of Cr and ameliorants on photosynthetic rate ($\mu M\ CO_2\ m^{-2}\ s^{-1}$) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	22.30	23.65	29.90	32.14	26.40	27.10
T_2	20.10	19.82	24.40	26.59	20.68	23.76
T_3	17.57	17.71	20.59	23.24	17.59	19.02
T_4	20.70	20.42	25.13	27.38	21.30	24.47
T_5	17.93	18.06	21.00	23.70	17.94	19.40
T_6	21.11	20.81	25.62	27.92	21.72	24.95
T_7	18.10	18.24	21.21	23.93	18.12	19.59
T_8	21.33	21.03	25.88	28.21	21.94	25.21
T_9	18.10	18.24	21.21	23.94	18.12	19.59
T_{10}	20.28	20.00	24.62	26.83	20.87	23.97
T_{11}	17.73	17.87	20.77	23.45	17.75	19.19
T_{12}	20.89	20.60	25.35	27.63	21.50	24.69
T_{13}	18.09	18.22	21.19	23.92	18.10	19.57
CD(0.05)	0.51	0.52	0.72	0.81	0.60	0.68

Table 4.18 Effect of different sources of Cr and ameliorants on stomatal conductance (m mol m^{-2} s^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	0.90	1.02	0.82	0.86	0.71	0.73
T_2	0.81	0.86	0.67	0.71	0.56	0.64
T_3	0.71	0.76	0.56	0.62	0.47	0.51
T_4	0.84	0.88	0.69	0.73	0.57	0.66
T_5	0.72	0.78	0.58	0.63	0.48	0.52
T_6	0.85	0.90	0.70	0.75	0.58	0.67
T_7	0.73	0.79	0.58	0.64	0.49	0.53
T_8	0.86	0.91	0.71	0.75	0.59	0.68
T_9	0.73	0.79	0.58	0.64	0.49	0.53
T_{10}	0.82	0.86	0.68	0.72	0.56	0.65
T_{11}	0.72	0.77	0.57	0.63	0.48	0.52
T_{12}	0.84	0.89	0.70	0.74	0.58	0.67
T_{13}	0.73	0.79	0.58	0.64	0.49	0.53
CD(0.05)	0.03	0.03	0.02	0.02	0.02	0.02

Table 4.19 Effect of different sources of Cr and ameliorants on Fv/Fm ratio in sunflower and sorghum

Treatment	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	0.81	0.82	0.82	0.82	0.75	0.78
T_2	0.74	0.70	0.68	0.69	0.60	0.69
T_3	0.65	0.62	0.57	0.60	0.51	0.56
T_4	0.76	0.72	0.70	0.71	0.62	0.71
T_5	0.66	0.64	0.59	0.61	0.52	0.57
T_6	0.78	0.73	0.71	0.72	0.63	0.73
T_7	0.67	0.64	0.59	0.62	0.52	0.57
T_8	0.78	0.74	0.72	0.73	0.63	0.74
T_9	0.67	0.64	0.59	0.62	0.52	0.57
T_{10}	0.75	0.70	0.68	0.69	0.60	0.70
T_{11}	0.65	0.63	0.58	0.61	0.51	0.56
T_{12}	0.77	0.72	0.71	0.71	0.62	0.72
T_{13}	0.67	0.64	0.59	0.62	0.52	0.57
CD(0.05)	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.20 Effect of different sources of Cr and ameliorants on Fv/Fo ratio in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T^1	4.62	4.48	4.60	4.42	4.41	4.38
T_2	4.25	3.88	3.90	3.78	3.66	3.95
T_3	3.83	3.56	3.42	3.40	3.26	3.33
T_4	4.35	3.98	3.99	3.88	3.74	4.04
T_5	3.88	3.61	3.47	3.45	3.30	3.38
T_6	4.42	4.04	4.06	3.94	3.80	4.10
T_7	3.91	3.64	3.50	3.48	3.33	3.41
T_8	4.46	4.07	4.09	3.97	3.83	4.13
T_9	3.91	3.64	3.50	3.48	3.33	3.41
T_{10}	4.28	3.91	3.93	3.81	3.69	3.97
T_{11}	3.85	3.58	3.44	3.43	3.28	3.35
T_{12}	4.38	4.01	4.02	3.90	3.77	4.07
T_{13}	3.91	3.64	3.49	3.48	3.33	3.40
CD(0.05)	0.09	0.09	0.09	0.09	0.08	0.09

Table 4.21 Effect of different sources of Cr and ameliorants on chlorophyll a (mg g^{-1} fresh weight.) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	1.15	1.26	1.34	1.43	1.12	1.06
T_2	1.04	1.06	1.09	1.18	0.88	0.93
T_3	0.91	0.94	0.92	1.03	0.75	0.74
T_4	1.07	1.09	1.13	1.22	0.90	0.96
T_5	0.92	0.96	0.94	1.05	0.76	0.76
T_6	1.09	1.11	1.15	1.24	0.92	0.98
T_7	0.93	0.97	0.95	1.06	0.77	0.77
T_8	1.10	1.12	1.16	1.25	0.93	0.99
T_9	0.93	0.97	0.95	1.07	0.77	0.77
T_{10}	1.05	1.07	1.10	1.19	0.89	0.94
T_{11}	0.91	0.95	0.93	1.04	0.75	0.75
T_{12}	1.08	1.10	1.14	1.23	0.91	0.97
T_{13}	0.93	0.97	0.95	1.06	0.77	0.77
CD(0.05)	0.03	0.03	0.03	0.04	0.03	0.03

Table 4.22 Effect of different sources of Cr and ameliorants on chlorophyll b (mg g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	0.76	0.80	0.95	0.92	0.84	0.71
T_2	0.72	0.74	0.92	0.88	0.80	0.67
T_3	0.67	0.68	0.86	0.83	0.72	0.62
T_4	0.71	0.74	0.93	0.89	0.77	0.66
T_5	0.70	0.70	0.89	0.86	0.75	0.67
T_6	0.74	0.74	0.93	0.91	0.78	0.69
T_7	0.71	0.70	0.87	0.84	0.74	0.66
T_8	0.73	0.75	0.94	0.91	0.80	0.70
T_9	0.72	0.72	0.87	0.86	0.76	0.67
T_{10}	0.73	0.74	0.94	0.89	0.77	0.68
T_{11}	0.72	0.69	0.89	0.86	0.73	0.67
T_{12}	0.74	0.75	0.93	0.87	0.79	0.69
T_{13}	0.74	0.71	0.91	0.88	0.77	0.69
CD(0.05)	0.08	0.10	0.08	0.08	0.10	0.09

Table 4.23 Effect of different sources of Cr and ameliorants on chlorophyll a/b ratio in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	1.51	1.58	1.41	1.55	1.33	1.49
T_2	1.44	1.43	1.19	1.34	1.10	1.39
T_3	1.35	1.39	1.07	1.25	1.04	1.20
T_4	1.50	1.47	1.21	1.37	1.17	1.45
T_5	1.32	1.37	1.06	1.23	1.01	1.13
T_6	1.47	1.50	1.23	1.36	1.18	1.41
T_7	1.31	1.39	1.09	1.27	1.04	1.16
T_8	1.51	1.49	1.23	1.38	1.16	1.41
T_9	1.30	1.35	1.09	1.24	1.01	1.14
T_{10}	1.43	1.44	1.17	1.34	1.15	1.38
T_{11}	1.27	1.38	1.05	1.21	1.03	1.12
T_{12}	1.46	1.46	1.22	1.41	1.15	1.40
T_{13}	1.26	1.37	1.04	1.21	1.00	1.11
CD(0.05)	0.06	0.03	0.01	0.01	0.06	0.01

Table 4.24 Effect of different sources of Cr and ameliorants on nitrate reductase (μ M NO_2 g^{-1} h^{-1} fresh weight.) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	2.78	5.32	6.98	10.12	5.12	6.27
T_2	2.10	4.17	5.95	8.95	4.51	5.31
T_3	1.93	3.54	4.94	7.86	3.93	4.91
T_4	2.21	4.44	6.41	9.21	4.61	5.29
T_5	1.74	3.55	5.22	7.85	4.10	4.70
T_6	2.43	4.80	6.20	10.54	4.69	5.53
T_7	1.67	3.31	5.04	7.71	4.01	4.60
T_8	2.39	4.79	6.92	9.94	4.98	5.71
T_9	1.84	3.77	5.54	8.32	4.34	4.99
T_{10}	2.14	4.25	6.06	9.13	4.60	5.41
T_{11}	1.97	3.31	5.04	7.71	4.01	4.60
T_{12}	2.26	4.53	6.54	9.39	4.70	5.39
T_{13}	1.77	3.62	5.33	8.00	4.18	4.80
CD(0.05)	0.10	0.34	0.31	0.42	0.35	0.36

Table 4.25 Effect of different sources of Cr and ameliorants on catalase activity (enzyme units g^{-1} min^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	3.32	4.68	4.10	5.34	3.10	4.21
T_2	3.34	4.71	4.13	5.38	3.12	4.24
T_3	3.36	4.74	4.15	5.39	3.14	4.26
T_4	3.36	4.72	4.11	5.35	3.14	4.28
T_5	3.37	4.71	4.14	5.36	3.15	4.26
T_6	3.37	4.75	4.16	5.42	3.14	4.27
T_7	3.38	4.76	4.17	5.43	3.16	4.29
T_8	3.34	4.70	4.12	5.37	3.12	4.23
T_9	3.34	4.72	4.13	5.35	3.15	4.29
T_{10}	3.36	4.67	4.15	5.37	3.13	4.28
T_{11}	3.38	4.75	4.18	5.32	3.17	4.38
T_{12}	3.32	4.72	4.17	5.37	3.13	4.24
T_{13}	3.36	4.69	4.18	5.36	3.10	4.23
CD(0.05)	0.06	0.06	0.07	0.08	0.06	0.07

Table 4.26 Effect of different sources of Cr and ameliorants on SOD activity (enzyme units mg^{-1} protein h^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	78.30	61.40	52.20	41.30	46.42	38.31
T_2	81.40	66.50	54.30	45.10	46.20	39.00
T_3	82.50	66.70	55.00	46.00	48.00	40.00
T_4	81.20	67.00	54.90	45.30	47.10	39.90
T_5	83.00	67.20	56.00	46.50	49.00	41.30
T_6	82.13	67.10	54.79	45.51	46.62	39.35
T_7	83.33	67.37	55.55	46.46	48.48	40.40
T_8	83.08	67.17	55.39	46.32	48.34	40.28
T_9	83.66	67.74	56.45	46.87	49.39	41.63
T_{10}	87.10	71.16	58.10	48.26	49.43	41.73
T_{11}	89.10	72.04	59.40	49.68	51.84	43.20
T_{12}	85.26	70.35	57.65	47.57	49.46	41.90
T_{13}	88.81	71.90	59.92	49.76	52.43	44.19
CD(0.05)	2.56	2.79	2.63	2.10	2.32	2.51

Table 4.27 Effect of different sources of Cr and ameliorants on ascorbate peroxidase activity (mU g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	3568.82	3247.74	3376.46	3146.73	3248.43	2987.93
T_2	3710.12	3270.48	3512.29	3168.76	3333.03	3008.85
T_3	3760.26	3296.83	3557.57	3198.26	3358.99	3023.89
T_4	3701.00	3275.50	3551.10	3152.62	3296.01	3037.62
T_5	3783.04	3268.56	3622.25	3158.52	3428.97	3023.42
T_6	3743.51	3283.37	3543.90	3190.94	3337.13	3029.91
T_7	3797.86	3301.55	3593.14	3197.33	3372.58	3042.04
T_8	3736.58	3273.98	3582.47	3162.46	3312.51	3032.87
T_9	3813.31	3275.50	3651.23	3152.62	3446.40	3044.71
T_{10}	3769.83	3270.80	3558.15	3164.41	3359.34	3037.62
T_{11}	3818.08	3296.32	3612.17	3174.94	3387.71	3028.59
T_{12}	3759.05	3285.50	3598.65	3164.41	3320.81	3049.23
T_{13}	3847.86	3274.68	3670.81	3158.52	3469.00	3030.13
CD(0.05)	57.96	21.34	45.13	27.60	24.21	32.14

Table 4.28 Effect of different sources of Cr and ameliorants on monodehydro-ascorbate reductase activity (mU g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	283.50	247.00	256.00	204.00	243.00	184.00
T_2	294.73	248.73	266.30	205.43	249.33	185.29
T_3	298.71	250.73	269.73	207.34	251.27	186.22
T_4	294.00	249.11	269.24	204.38	246.56	187.06
T_5	300.52	248.58	274.64	204.76	256.51	186.19
T_6	297.38	249.71	268.70	206.87	249.64	186.59
T_7	301.70	251.09	272.43	207.28	252.29	187.33
T_8	296.83	249.00	271.62	205.02	247.79	186.77
T_9	302.92	249.11	276.83	204.38	257.81	187.50
T_{10}	299.47	248.76	269.78	205.15	251.30	187.06
T_{11}	303.30	250.70	273.87	205.83	253.42	186.50
T_{12}	298.61	249.87	272.85	205.15	248.42	187.78
T_{13}	305.67	249.05	278.32	204.76	259.50	186.60
CD(0.05)	3.67	1.98	3.41	1.87	1.92	2.18

Table 4.29 Effect of different sources of Cr and ameliorants on dehydroascorbate reductase activity (mU g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	240.06	232.03	221.02	220.02	203.01	207.08
T_2	241.74	233.65	222.57	221.56	204.43	208.53
T_3	242.95	234.82	223.46	222.23	205.46	209.58
T_4	242.95	234.01	221.56	220.44	205.63	210.53
T_5	243.67	233.52	223.18	220.85	206.29	209.54
T_6	243.43	235.29	224.13	223.12	205.86	209.99
T_7	244.40	236.23	224.80	223.56	206.69	210.83
T_8	241.26	233.19	222.13	221.12	204.03	208.12
T_9	241.50	234.01	222.64	220.44	206.29	211.02
T_{10}	242.95	231.53	223.72	221.26	204.98	210.53
T_{11}	244.40	235.50	225.34	219.20	207.60	213.45
T_{12}	240.06	234.01	224.80	221.26	204.98	208.56
T_{13}	242.95	232.52	225.34	220.85	203.01	208.07
CD(0.05)	2.92	2.64	3.24	3.18	3.32	3.89

Table 4.30 Effect of different sources of Cr and ameliorants on glutathione reductase activity (mU g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	356.00	339.26	333.00	320.00	293.00	304.00
T_2	370.10	341.64	346.40	322.24	300.63	306.13
T_3	375.10	344.39	350.86	325.24	302.97	307.66
T_4	369.19	342.16	350.22	320.60	297.29	309.06
T_5	377.37	341.44	357.24	321.20	309.29	307.61
T_6	373.43	342.99	349.51	324.50	301.00	308.27
T_7	378.85	344.89	354.37	325.15	304.20	309.51
T_8	372.74	342.01	353.32	321.60	298.78	308.57
T_9	380.39	342.16	360.10	320.60	310.86	309.78
T_{10}	376.05	341.67	350.92	321.80	303.01	309.06
T_{11}	380.87	344.34	356.25	322.87	305.57	308.14
T_{12}	374.98	343.21	354.91	321.80	299.53	310.24
T_{13}	383.84	342.08	362.03	321.20	312.90	308.29
CD(0.05)	4.89	2.31	4.42	2.83	2.31	2.13

Table 4.31 Effect of different sources of Cr and ameliorants on ascorbic acid content (μg g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	470.00	421.00	432.00	410.00	425.00	403.00
T_2	486.33	423.95	449.38	412.87	436.07	405.82
T_3	487.84	427.37	455.18	416.72	439.47	407.85
T_4	480.15	424.60	454.35	410.77	431.23	409.70
T_5	490.79	423.70	463.45	411.54	448.62	407.79
T_6	485.67	425.62	453.43	415.76	436.61	408.66
T_7	492.72	427.98	459.73	416.60	441.25	410.30
T_8	484.77	424.40	458.36	412.05	433.39	409.06
T_9	494.72	424.60	467.16	410.77	450.91	410.66
T_{10}	489.08	423.99	455.25	412.31	439.51	409.70
T_{11}	495.34	427.30	462.16	413.68	443.23	408.48
T_{12}	487.68	425.90	460.43	412.31	434.47	411.27
T_{13}	499.20	424.50	469.67	411.54	453.86	408.69
CD(0.05)	6.45	2.89	5.64	2.82	3.12	2.61

Table 4.32 Effect of different sources of Cr and ameliorants on total acid soluble thiols (μM g^{-1} dry weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	0.09	0.43	0.03	0.33	0.02	0.15
T_2	4.95	0.65	4.89	0.25	4.08	0.68
T_3	5.02	0.64	4.99	0.25	4.11	0.81
T_4	5.00	0.66	4.94	0.27	4.11	0.70
T_5	4.99	0.61	4.96	0.22	4.09	0.78
T_6	4.99	0.66	4.90	0.34	4.11	0.68
T_7	5.03	0.65	5.01	0.28	4.16	0.88
T_8	5.09	0.56	4.91	0.28	4.15	0.77
T_9	5.12	0.61	4.99	0.24	4.13	0.84
T_{10}	4.86	0.49	4.66	-0.12	3.85	0.51
T_{11}	4.82	0.57	4.73	-0.08	3.86	0.50
T_{12}	4.85	0.63	4.77	-0.04	3.91	0.53
T_{13}	4.86	0.57	4.75	-0.04	3.93	0.51
CD(0.05)	0.12	0.22	0.16	0.19	0.15	0.17

Table 4.33 Effect of different sources of Cr and ameliorants on total glutathione (μM g^{-1} dry weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	3.57	4.38	3.35	4.26	3.31	4.17
T_2	3.89	4.76	3.68	4.60	3.52	4.31
T_3	3.94	4.82	3.71	4.66	3.58	4.45
T_4	3.92	4.80	3.70	4.63	3.55	4.34
T_5	3.94	4.86	3.74	4.69	3.59	4.40
T_6	3.92	4.65	3.73	4.64	3.57	4.37
T_7	3.96	4.88	3.78	4.67	3.59	4.43
T_8	3.86	4.92	3.74	4.60	3.58	4.45
T_9	3.84	4.93	3.77	4.70	3.60	4.41
T_{10}	4.18	5.17	4.12	5.10	4.00	4.83
T_{11}	4.25	5.14	4.08	5.07	4.00	4.86
T_{12}	4.21	5.10	4.08	5.06	3.99	4.87
T_{13}	4.21	5.17	4.10	5.05	3.98	4.87
CD(0.05)	0.27	0.28	0.32	0.34	0.26	0.28

Table 4.34 Effect of different sources of Cr and ameliorants on reduced glutathione (μM g^{-1} dry weight)in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	3.41	4.20	3.21	4.10	3.18	4.03
T_2	3.72	4.56	3.52	4.42	3.38	4.15
T_3	3.76	4.62	3.54	4.48	3.41	4.20
T_4	3.73	4.58	3.53	4.43	3.40	4.16
T_5	3.78	4.64	3.56	4.50	3.43	4.22
T_6	3.75	4.63	3.56	4.46	3.40	4.21
T_7	3.78	4.65	3.60	4.48	3.43	4.26
T_8	3.70	4.71	3.59	4.43	3.41	4.27
T_9	3.68	4.68	3.58	4.51	3.45	4.25
T_{10}	4.01	4.93	3.96	4.92	3.83	4.65
T_{11}	4.06	4.90	3.92	4.90	3.84	4.69
T_{12}	4.05	4.87	3.92	4.87	3.82	4.68
T_{13}	4.04	4.91	3.95	4.87	3.82	4.69
CD(0.05)	0.25	0.24	0.29	0.03	0.04	0.38

Table 4.35 Effect of different sources of Cr and ameliorants on oxidised glutathione (μM g^{-1} dry weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	0.16	0.18	0.14	0.16	0.13	0.14
T_2	0.17	0.20	0.16	0.18	0.14	0.16
T_3	0.18	0.20	0.17	0.18	0.15	0.17
T_4	0.19	0.22	0.17	0.20	0.15	0.17
T_5	0.16	0.22	0.18	0.19	0.16	0.18
T_6	0.17	0.02	0.17	0.18	0.17	0.16
T_7	0.18	0.23	0.18	0.19	0.16	0.17
T_8	0.16	0.21	0.15	0.17	0.17	0.18
T_9	0.16	0.25	0.19	0.19	0.15	0.16
T_{10}	0.17	0.24	0.16	0.18	0.17	0.18
T_{11}	0.19	0.24	0.16	0.17	0.16	0.17
T_{12}	0.16	0.23	0.16	0.19	0.17	0.19
T_{13}	0.17	0.26	0.15	0.18	0.16	0.18
CD(0.05)	0.08	0.07	0.07	0.05	0.06	0.05

Table 4.36 Effect of different sources of Cr and ameliorants on phytochelatin S-H (μM g^{-1} dry weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	3.50	4.63	3.24	4.43	3.20	4.18
T_2	8.67	5.21	8.41	4.67	7.46	4.83
T_3	8.78	5.26	8.53	4.73	7.52	5.01
T_4	8.73	5.25	8.47	4.70	7.51	4.86
T_5	8.77	5.25	8.52	4.73	7.51	5.00
T_6	8.74	5.29	8.46	4.80	7.51	4.89
T_7	8.81	5.30	8.61	4.76	7.59	5.14
T_8	8.79	5.27	8.50	4.71	7.56	5.04
T_9	8.80	5.29	8.57	4.75	7.58	5.09
T_{10}	8.87	5.42	8.62	4.80	7.68	5.16
T_{11}	8.88	5.47	8.65	4.82	7.70	5.19
T_{12}	8.90	5.50	8.69	4.83	7.73	5.21
T_{13}	8.90	5.48	8.70	4.83	7.75	5.20
CD(0.05)	0.13	0.22	0.12	0.13	0.18	0.19

Table 4.37 Effect of different sources of Cr and ameliorants on root ethylene content (µ mole g^{-1}) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	1.71	1.93	2.16	2.41	2.26	2.63
T_2	1.91	2.14	2.32	2.61	2.45	2.81
T_3	2.01	2.23	2.42	2.78	2.50	2.90
T_4	1.85	2.06	2.23	2.51	2.40	2.77
T_5	1.95	2.18	2.38	2.66	2.44	2.81
T_6	1.92	2.12	2.34	2.57	2.43	2.80
T_7	1.98	2.14	2.40	2.68	2.51	2.92
T_8	1.87	2.04	2.32	2.76	2.51	2.94
T_9	1.97	2.19	2.41	2.69	2.49	2.76
T_{10}	1.90	2.13	2.30	2.60	2.41	2.79
T_{11}	1.94	2.15	2.34	2.68	2.41	2.80
T_{12}	1.80	1.99	2.19	2.43	2.33	2.67
T_{13}	1.89	2.15	2.30	2.56	2.39	2.69
CD(0.05)	0.20	0.21	0.15	0.19	0.18	0.18

Table 4.38 Effect of different sources of Cr and ameliorants on total free amino acids (μg g^{-1} fresh weight) in sunflower and sorghum

Treatments	Stage I		Stage II		Stage III	
	Sunflower 30 DAS	Sorghum 20 DAS	Sunflower 50 DAS	Sorghum 45 DAS	Sunflower 80 DAS	Sorghum 65 DAS
T_1	186.72	173.70	248.95	223.00	208.00	196.00
T_2	192.11	187.25	257.97	243.52	207.01	198.34
T_3	197.73	187.93	262.69	248.38	215.08	204.65
T_4	194.65	189.58	260.83	244.60	211.05	204.13
T_5	196.73	189.82	267.32	251.08	219.56	211.96
T_6	196.53	188.06	261.30	245.71	208.88	200.33
T_7	198.43	189.28	264.91	250.86	217.23	205.37
T_8	197.11	188.25	264.74	250.12	216.59	206.25
T_9	198.61	190.42	269.69	253.09	221.32	212.00
T_{10}	206.70	200.28	276.10	260.57	221.51	212.50
T_{11}	212.09	202.13	283.74	268.25	232.29	221.65
T_{12}	204.31	198.14	275.92	256.83	221.60	214.63
T_{13}	211.86	201.60	285.24	268.65	234.93	225.09
CD(0.05)	5.32	12.35	4.67	4.82	7.63	6.21

Table 4.39 Effect of different sources of Cr and ameliorants on nitrogen, phosphorus and potassium content (percentage DW) and uptake (g plant^{-1}) in sunflower and sorghum at stage III.

Treatments	Sunflower			Sorghum		
	N	P	K	N	P	K
T_1	1.124 (0.7133)	0.072 (0.0460)	0.747 (0.4741)	1.182 (0.8551)	0.079 (0.0577)	0.791 (0.5724)
T_2	1.085 (0.6036)	0.062 (0.0347)	0.710 (0.3950)	1.120 (0.6348)	0.068 (0.0389)	0.740 (0.4194)
T_3	1.010 (0.4498)	0.053 (0.0238)	0.629 (0.2801)	1.020 (0.4916)	0.052 (0.0253)	0.630 (0.3037)
T_4	1.120 (0.6418)	0.070 (0.0404)	0.753 (0.4315)	1.185 (0.6917)	0.080 (0.0468)	0.795 (0.4644)
T_5	1.126 (0.5118)	0.070 (0.0322)	0.757 (0.3441)	1.192 (0.5861)	0.080 (0.0396)	0.800 (0.3935)
T_6	1.139 (0.6656)	0.073 (0.0382)	0.745 (0.4355)	1.175 (0.6992)	0.092 (0.0429)	0.777 (0.4624)
T_7	1.050 (0.4818)	0.063 (0.0255)	0.654 (0.3000)	1.060 (0.5266)	0.060 (0.0271)	0.655 (0.3253)
T_8	1.199 (0.7077)	0.075 (0.0445)	0.805 (0.4756)	1.269 (0.7630)	0.085 (0.0515)	0.853 (0.5131)
T_9	1.194 (0.5479)	0.075 (0.0345)	0.803 (0.3684)	1.263 (0.6275)	0.085 (0.0424)	0.848 (0.4213)
T_{10}	1.088 (0.6109)	0.062 (0.0351)	0.712 (0.3999)	1.123 (0.6424)	0.069 (0.0399)	0.741 (0.4239)
T_{11}	1.013 (0.4552)	0.053 (0.0242)	0.630 (0.2835)	1.025 (0.4985)	0.052 (0.0256)	0.632 (0.3078)
T_{12}	1.130 (0.6534)	0.071 (0.0412)	0.759 (0.4393)	1.195 (0.7043)	0.081 (0.0482)	0.802 (0.4728)
T_{13}	1.142 (0.5235)	0.071 (0.0327)	0.761 (0.3490)	1.197 (0.5938)	0.080 (0.0401)	0.814 (0.4040)
CD(0.05)	0.0389	0.009	0.037	0.059	0.001	0.004

Date in parenthesis represents uptake

Table 4.40 Effect of different sources of Cr and ameliorants on iron (ppm), sulphur (percentage DW) and boron (μg g^{-1}DW) content in sunflower and sorghum at stage III

Treatments	Sunflower			Sorghum		
Nutrients	Fe	S	B	Fe	S	B
T_1	119.54	0.090	6.47	113.25	0.060	6.89
T_2	113.63	0.085	5.35	106.63	0.057	5.39
T_3	113.27	0.079	4.68	105.62	0.051	4.59
T_4	113.33	0.084	5.52	105.34	0.055	5.56
T_5	114.02	0.082	4.77	106.71	0.053	4.68
T_6	114.72	0.087	5.62	107.01	0.055	5.66
T_7	116.50	0.084	4.82	108.38	0.052	4.72
T_8	116.13	0.086	5.68	109.35	0.057	5.72
T_9	117.01	0.085	4.82	110.61	0.054	4.73
T_{10}	122.14	0.092	5.40	115.86	0.060	5.44
T_{11}	125.13	0.095	4.72	118.08	0.059	4.63
T_{12}	119.40	0.092	5.57	112.14	0.060	5.61
T_{13}	124.70	0.090	4.82	117.75	0.060	4.72
CD(0.05)	5.8610	0.0063	1.0980	6.5900	0.004	0.1430

Table 4.41 Effect of different sources of Cr and ameliorants on chromium content (µg g^{-1} DW) in sunflower and sorghum at stage III

Treatments	Sunflower			Sorghum		
	Root	Shoot	Root/ Shoot	Root	Shoot	Root/ Shoot
T_1	3.45	trace	5.60	3.40	trace	
T_2	187.43	7.10	26.40	214.78	4.17	51.51
T_3	210.00	6.87	30.57	204.00	4.23	48.23
T_4	124.58	4.76	26.19	141.90	2.79	50.79
T_5	135.08	5.04	26.80	150.49	2.96	50.83
T_6	205.55	7.02	29.28	231.81	4.06	57.07
T_7	228.90	5.49	41.71	222.36	4.12	53.96
T_8	132.60	4.45	29.82	148.66	2.66	55.91
T_9	139.73	5.04	27.73	161.12	2.91	55.35
T_{10}	184.55	7.14	25.84	214.07	4.20	51.03
T_{11}	210.56	6.92	30.44	204.43	4.26	47.99
T_{12}	127.54	4.50	28.34	145.54	2.71	53.70
T_{13}	135.00	5.12	26.37	148.98	2.83	52.64
CD(0.05)	16.46	6.43	24.34	9.63	4.12	3.12

Table 4.42 Effect of different sources of Cr and ameliorants on chromium uptake (µg plant^{-1}) in sunflower and sorghum at stage III.

Treatments	Sunflower			Sorghum		
	Root	Shoot	Root/Shoot	Root	Shoot	Root/Shoot
T_1	72.93		0.00	81.31		0.00
T_2	3493.21	262.69	13.30	3792.46	162.70	23.31
T_3	3117.16	203.95	15.28	3442.31	132.51	25.98
T_4	2138.44	190.94	11.20	2608.58	111.73	23.35
T_5	2095.35	150.77	13.90	2466.27	117.04	25.42
T_6	3899.80	276.90	14.08	4598.34	161.15	28.54
T_7	3563.18	166.30	21.43	3640.09	137.13	26.54
T_8	2456.18	180.12	13.64	2994.31	106.32	28.16
T_9	2042.56	157.52	12.97	2666.79	116.36	27.68
T_{10}	3433.64	268.07	12.81	4248.48	156.64	27.12
T_{11}	3174.65	206.54	15.37	3314.00	138.11	24.00
T_{12}	2513.62	171.50	14.66	2798.99	107.50	26.04
T_{13}	2062.36	156.43	13.18	2463.43	93.59	26.32
CD(0.05)	412.34	12.35	4.470	487.56	7.67	6.19

Table 4.43 Effect of different sources of Cr and ameliorants on yield and yield components of sunflower and green fodder yield (GFY) of sorghum

Treatments	Sunflower				Sorghum
	No. of seeds head^{-1}	Filled Seeds Head^{-1}	Percentage Filled Seeds	100 Seed weight (g)	G FY (g plant^{-1})
T_1	543.00	461.55	0.85	4.23	109.4500
T_2	432.00	280.80	0.65	4.17	90.67
T_3	392.00	239.12	0.61	4.15	88.96
T_4	442.00	309.40	0.70	4.18	93.87
T_5	400.00	288.00	0.72	4.19	93.87
T_6	452.00	315.67	0.69	4.18	94.78
T_7	412.00	267.80	0.65	4.15	90.23
T_8	463.00	344.28	0.75	4.19	95.21
T_9	421.00	319.96	0.76	4.18	92.21
T_{10}	438.00	293.46	0.67	4.14	91.23
T_{11}	400.00	252.00	0.63	4.15	89.32
T_{12}	446.00	312.20	0.70	4.16	94.00
T_{13}	403.00	290.16	0.72	4.18	94.76
CD(0.05)	18.78	23.46	0.04	0.06	3.68

Table 2.2. Relationship between Cr concentration in growth medium and its uptake in crops

CR CONCENTRATION IN MEDIUM	**Uptake and accumulation pattern**	**Crop**	**Reference**
0, 5, 30, 45, 60, 75, 90, 105, 120 and 135 mg kg^{-1} Cr (III) and Cr(VI)	2.8 Cr(III) and 3.14 Cr(VI) μg g^{-1}	Spinach	Singh (2001)
0, 5, 10, 20 and 40 ppm Cr(IV)	Progressive increase with more Cr in roots than shoots	Lucerne	Peralta *et al*. (2001)
0, 5, 10, 20 and 40 mgL^{-1} Cr(VI)	3.4 mg kg^{-1} in tops	Soybean	del Castilhos *et al*. (2001)
Total Cr 1 ppm	10-200 times in roots	*Veronica beccabunga* and several hydrophytes	Zurayk *et al*. (2001a)
0, 100, 300, 500, 1000 mg kg^{-1} Cr (III)	Mobile soil Cr X Plant Cr (r = 0.965) Total soil Cr X Plant Cr (r = 0.629)	*Medicago sativa*	Zlaterava *et al*. (1999)
50, 100, 200 μ M Cr(VI)	Progressive increase with more Cr in roots than shoots	*Nelumbo nucifera*	Vajpayee *et al*. (1999)
6, 12, 24 mg L^{-1} Cr	Cr more in roots than shoots in A and more in shoots than roots in B	A- *Dactylis glomerate* B- *Medicago sativa*	Tena *et al*. (1999)
1mg L^{-1} for 10 days Cr	Shoot : 44 mg kg^{-1} DW Root: 2980 mg kg^{-1} DW	Smart weed	JinHong *et al*. (1999)
0.5,1,5, 25 μg ml^{-1} ^{51} Cr radio labelled	Progressive increase with more Cr in roots than shoots	Rice	Mishra *et al*. (1997)
0, 50, 100 mg L^{-1} Cr(III)	Roots took up more than shoots and not detected in fruits	Tomato	Moral *et al*. (1996)
0.01 to 1.0mM Cr(VI)	Uptake followed Michelis Menton kinetics	Wheat	Sharma *et al*. (1995)
0- 200 mg kg^{-1}	Progressive increase with more Cr in roots than shoots	Sunflower, maize and *Vicia faba*	Kocik and IIlavsky (1994)

Table 2.2. (Contd..)

Cr Concentration in medium	Uptake and accumulation pattern	Crop	Reference
0.25 and 1.0 mg L^{-1} L	75- 100 per cent steady state removal.1-2 mg kg^{-1} DW at the rate of 250-667 mg day^{-1} m^2	*Lemna minor*	Wahaab *et al*. (1995)
Tannery effluent	Progressive increase with more Cr in roots than shoots, 38 ppm accumulation	*Eichornia crassipes*	Singaram (1994)
Tannery effluent	38-50 per cent removal of Cr	*Hydrilla verticiliata*	Vajpayee *et al*. (1995)
10 ppm r	105-156 μg g^{-1} accumulation	*Eichornia crassipes*	Saltabas and Akcin (1994)
0, 5, 50, 150 and 300 μg ml^{-1} Cr(III) and Cr(VI)	70- 90 per cent accumulation in roots	*Allium cepa*	Srivastava *et al*. (1994)
Tannery effluent 5,10 and 15 per cent	High Cr removal from 10 and 15 per cent	Swiss Chard	Grubinger *et al*. (1994)
0.091-0.24 mg kg^{-1} in surface soil and 0.0107 -0.31 mg kg^{-1}	0.00081- 0.000122μ g kg^{-1} 100-200 times lower in soil	*Digitalis sp*	Shuvalov 1989
0,2,4,6,8 mg L^{-1}	6700 mg kg^{-1} in roots	*Veronica beccabanga*	Zurayk *et al*. (2001b)
0, 100, 500 Cr(VI) and Cr (VI)	2.4 mg kg^{-1} shoot and 115.6 mg kg^{-1} in root in A 5.8 mg kg^{-1} shoot and 212 mg kg^{-1} in root in B	A- Sorghum B- Sunflower	Shahendah and Hossner (2000a)
19.2 μM Cr(VI) and 19.2 μM Cr(III)	350 mg kg^{-1} roots and 2mg kg^{-1} shoots	Cauliflower, kale, and cabbage	Zayed *et al*. (1998)
0,0.05, 0.10,0.50,1.00 and 5.00 ppm	11.9 -32.8 ppm in tops	Soybean	Turner and Rust (1971)
0. 0.2, 2 and 10 ppm Total Cr	Progressive increase with increase in C concentration	Cabbage	Hara and Sonoda (1979)

Table 4.1 Effect of chromium levels in nutrient media on germination percentage of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	94.00	58.98	39.61	39.77	8.54	8.78	4.43	36.30
	CO-4	92.26	57.87	39.77	40.00	7.91	8.62	4.66	35.87
	CO-5	93.53	56.92	40.48	40.32	9.57	8.54	5.30	36.38
	Mean	93.26	57.92	39.95	40.03	8.67	8.64	4.80	
	CD(P=0.05)			T=25.98		V=12.13		TxV=27.32	
Rice	TRY-1	95.66	60.08	41.43	41.35	7.91	7.83	5.14	37.06
	ADT-37	94.17	58.74	42.85	41.98	7.12	7.98	4.59	37.63
	CO-43	94.87	59.77	41.19	41.11	7.98	8.85	5.93	37.10
	Mean	96.90	59.53	41.82	41.48	7.67	8.22	5.22	
	CD(P=0.05)			**T=24.76**		V=9.56		TxV=26.12	
Sunflower	Modern	94.87	66.80	47.59	48.38	8.85	9.57	7.04	40.44
	CO-2	98.27	64.91	49.10	47.51	8.85	9.41	6.88	40.70
	CO-4	97.40	69.41	51.39	50.99	10.20	10.59	8.46	42.63
	Mean	96.85	67.04	49.36	48.96	9.30	9.86	7.46	
	CD(P=0.05)			T=29.32		V=8.67		TxV=28.93	
Sorghum	CO-26	97.56	68.54	48.15	49.33	10.44	10.12	7.19	41.62
	K-10	96.53	67.52	48.30	49.10	10.36	10.04	7.04	41.27
	CO-27	98.51	70.28	49.10	50.52	12.02	11.31	8.70	42.92
	Mean	97.53	68.78	48.52	49.65	10.94	10.49	7.64	
	CD(P=0.05)			T=30.12		V=9.13		TxV=28.12	

Table 4.2 Effect of chromium levels in nutrient media on vigour index of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	1189	746	501	503	108	111	56	459.14
	CO-4	1167	732	503	506	100	109	59	453.71
	CO-5	1183	720	512	510	121	108	67	460.14
	Mean	1179.67	732.67	505.33	506.33	109.67	109.33	60.67	457.67
	CD(P=0.05)	T=25.98			V=12.13			TxV=27.32	
Rice	TRY-1	1210	760	524	523	100	99	65	468.71
	ADT-37	1267	743	542	531	90	101	58	476.00
	CO-43	1200	756	521	520	101	112	75	469.29
	Mean	1225.67	753.00	529.00	524.67	97.00	104.00	66.00	
	CD(P=0.05)	T=24.76			V=9.56			TxV=26.12	
Sunflower	Modern	1200	845	602	612	112	121	89	511.57
	CO-2	1243	821	621	601	112	119	87	514.86
	CO-4	1232	878	650	645	129	134	107	539.29
	Mean	1225.00	848.00	624.33	619.33	117.67	124.67	94.33	
	CD(P=0.05)	T=29.32			V=8.67			TxV=28.93	
Sorghum	CO-26	1234	867	609	624	132	128	91	526.43
	K-10	1221	854	611	621	131	127	89	522.00
	CO-27	1246	889	621	639	152	143	110	542.86
	Mean	1233.67	870.00	613.67	628.00	138.33	132.67	96.67	
	CD(P=0.05)	T=30.12			V=9.13			TxV=28.12	

Table 4.3 Effect of chromium levels in nutrient media on promptness index of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	238.76	149.80	100.60	101.00	21.69	22.29	11.24	92.20
	CO-4	234.34	146.99	101.00	101.61	20.08	21.89	11.85	91.11
	CO-5	237.55	144.58	102.81	102.41	24.30	21.69	7.43	91.54
	Mean	236.88	147.12	101.47	101.67	22.02	21.95	10.17	91.61
	CD(P=0.05)			T=5.67		V=2.13		TxV=10.34	
Rice	TRY-1	242.97	152.61	105.22	105.02	20.08	19.88	13.05	94.12
	ADT-37	254.42	149.20	108.84	106.63	18.07	20.28	11.65	95.58
	CO-43	240.96	151.81	104.62	104.42	20.28	22.49	10.21	93.54
	Mean	246.12	151.20	106.22	105.35	19.48	20.88	11.64	94.41
	CD(P=0.05)			T=4.57		V=2.56		TxV=13.12	
Sunflower	Modern	240.96	169.68	120.88	122.89	22.49	24.30	17.87	102.73
	CO-2	249.60	164.86	124.70	120.68	22.49	23.90	17.47	103.38
	CO-4	247.39	176.31	130.52	134.60	25.90	30.30	26.01	110.15
	Mean	245.98	170.28	125.37	126.06	23.63	26.16	20.45	105.42
	CD(P=0.05)			T=5.71		V=3.03		TxV=15.78	
Sorghum	CO-26	247.79	174.10	122.29	125.30	26.51	25.70	18.27	105.71
	K-10	245.18	171.49	122.69	124.70	26.31	25.50	17.87	104.82
	CO-27	250.20	178.51	124.70	128.31	30.52	28.71	25.87	109.55
	Mean	247.72	174.70	123.23	126.10	27.78	26.64	20.67	106.69
	CD(P=0.05)			***T=7.34***		V=3.78		TxV=18.12	

Table 4.4 Effect of chromium levels in nutrient media on germination stress index of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	238.76	62.74	42.14	42.30	9.08	9.34	4.71	28.39
	CO-4	234.34	62.72	43.10	43.36	8.57	9.34	5.06	28.69
	CO-5	237.55	60.86	43.28	43.11	10.23	9.13	3.13	28.29
	Mean	236.88	62.11	42.84	42.92	9.29	9.27	4.30	
	CD(P=0.05)	T=10.23			V=2.13		TxV=13.32		
Rice	TRY-1	242.97	62.81	43.31	43.22	8.26	8.18	5.37	28.53
	ADT-37	254.42	58.64	42.78	41.91	7.10	7.97	4.58	27.16
	CO-43	240.96	63.00	43.42	43.33	8.42	9.33	4.24	28.62
	Mean	246.12	61.48	43.17	42.82	7.93	8.50	4.73	
	CD(P=0.05)								
Sunflower	Modern	240.96	70.42	50.17	51.00	9.33	10.08	7.42	33.07
	CO-2	249.60	66.05	49.96	48.35	9.01	9.57	7.00	31.66
	CO-4	247.39	71.27	52.76	54.41	10.47	12.25	10.51	35.28
	Mean	245.98	69.24	50.96	51.25	9.60	10.63	8.31	
	CD(P=0.05)	T=13.32			V=2.67		TxV=12.01		
Sorghum	CO-26	247.79	70.26	49.35	50.57	10.70	10.37	7.37	33.10
	K-10	245.18	69.94	50.04	50.86	10.73	10.40	7.29	33.21
	CO-27	250.20	71.35	49.84	51.28	12.20	11.48	10.34	34.41
	Mean	247.72	70.52	49.74	50.90	11.21	10.75	8.33	
	CD(P=0.05)	T=16.26			V=2.74		TxV=9.97		

Table 4.5 Effect of chromium levels in nutrient media on root length (cm) of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	8.40	7.10	7.00	5.10	4.10	5.20	4.00	5.84
	CO-4	8.90	7.20	7.20	5.30	4.30	5.50	4.30	6.10
	CO-5	8.60	7.20	7.10	5.10	4.50	5.10	4.40	6.00
	Mean	8.63	7.17	7.10	5.17	4.30	5.27	4.23	
	CD(P=0.05)			T=1.45		V=0.557		TxV=2.67	
Rice	TRY-1	9.20	7.30	7.20	5.00	4.00	5.10	4.00	5.97
	ADT-37	9.00	7.60	7.50	5.20	4.00	5.20	4.10	6.09
	CO-43	8.60	7.50	7.50	5.30	4.30	5.40	4.20	6.11
	Mean	8.93	7.47	7.40	5.17	4.10	5.23	4.10	
	CD(P=0.05)			T=1.06		V=0.534		TxV=2.89	
Sunflower	Modern	7.30	6.00	6.10	5.40	5.00	5.30	4.80	5.70
	CO-2	7.30	6.10	6.30	5.50	4.90	5.50	4.80	5.77
	CO-4	8.10	7.10	7.30	6.40	5.40	6.20	5.60	6.59
	Mean	7.57	6.40	6.57	5.77	5.10	5.67	5.07	
	CD(P=0.05)			T=0.93		V=0.678		TxV=3.18	
Sorghum	CO-26	10.10	8.90	8.70	8.10	6.90	8.00	6.70	8.20
	K-10	10.00	8.80	8.80	8.00	6.80	7.70	6.50	8.09
	CO-27	10.70	9.40	9.10	8.60	7.40	8.40	7.00	8.66
	Mean	10.27	9.03	8.87	8.23	7.03	8.03	6.73	
	CD(P=0.05)			T=0.95		V=0.553		TxV=3.34	

Table 4.6 Effect of chromium levels in nutrient media on anthocyanin content (μg g^{-1}fresh weight) of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	0.0034	0.0036	0.0037	0.0041	0.0029	0.0028	0.0017	0.0032
	CO-4	0.0033	0.0036	0.0036	0.0041	0.0029	0.0028	0.0017	0.0031

	CO-5	0.0033	0.0037	0.0037	0.0041	0.0028	0.0030	0.0015	0.0032
	Mean	0.0034	0.0037	0.0037	0.0041	0.0029	0.0028	0.0016	
	CD(P=0.05)			T=0.00024		V=0.00029		TxV=0.00056	
Rice	TRY-1	0.0027	0.0029	0.0030	0.0038	0.0018	0.0018	0.0010	0.0024
	ADT-37	0.0026	0.0028	0.0028	0.0038	0.0015	0.0015	0.0009	0.0023
	CO-43	0.0026	0.0027	0.0027	0.0038	0.0016	0.0015	0.0009	0.0022
	Mean	0.0027	0.0028	0.0028	0.0038	0.0017	0.0016	0.0009	
	CD(P=0.05)			T=0.00021		V=0.00023		VxT=0.00048	
Sunflower	Modern	0.0040	0.0044	0.0045	0.0049	0.0035	0.0034	0.0020	0.0038
	CO-2	0.0042	0.0045	0.0045	0.0049	0.0035	0.0034	0.0020	0.0039
	CO-4	0.0041	0.0047	0.0048	0.0055	0.0038	0.0038	0.0025	0.0042
	Mean	0.0041	0.0046	0.0046	0.0051	0.0036	0.0035	0.0022	
	CD(P=0.05)			T=0.00032		V=0.00027		TxV=0.00059	
Sorghum	CO-26	0.0035	0.0040	0.0041	0.0046	0.0032	0.0031	0.0019	0.0035
	K-10	0.0035	0.0041	0.0041	0.0046	0.0032	0.0031	0.0019	0.0035
	CO-27	0.0037	0.0043	0.0044	0.0051	0.0035	0.0035	0.0023	0.0038
	Mean	0.0036	0.0041	0.0042	0.0048	0.0033	0.0032	0.0020	
	CD(P=0.05)			T=0.00029		V=0.00026		TxV=0.00051	

Table 4.7 Effect of chromium levels in nutrient media on lipid peroxidation (μM g^{-1}fresh weight) of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	6.820	7.320	8.076	9.435	11.080	11.156	11.254	9.306
	CO-4	6.800	7.300	8.056	9.415	11.060	11.136	11.234	9.286
	CO-5	6.800	7.310	8.066	9.425	11.060	11.136	11.234	9.290
	Mean	6.807	7.310	8.066	9.425	11.067	11.143	11.241	
	CD(P=0.05)			T=0.48		V=0.052		TxV=1.83	
Rice	TRY-1	5.650	6.240	6.996	8.355	9.910	9.986	10.084	8.174
	ADT-37	5.680	6.260	7.016	8.375	9.940	10.016	10.114	8.200
	CO-43	5.690	6.270	7.026	8.385	9.950	10.026	10.124	8.210
	Mean	5.673	6.257	7.013	8.371	9.933	10.009	10.107	
	CD(P=0.05)			T=0.51		V=0.054		TxV=1.89	
Sunflower	Modern	7.140	7.150	7.795	8.923	10.930	11.006	11.104	9.150
	CO-2	7.150	7.210	7.855	8.944	10.940	11.016	11.114	9.176
	CO-4	7.160	7.190	7.835	8.768	10.750	10.826	10.924	9.065
	Mean	7.150	7.183	7.828	8.878	10.873	10.949	11.047	
	CD(P=0.05)			T=0.53		V=0.063		TxV=2.07	
Sorghum	CO-26	6.560	6.920	7.565	8.654	10.450	10.526	10.624	8.757
	K-10	6.680	7.010	7.655	8.744	10.470	10.546	10.644	8.821
	CO-27	6.660	6.980	7.625	8.714	10.250	10.326	10.424	8.711
	Mean	6.633	6.970	7.615	8.704	10.390	10.466	10.564	
	CD(P=0.05)			*T=0.58*		V=0.059		TxV=2.16	

Table 4.8 Effect of chromium levels in nutrient media on SOD activity (units mg^{-1} protein $hour^{-1}$) of selected crops

CROP	Variety	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	66.50	70.50	71.50	78.70	56.30	54.17	33.16	61.55
	CO-4	64.30	70.00	70.30	78.90	56.00	53.84	32.87	60.89
	CO-5	64.00	72.00	72.00	80.00	53.70	52.33	28.57	60.37
	Mean	64.93	70.83	71.27	79.20	55.33	53.45	31.53	
	CD(P=0.05)	T=6.34				V=4.86		TxV=12.35	
Rice	TRY-1	53.20	56.90	58.00	73.10	35.60	34.23	20.19	47.32
	ADT-37	51.00	54.30	55.00	74.50	30.00	28.86	17.67	44.48
	CO-43	50.50	51.80	52.20	73.00	31.70	28.47	17.31	43.57
	Mean	51.57	54.33	55.07	73.53	32.43	30.52	18.39	
	CD(P=0.05)	T=6.34				V=4.86		TxV=12.35	
Sunflower	Modern	78.00	86.20	87.50	95.60	68.00	65.38	39.37	74.29
	CO-2	81.30	87.40	87.30	94.50	67.90	65.33	39.65	74.77
	CO-4	79.30	91.70	93.70	106.00	74.20	74.36	48.89	81.16
	Mean	79.53	88.43	89.50	98.70	70.03	68.36	42.64	
	CD(P=0.05)	T=7.13				V=6.17		TxV=13.23	
Sorghum	CO-26	68.30	77.20	78.60	90.10	62.30	59.90	36.69	67.59
	K-10	68.10	79.20	79.70	90.00	62.50	60.13	36.81	68.06
	CO-27	71.90	83.90	86.00	99.70	67.70	68.00	44.41	74.52
	Mean	69.43	80.10	81.43	93.27	64.17	62.68	39.31	
	CD(P=0.05)	T=7.67				V=6.06		TxV=14.44	

Table 4.9 Effect of chromium levels in nutrient media on plasma membrane H^+ATPase activity (μM P_i mg $protein^{-1}$ h^{-1}) of selected crops

CROP	*Variety*	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	17.60	16.30	16.40	12.30	10.00	9.00	7.20	12.69
	CO-4	17.30	16.30	16.40	12.20	10.20	9.00	7.40	12.69
	CO-5	17.00	16.20	16.30	12.20	10.00	10.00	7.70	12.77
	Mean	17.30	16.27	16.37	12.23	10.07	9.33	7.43	
	CD(P=0.05)			T=1.89		V=0.56		TxV=5.46	
Rice	TRY-1	19.60	18.10	18.00	14.20	12.00	12.00	7.50	14.49
	ADT-37	19.90	18.10	18.00	14.20	12.00	11.50	7.90	14.51
	CO-43	19.40	18.50	18.00	14.30	12.30	12.10	7.30	14.56
	Mean	19.63	18.23	18.00	14.23	12.10	11.87	7.57	
	CD(P=0.05)			T=2.03		V=0.49		TxV=5.87	
Sunflower	Modern	17.00	16.80	16.50	14.10	14.30	14.20	9.10	14.57
	CO-2	17.50	16.80	16.60	14.30	14.30	14.20	9.20	14.70
	CO-4	17.20	16.80	16.90	14.80	15.20	14.80	9.80	15.07
	Mean	17.23	16.80	16.67	14.40	14.60	14.40	9.37	
	CD(P=0.05)			T=1.96		V=0.63		TxV=5.33	
Sorghum	CO-26	17.80	16.10	16.00	14.00	14.10	14.00	9.20	14.46
	K-10	17.40	16.70	16.50	14.00	14.30	14.10	9.10	14.59
	CO-27	17.40	16.90	16.40	14.70	15.10	14.80	9.87	15.02
	Mean	17.53	16.57	16.30	14.23	14.50	14.30	9.39	
	CD(P=0.05)			T=2.01		V=0.67		TxV=5.46	

Table 4.10 Effect of chromium levels in nutrient media on root Fe(III) reductase (μM P_i mg protein^{-1} h^{-1}) of selected crops

CROP	*Variety*	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	1.40	1.35	1.30	1.15	1.12	1.15	0.78	1.18
	CO-4	1.38	1.34	1.30	1.15	1.12	1.15	0.65	1.16
	CO-5	1.40	1.35	1.28	1.18	1.13	1.16	0.79	1.18
	Mean	1.39	1.35	1.29	1.16	1.12	1.15	0.74	
	CD(P=0.05)			T=0.0432		V=0.23		TxV=1.85	
Sunflower	Modern	1.71	1.66	1.60	1.42	1.38	1.42	0.92	1.44
	CO-2	1.70	1.65	1.60	1.42	1.38	1.42	0.80	1.43
	CO-4	1.73	1.67	1.63	1.46	1.40	1.44	0.98	1.47
	Mean	1.71	1.66	1.61	1.43	1.39	1.43	0.90	
	CD(P=0.05)			T=0.0563		V=0.184		TxV=1.32	

Table 4.11 Effect of chromium levels in nutrient media on chromium content (µg g^{-1} DW) of selected crops

CROP	*Variety*	TREATMENTS							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
Green Gram	CO-6	ND	275.00	291.40	116.24	276.56	143.50	314.50	236.20
	CO-4	ND	283.00	299.80	121.50	285.43	156.00	327.30	245.51
	CO-5	ND	252.00	284.00	118.00	277.30	149.00	319.00	233.22
	Mean		270.00	291.73	118.58	279.76	149.50	320.27	
	CD(P=0.05)			T=43.34		V=24.86		TxV=78.63	
Rice	TRY-1	ND	283.55	314.32	121.47	284.51	149.96	328.65	247.08
	ADT-37	ND	292.11	329.64	126.97	293.77	163.02	342.03	257.92
	CO-43	ND	268.94	286.91	123.31	285.28	155.71	333.36	242.25
	Mean		281.53	310.29	123.92	287.85	156.23	334.68	
	CD(P=0.05)			T=47.32		V=23.41		TxV=84.41	
Sunflower	Modern	ND	223.50	293.76	113.00	264.00	139.40	302.21	222.64
	CO-2	ND	246.60	308.07	117.00	267.00	145.80	313.04	232.92
	CO-4	ND	235.80	268.14	104.00	242.40	126.00	282.83	209.86
	Mean		235.30	289.99	111.33	257.80	137.07	299.36	
	CD(P=0.05)			T=46.66		V=24.65		TxV=76.88	
Sorghum	CO-26	ND	248.70	304.51	120.91	275.48	149.16	317.76	236.09
	K-10	ND	257.70	313.29	125.19	278.69	156.01	329.35	243.37
	CO-27	ND	246.41	296.78	111.28	252.37	134.82	297.03	223.11
	Mean		250.94	304.86	119.13	268.85	146.66	314.71	
	CD(P=0.05)			T=44.34		V=24.26		TxV=87.81	

Table 4.12 Bioassay (mm) of different concentration of tannery effluent using wheat coleoptile

Effluent Percentage	Organic fraction					Inorganic fraction				
	0hrs	5hrs	10hrs	24hrs	30hrs	0hrs	5hrs	10hrs	24hrs	30hrs
Control	4.00	4.32	4.44	5.12	5.31	4.00	4.31	4.45	5.10	5.28
Five	4.00	4.30	4.37	5.07	5.14	4.00	4.20	4.23	4.42	4.60
Ten	4.00	4.21	4.28	4.76	4.97	4.00	4.20	4.21	4.30	4.40
Fifteen	4.00	4.01	4.18	4.26	4.43	4.00	4.00	4.00	4.00	4.00
Twenty	4.00	4.20	4.20	4.20	4.20	4.00	4.00	4.00	4.00	4.00

Table 4.44 Effect of chromium speciation, VAM and organic acid amendments on collar diameter (cm) of different tree seedlings

TREATMENTS	*L. luecocephala*	*C. equisetifolia*	*T. grandis*	*A.amara*
T_1	1.65	2.15	1.09	1.29
T_2	1.43	1.86	0.65	1.24

T_3	1.38	1.85	0.64	1.24
T_4	1.45	2.00	0.76	1.25
T_5	1.45	1.97	0.79	1.26
T_6	1.40	1.90	0.68	1.25
T_7	1.50	1.92	0.75	1.23
T_8	1.47	1.90	0.78	1.26
T_9	1.40	1.90	0.69	1.27
T_{10}	1.47	2.00	0.65	1.28
T_{11}	1.45	2.05	0.66	1.27
T_{12}	1.47	1.86	0.63	1.26
T_{13}	1.48	1.98	0.75	1.25
CD(0.05)	0.0723	0.09121	0.09376	0.09258

Table 4.45 Effect of chromium speciation. VAM and organic acid amendments on chromium content (µg g^{-1} DM) of different tree seedlings

Treatments	*L. luecocephala*		*C. equisetifolia*		*T. grandis*		*A.amara*	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
T_1	4.56	5.64	3.44	trace	5.78	5.45	trace	trace
T_2	476.00	46.00	524.00	56.00	532.00	78.00	623.00	64.00
T_3	425.29	47.10	464.17	53.03	475.98	67.69	599.62	57.20
T_4	344.37	35.28	379.71	42.51	384.44	56.43	451.99	47.30
T_5	472.80	49.30	555.20	54.80	559.60	81.90	657.15	69.20
T_6	450.80	49.24	495.02	56.60	507.54	71.96	603.20	62.63
T_7	358.15	36.13	397.90	47.21	399.76	59.69	470.86	49.35
T_8	509.80	51.29	566.30	54.90	571.79	82.54	673.29	70.59
T_9	463.56	51.39	504.95	57.64	516.82	73.46	639.17	63.34
T_{10}	375.03	37.51	412.49	43.07	417.51	57.82	489.11	50.00
T_{11}	514.08	47.68	562.92	60.48	577.56	82.24	671.84	69.12
T_{12}	459.31	50.40	498.30	55.28	514.06	73.03	602.39	61.54
T_{13}	371.92	38.06	409.08	45.92	415.20	60.60	485.15	51.90
CD(0.05)	30.13	5.32	33.18	5.76	32.76	6.41	35.48	6.97

Table 4.46 Effect of chromium (VI) speciation. VAM and organic acid amendments on accumulation factor (ACF) of different tree seedlings

TREATMENTS	*L. luecocephala*		**C. equisetifolia**		*T. grandis*		*A.amara*	
	Roots	Shoots	Roots	Shoots	**Roots**	Shoots	Roots	Shoots
T_3	1.36	0.13	1.50	0.16	1.52	0.22	1.78	0.18
T_4	3.44	0.35	3.80	0.43	3.84	0.56	4.52	0.47
T_6	1.35	0.14	1.59	0.16	1.60	0.23	1.88	0.20
T_7	4.51	0.49	4.95	0.57	5.08	0.72	6.03	0.63
T_9	1.46	0.15	1.62	0.16	1.63	0.24	1.92	0.20
T_{10}	4.64	0.51	5.05	0.58	5.17	0.73	6.39	0.63
T_{12}	1.47	0.14	1.61	0.17	1.65	0.23	1.92	0.20
T_{13}	4.59	0.50	4.98	0.55	5.14	0.73	6.02	0.62
CD(0.05)	0.98	0.08	1.02	0.09	1.12	0.09	1.21	0.96

Table 4.47 Effect of soil added Cr(III) ,Cr(VI), VAM and organic acid amendments on bio magnification ratio (BMR) of different tree seedlings

Treatments	*L. luecocephala*		*C. equisetifolia*		*T. grandis*		*A.amara*	
	Roots BMR	Shoots BMR	Roots BMR	Shoots BMR	Roots BMR	Shoots BMR	Roots BMR	Shoots BMR
T_4	11.31	1.25	12.34	1.41	12.66	1.80	15.95	1.52
T_7	11.99	1.31	13.17	1.51	13.50	1.91	16.04	1.67
T_{10}	12.33	1.37	13.43	1.53	13.75	1.95	17.00	1.68
T_{13}	12.22	1.34	13.25	1.47	13.67	1.94	16.02	1.64
CD(0.05)	0.61	0.16	0.72	0.18	0.79	0.83	0.51	0.82

Table 4.48 Effect of VAM as an ameliorant on field performance of 10 per cent and 15 per cent raw tannery effluent irrigated sorghum var. CO-27

Treatment	Leaf area (cm^2 $plant^{-1}$)	Green Fodder Yield (Tons Ha^{-1})	Shoot Cr (µg g^{-1}DW)	N (%)	P (%)	K (%)
T_1	2835	30.43	Trace	1.21	0.081	0.79
T_2	2640	26.57	4.56	1.19	0.075	0.76
T_3	2743	28.54	5.78	1.23	0.082	0.78
T_4	2525	25.78	6.35	1.2	0.072	0.75
T_5	2611	27.43	7.21	1.23	0.081	0.78
CD (0.05)	89.74	1.76	1.02	0.028	0.006	0.006

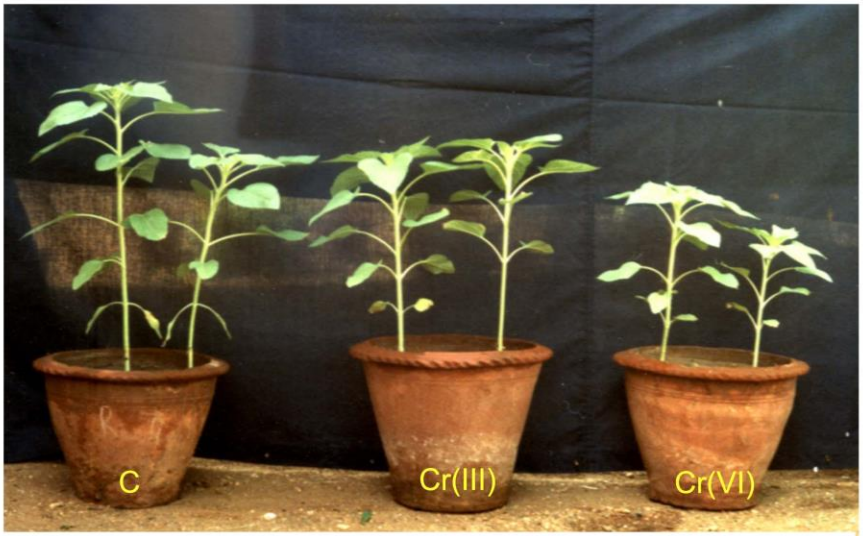


Plate 1. Effect of Cr speciation on shoot growth of sunflower var. CO 4

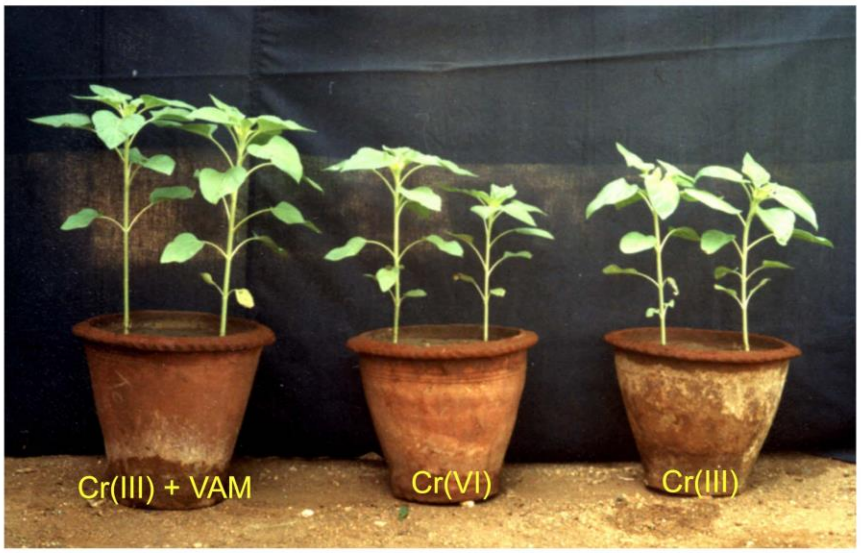


Plate 2. Effect of VAM on shoot growth of Cr(III) treated sunflower var. CO 4

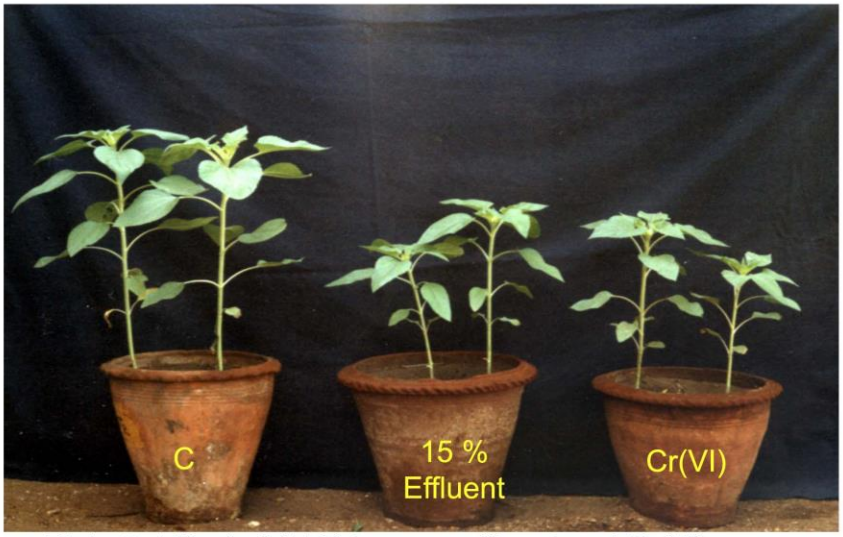
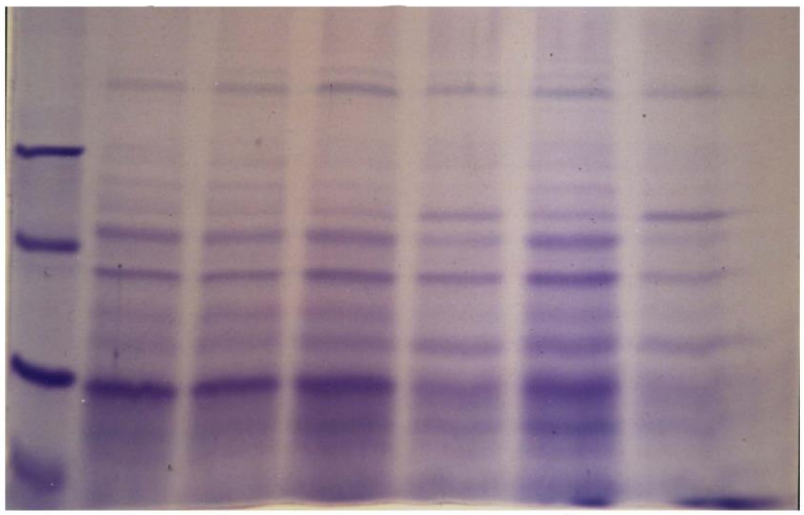


Plate 3. Effect of 15 % tannery effluent and Cr(VI) on shoot growth of sunflower var. CO 4

Plate 4. Ameliorative effect of VAM on Cr(III) and 10% tannery effluent treated sorghum var. CO 27

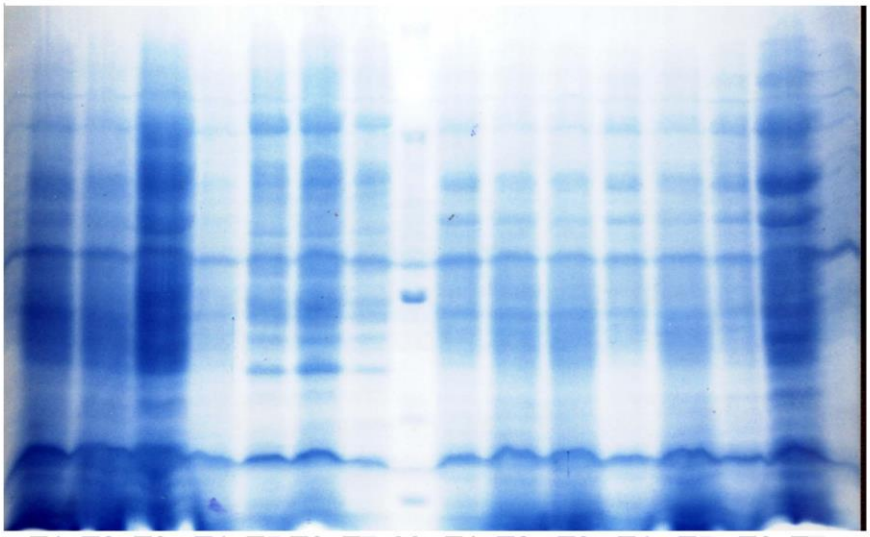
Plate 5. Leaf scorching in sunflower due to Cr(VI)

Plate 6. Leaf scorching in teak due to Cr(VI)



1- Marker
2- sunflower var. Modern
3- sunflower var. CO 2
4- sunflower var. CO 4
5- sorghum var. K 10
6- sorghum var. CO 27
7. sorghum var. CO 26

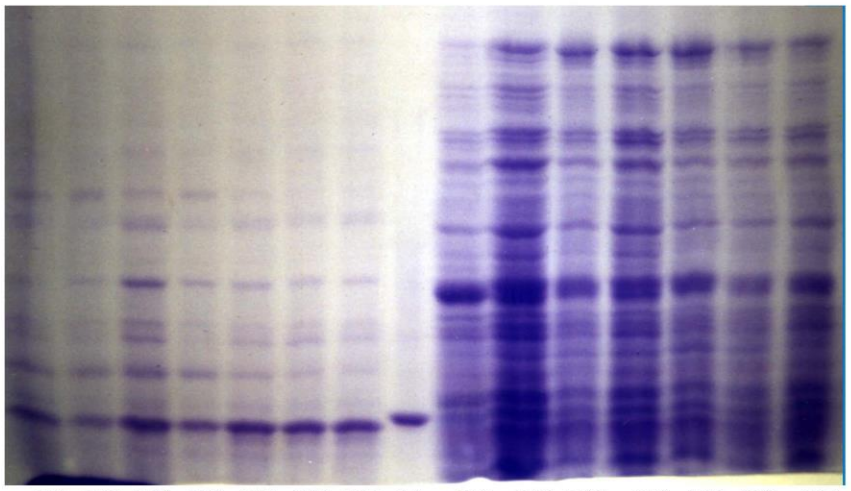
Plate 7. Leaf protein profile (SDS-PAGE) of Cr(III) 100 micro M treated sunflower and sorghum varieties



T1 T2 T3 T4 T5 T6 T7 M T1 T2 T3 T4 T5 T6 T7

Green gram var. CO 5 Rice var. CO 43

Plate 8. Leaf protein profile (SDS-PAGE) of Cr treated green gram and rice



T1 T2 T3 T4 T5 T6 T7 M T1 T2 T3 T4 T5 T6 T7

Root Leaf

Plate 9. Root and leaf protein profile (SDS-PAGE) of Cr Treated sunflower var. CO 4

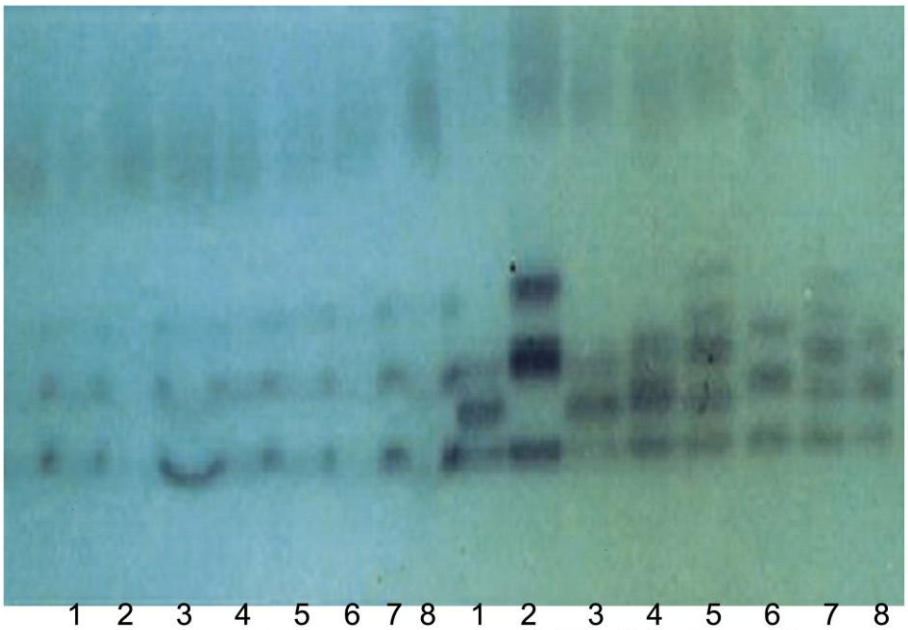
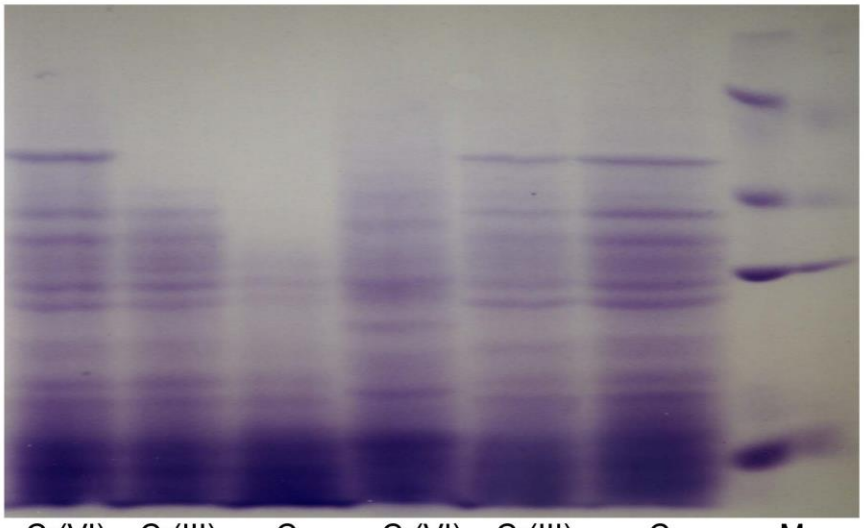


Plate 10. Leaf GR isozyme pattern of sunflower, green gram, sorghum and rice

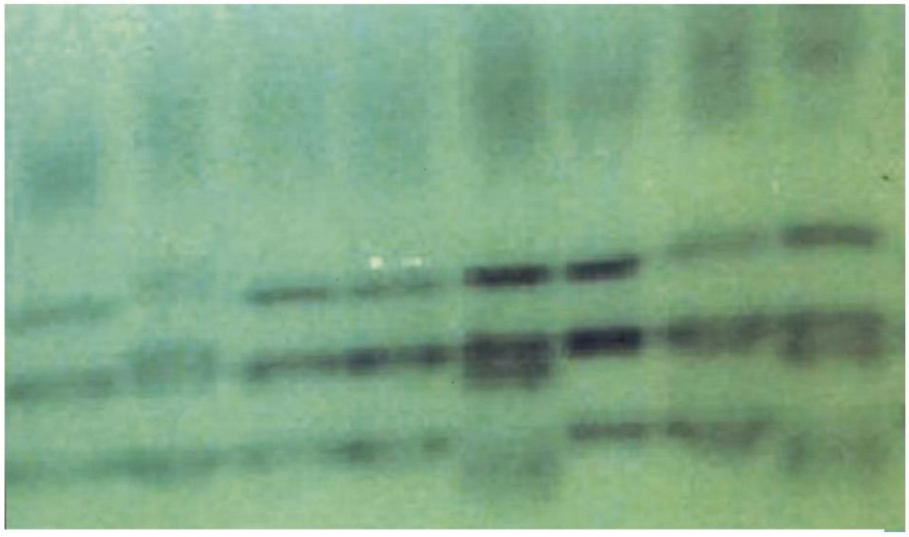
1 - Sunflower var. Modern
2 - Sunflower var. CO 4
3 - Green gram var. CO 4
4 - Green gram var. CO 5
5 - Sorghum var. CO 27
6 - Sorghum var. K 10
7 - Rice var. CO 43
8 - Rice var. TRY 1



Cr(VI) Cr(III) C Cr(VI) Cr(III) C M

Sunflower var. CO 4 Sorghum var. CO 27

Plate 11. Leaf protein profile (SDS-PAGE) of Cr(III) and Cr(VI) treated sorghum and sunflower



C C T4 T5 T3 T4 T5 T3

Sorghum Sunflower

Plate 12. Leaf GR isozyme pattern of Cr(VI) and effluent treated sorghum and sunflower

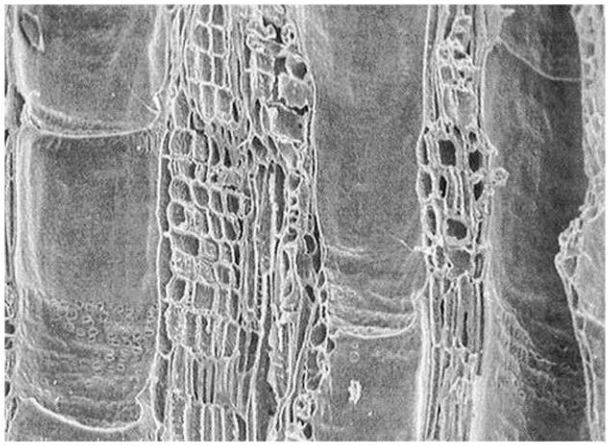


Plate 13. Vasular system of Cr(VI) treated sorghum

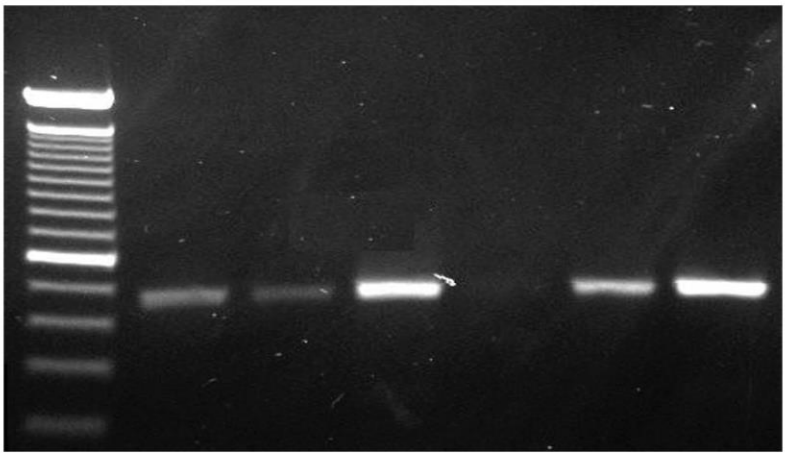


Plate 14. MT3 gene expression in sorghum cultivars

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