

**Effect of chromium on phytotoxicity in okra
(*Abelmoschus esculentus* L. Moench)**

A

***Thesis submitted to the Orissa University of Agriculture and
Technology in partial fulfilment of the
requirements for the degree of
Master of Science in Agriculture
(Plant Physiology)***

By

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This is to certify that the thesis entitled “**Effect of chromium on phytotoxicity in Okra (*Abelmoschus esculentus* L. Moench)**” submitted in partial fulfilment of the requirements for the award of degree of **Master of science in agriculture** (Plant Physiology) to the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of *bonafide* and original research work carried out by **Anwesa Pradhan, Adm. No. 05PP/16** under my guidance and supervision. No part of thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled “**Effect of chromium on phytotoxicity in Okra (*Abelmoschus esculentus* L. Moench)**” submitted by **Anwesa Pradhan, Adm. No. 05PP/16** in partial fulfilment of the requirements for the degree of **Master of science in agriculture (Plant Physiology)** to the Orissa University of Agriculture and Technology, Bhubaneswar has been approved by the Student’s Advisory Committee and the External Examiner.

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ABBREVIATIONS USED

%	: Percentage
@	: At the rate
⁰ C	: Degree Celsius
BSH	: Bright sunshine hour
CD	: Critical difference
Cm	: Centimetre
Cv	: Coefficient of variation
DOS	: Date of sowing
DOT	: Date of treatment
et al.	: Co-workers
CRD	: Completely randomized design
Fig	: Figure
FW	: Fresh weight
G	: Gram
Max	: Maximum
Mg	: miligram
Min	: Minimum
RH	: Relative humidity
S.E.m	: Standard error of mean
ng	: nanogram

ABSTRACT

A pot experiment entitled “Effect of chromium on phytotoxicity in okra (*Abelmoschus esculentus* L. Moench) was conducted collaborately by AICRP on micronutrient, Department of Soil Science and Agril. Chemistry and Department of Plant Physiology, College of Agriculture, OUAT, Bhubaneswar during *Rabi* 2018. The experiment was laid out in completely randomized design (CRD) and were replicated thrice. Okra cultivar ‘Kajari’ was sown in pots and treated with different concentrations of chromium (K_2CrO_4), available form of Cr (VI), treated as chemical formulation of Cr (0, 2.5, 5, 10, 15, 25, 50, 75, 100, 200, 300 mg/kg soil) as basal application. Germination test of okra seeds at different concentration of chromium (Cr) was conducted. At 300ppm of Cr the germination was completely failed and no crop established in pots also. Observations of Cr at a range of 2.5 to 200ppm on plant height, root length, total leaf number per plant, leaf area per plant were recorded from 25 DAS in 15 days interval till harvest. Other observations on Chlorophyll index by SPAD, proline content, protein content, catalase activity, SOD activity, total biomass, Cr accumulation in plant parts and concentration of other micronutrients (Zn, Fe, Mn, Cu) during metal stress and yield attributes were determined during the course of investigation and were statistically analyzed. The data revealed that the growth parameters like plant height, root length, total leaf number, leaf area per plant, total biomass decreased significantly with the increase in the Cr concentration. There was significant decrease in biochemical traits like chlorophyll index, protein content and significant increase in proline content, catalase activity and SOD activity in treated plants as compared to untreated plants with increase in the metal concentration. As the uptake was not hindered at root region, thereby, the translocation and accumulation of Cr in different plant parts increased with the increase in metal concentration at root zone. In order of passive movement of Cr, as Cr is not an essential element its accumulation was in order of roots > leaves > fruits > seeds. The amount of Cr uptake by different parts of plant caused improper nutrient mobilization as evident from the study. Consequently, this study will help to understand the phytoremediation technology and molecular mechanism in okra crop growth and productivity under heavy metal stress.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Vegetables play an important role in Indian agriculture by providing food, nutritional and economic security and more importantly producing higher return per unit area and time. Most of the vegetables, being short duration crops, fit very well in the intensive cropping system and are capable of giving very high yield and very high economic returns to the grower.

Okra (*Abelmoschus esculentus* L. Moench) is one of the most popular, tasty and gelatinous vegetables and it plays a major role in human diet. It contains protein, carbohydrates and vit C. It also has anti-oxidant and anti-diabetic properties. Several workers have reported that okra has found medical application as a plasma replacement or blood volume expander (Kumar *et al.*, 2010). Raw okra is 90% water, 2% protein, 7% carbohydrates and negligible in fat. In a 100 gram amount, raw okra is rich (20% or more of the Daily Value, DV) in dietary fiber, vitamin C and vitamin K, with moderate contents of thiamin, folate and magnesium.

It is mainly grown in India, Nigeria, Sudan, Pakistan, Ghana, Egypt, Benin, Saudi Arabia, Mexico and Cameroon. Largest area and production is in India having an area of 498.0 thousand ha and the production of 5784.0 thousand tons, followed by Nigeria. The crop is cultivated widely in Uttar Pradesh, Bihar, West Bengal, Odisha, Assam, Andhra Pradesh, Karnataka and Tamil Nadu and consumed in all the states. Andhra Pradesh is the leading okra producing state which has production of around 1184.2 thousand tons followed by West Bengal (862.1 thousand tons), then comes Bihar (788.3 thousand tons).

Knowledge about responses to abiotic stresses may play significant role in cultivation and production of okra. Heavy metal toxicity is one of the major abiotic stress which leads to hazardous effects in plants. Because of their high reactivity, they can directly influence growth, senescence and energy synthesis processes. Heavy metals could interact with many metabolic functions of plants. For instance; inhibition of growth processes or decrease in the activity of the photosynthetic apparatus often correlated with progression of senescence processes etc. (Duquesnay *et al.* , 2010) as well as shortened and thickened or poorly developed roots(Khudsar *et al.* , 2004). Growth inhibition and senescence stimulation, caused by heavy metals in excess are

intriguing effects, more so, as the knowledge of their mechanisms can have a great significance in ecophysiology and medicine (Amat *et al.* , 2010).

Heavy metals have recently received the attention of researchers all over the world, mainly due to their harmful effects on plant. Heavy metals are defined as metals with a density higher than 5g/cm^3 . 53 of the 90 naturally occurring elements are heavy metals (Weast, 1984), but not all of them are of biological importance. Chromium (Cr) is the 21st most abundant element in the earth's crust (Krauskopf, 1979). It occurs in nature in bound forms that constitute 0.1–0.3 mg/kg of the earth's crust. Cr has several oxidation states ranging from Cr (-II) to Cr (+VI). Cr exists predominantly in the +III and +VI oxidation states. The most stable oxidation state of Cr is +III, and under most prevailing environmental conditions Cr (VI) is rapidly reduced to Cr (III). The intermediate states of +IV and +V are metastable and rarely encountered (Zayed and Terry, 2003).

The stable forms of Cr are trivalent Cr (III) and hexavalent Cr (VI) species, although there are various other valence states which are unstable and transient in biological systems. Cr (VI) is conceived as the most toxic form of Cr, highly soluble in water, which usually occurs amalgamated with oxygen as chromate (CrO_4^{2-})or dichromate ($\text{Cr}_2\text{O}_7^{2-}$) oxyanion. Both Cr (III) and Cr (VI) differ in terms of mobility, bioavailability, and toxicity. Cr (III), on the other hand, is less toxic, less mobile, and is mainly found coalesced to organic matter in soil and aquatic environment (Becquer *et al.*, 2003). Since trivalent and hexavalent Cr may interconvert in the soil and soil immobilizes both trivalent and hexavalent chromium, it is difficult to valuate separately the consequence of the two types of Cr on plants. Consequently, it might be appropriate to use the term Cr toxicity in plants (Arun *et al.*, 2005) rather than toxicity of trivalent or hexavalent Cr.

Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma *et al.*, 1995) .Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations, and nutritional imbalance. The amount and fate of Cr taken up by plants varies among species due to differences in absorption, transportation, tolerance mechanism and storage of metals. Although, most plants studied store the majority of Cr in their roots, translocation to

all other parts of a plant does occur. Many studies have found low plant ability to transport Cr from root to other plant parts. The occurrence of Cr in the soils is generally dependent on its content in bedrock. The greatest amounts of chromium occur in soils formed from ultra-alkaline rock as well as some metamorphic rock.

Even though vegetables are an important component of our daily diet, there is little information available as to its contamination by heavy metals. A common example of contamination includes bioaccumulation of heavy metals in vegetables. Odisha, being a state surrounded by many mining areas, the chances of stress due to heavy metals are much high. Okra being a hardy crop and suitable for various climatic conditions and soil types it is used in the present experiment.

Thus, the aim of this work was to investigate the “**Effect of chromium on phytotoxicity in Okra (*Abelmoschus esculentus* L. Moench)**”, the effect of different concentrations of Cr on the growth, biochemical constituents and enzyme antioxidant system in a pot culture of okra as an approach to understand the plant’s response to heavy metals and the effects of pH on solubility and its interaction with other transition metals (Fe and Cu) available in soil with following objectives.

Objectives:

- Screening and identify the toxic level of chromium.
- Morphological and physiological changes in okra grown in different concentration of chromium.
- Analysis of Cr and its interaction with other micronutrients (like Fe and Cu).

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Naturally plants are exposed to many biotic and abiotic stresses. Among all heavy metal stress is one of the great importance which has many notable adverse effects on crop productivity and growth. Their contamination in soil is due to industrialization, urbanization and agricultural activities and cause serious problem for environment.

Chromium is known to decrease the crop yield significantly. Although a lot of work has been done on physiological and biochemical aspects of heavy metals toxicity, very little is known with regard to their effect on reproductive functions, which is central to crop (fruit, seed) production. This review is a compilation of literature in relation to the effect of heavy metals mainly chromium on the following aspects relevant to the topic of present investigation.

2.1 Seed germination and seedling growth

Seed germination constitutes an important phase in the life cycle of angiosperms. Proper seed germination is a pre-requisite for establishment of healthy seedling. Seed germination is a complex process and mobilization of reserve material is one of the early and important events because hydrolysis of complex biomolecules into simple molecules provides substrate for respiratory activity as well as for the synthesis of new cellular material. Germination of seed is known to be quite sensitive to the HMs (heavy metal).

Parr and Taylor (1982) conducted an experiment and resulted that high levels (500ppm) of hexavalent chromium in soil reduced germination up to 48% in bush bean(*Phaseolus vulgaris*).

Khan and Khan (1983) reported that low concentration of lead and cadmium promoted seed germination as well as the initial seedling growth of tomato and egg plants, but its higher concentration were inhibitory.

Inhibition of seed germination in rice (Bai, 1988) by high (100 nM) as well by low concentration (25 (xM) of cadmium has also been reported.

Patra and Patro (1994) reported that the effluent water which contained both trivalent and hexavalent chromium to a considerable amount (182.5 mg/l and 156 mg/l), resulted in a significant reduction in seed germination, seedling growth and biochemical lesion.

Chromium interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced seed germination or early seedling development (Sharma *et al.*, 1995).

Mahadeswara Swamy (1996) reported that the application of 1500 ppm Cr in *Vigna mungo* caused maximum retardation of growth i.e. upto 90 per cent both in root and shoot supplementation of chromium (above 0.2 mM) to the germination medium completely inhibited it in pea (Kajal *et al.*, 1996).

Rout *et al.*, (1997) suggested that sensitive mung bean cultivars also showed decreased root growth when exposed to Cr (VI).

Inhibitory effects of heavy metal (HM) ions like cadmium, chromium etc. on seed germination have been reported by a number of investigators (Divya, 1999) and the effect has been reported to be concentration dependent.

Jain *et al.*, (2000) found that the reduction of 37-52% of sugarcane bud germination were observed in 20 and 80ppm of Cr respectively.

Seed germination of the weed *Echinochloa colona* was reduced to 25% with 200µM Cr.(Rout *et al.*, 2000).

Peralta *et al.*, (2001) found that 40ppm of Cr(VI) reduced the ability of the seeds of Lucerne (*Medicago sativa* cv. Malone) by 23%.

The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and the subsequent translocation of sugars to the embryonic axis.(Zeid,2001).

Samantary *et al.*, (2002) observed that the effect of Cr contamination on the germination medium was also tested in mungbean (*Vigna radiata* L.) tolerant/sensitive cultivars and results showed that in sensitive plants, germination

rate decreased in plants exposed to 96 or 192 μ M Cr(VI), while in tolerant plants germination was not affected.

Labra *et al.*, (2006) observed that the maize seeds exposed to Cr(VI) also presented decreased rates of germination when exposed to concentrations of 100–300mgL⁻¹ of Cr (VI).

L'opez-Lunar *et al.*, (2009) found that oat seed germination was severely diminished (84%, resp. to the control) in tannery sludge soil with 4000mg Crkg⁻¹, while in tannery sludge soil containing 8000mg Crkg⁻¹ both oat and sorghum seed germination was suppressed. When comparing the sensitivity of sorghum, wheat, and oat germination to Cr they also found that germination of sorghum and wheat were markedly affected at 500–1000mg Cr (III)kg⁻¹ soil respectively, while oat germination was not affected in levels of Cr(III) below 4000mgkg⁻¹ soil. With respect to Cr (VI) it affected wheat and sorghum germination at the maximum concentration of 500mgkg⁻¹ soil.

Smiri *et al.*, (2009) suggested that decrease in germination is a common response upon exposure to heavy metals, such as Cd, Pb, and Hg.

Akinci *et al.*, (2010) concluded that excess chromium was limited to germination rate, germination index, mean germination time and germination uniformity index values in germination level. Radicle length, radicle fresh and dry weight, hypocotyl length, hypocotyl fresh and dry weight, growth tolerance index and seedling relative growth rate was negatively affected by the increased chromium concentrations at the seedling stage. Response of seedlings to chromium was more than that of seed germination. This event is based on the impermeability of seed coats and selectivity of embryos against chromium.

Kumar *et al.*, (2010) studied the effect of sponge iron factory effluent on fenugreek (*Trigonella foenum graecum* L.) i.e. germination, vegetative, flowering, fruiting and maturity attributes were affected.

Sundaramoorthy *et al.*, (2010) observed that Cr (VI) in concentrations up to 200mgL⁻¹ decreased growth of paddy (*Oriza sativa* L.).

Chopra *et al.*, (2011) conducted a comparative study on agro-potentiality of paper mill effluent and synthetic nutrient (DAP) on *Vigna unguiculata* L. (Walp) cowpea. They reported the higher contents of Cr (0.40 mg L⁻¹) in the absolute paper mill effluent. The paper mill effluent irrigation significantly increased the contents of Cr in the soil (0.90 mg L⁻¹) and *V. unguiculata* (0.78 mg L⁻¹). Moreover, the contents of Cr inhibited the seed mechanisms by which the gender may influence these germination, shoot height, root length, biomass and variables. *S. vulgaris* presented high diversity at chlorophyll content and yield attributes like number of genotypic level; the treatment with hexavalent Cr pods and crop yield of *V. unguiculata* at 100% increased the differences among genotypes so the use of concentration of paper mill effluent.

Dheeba and Kumar (2012) found the toxicological effects of hexavalent Cr (Cr) on germination, early seedling growth and chlorophyll content in maize (*Zea mays*), pearl millet (*Sorghum bicolor*), green gram (*Vigna radiata*) and ground nut (*Arachis hypogaea*).

Singh *et al.*, (2017) stated that the symptoms of Cr toxicity in plants are diverse and include decrease of seed germination, reduction of growth, decrease of yield, inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalance in green gram (*Phaseolus aureus* L.) and chickpea (*Cicer arietinum* L.).

2.2 Growth

The adverse effect of chromium on plant growth, first noted by Koenig (1910).

Hunter & Vergnano (1953) observed that growth of oat plants decreased with increased chromium concentration added to nutrient solution as potassium dichromate.

Foy *et al.*, (1992) observed the reduction in plant growth and that may be due to the formation of complex between metal and protein which inhibits protein synthesis and ultimately restricts the plant growth.

Maksimoviae *et al.*, (2000) reported that cadmium and nickel modified plant growth and development through their effect on root and above ground biomass, total leaf area and root length in maize .

Chromium also induces phytotoxic symptoms in plants like morphological changes, proline accumulation and alterations in antioxidant metabolism (Panda *et al.*, 2003).

There was no significant growth retardation of plants under 50 mM Cr (III) stress, while 100 mM Cr(VI) was most toxic of all the treatments in terms of root and leaf growth in Sorghum bicolor (Shankar and Pathmanabhan, 2004).

Cr stress has adverse effects on physio-biochemistry, absorption and accumulation of crops mainly rice, wheat and vegetables (He *et al.*, 2005).

The content of Cr in tea is on the rise (Shi *et al.*, 2006), which has severely inhibited the growth of tea.

Singh *et al.*, (2006) concluded that increasing doses of chromium caused reduction in growth and chlorophyll, sugar and protein in paddy (*Oryza sativa* L.) leaves.

Gyawali and Lekhak (2006), noted that an increase in Cr (VI) concentration 10-800 mg L⁻¹ in culture medium negatively limited the seedling growth of rice cultivars. These results were confirmed by Jun *et al.*, (2009).

The plants exposed to Cr stress exhibited decrease of growth and yield, as found in cauliflower (Chatterjee and Chatterjee 2000), *Amaranthus viridis* (Liu *et al.*, 2008).

Higher concentrations of heavy metals in surface soil have been reported to inhibit nutrient uptake, physiological and metabolic processes (Ahsan *et al.*, 2007) or reduce plant growth (Boussama *et al.*, 1999) and also cause chlorosis due to interference with photosynthesis and respiration processes (Ekmekci *et al.*, 2008), damage to cell membrane (Cunha *et al.*, 2008) and root tips (Sheldon and Menzies , 2005), as well as reduced water and nutrient uptake including damage to enzymes (Balestrasse *et al.*, 2001).

Early growth stages of seedling are very important indicators in determining toxicity impacts of heavy metals like chromium in plants (Pandey and Pandey, 2008).

Interaction of toxic metals can affect growth, biomass and photosynthesis of plants, such as Al and Cd in Cd and Cr in *Dalbergia sissoo* (Shah *et al.*, 2008).

Heavy metals decrease growth rate of plants by affecting various parts of root metabolism such as water and mineral uptake membrane function inhibition of enzyme activities oxidation and cross-linking of proteins inhibition of cell division induction of DNA damage and cell death but mainly and primarily disturb cellular redox environment causing oxidative stress both in roots and leaves and subsequently induces some of these above mentioned toxic symptoms (Tamas *et al.*, 2008).

Chromium stress also induced a significant decline in growth parameters of both barley genotypes. Similarly, reduction in growth of plants under Cr stress was observed in wheat, oat and sorghum (Lopez-Luna *et al.*, 2009).

Ghani (2010) suggested that increase in concentrations of chromium caused reduction in growth, chlorophyll contents and mineral nutrients of brassica. At increasing concentrations of chromium, all the attributes were found to be reduced.

An experiment was conducted by Sundaramoorthy *et al.*, (2010), with paddy irrigated with different concentration of chromium (control, 2.5, 5, 10, 25, 50, 75, 100, 200mg/ltr). The growth of shoot, root, total leaf area, fresh weight, dry weight and yield of paddy decreased with increase in Cr concentration. The uptake of macronutrient (N,P,K) and the micronutrients were gradually decreased. The Cr accumulation increased with the increase concentration of Cr.

Águeda *et al.*, (2012) evaluated the potential of Barley (*Hordium vulgare* L.) for the phytoremediation of soils contaminated by metals resulting in retardation of plant growth and interference in chlorophyll content.

P. Sundaramoorthy *et al.*, (2018) revealed that, the increase in chromium reduce the growth and yield in black gram plants.

2.3 Plant height

When Cr was added at 2, 10 and 25 ppm to nutrient solutions in sand cultures in oats. 11, 22 and 41% reduction in plant height, respectively, over control was observed (Anderson *et al.*, 1972).

Adverse effects of Cr on plant height and shoot growth (Rout *et al.*, 1980).

Sharma and Sharma (1993) reported that after 32 and 96 days, there was reduction in plant height in wheat cv. UP2003 in a glass house trial when sown in sand with 0.5 mM sodium dichromate.

Corradi *et al.*, (1993) recorded suppression of lateral shoots with a diminishing trend with the increase in the dose of chromium.

Garg *et al.*, (1994) observed the production of stunted, brown-coloured roots by chromium treated excised leaves in contrast to control in which thin, elongated green- coloured roots were formed.

Reduction in plant height due to Cr(VI) on *Curcumas sativus*, *Lactuca sativa* and *Panicum miliaceum* was reported by Joseph *et al.*, (1995).

Rout *et al.*, (1997) found the toxicity symptoms in corn and mung bean due to the application of chromium in excess by which the plants were severely stunted.

Samantaray *et al.*, (1996a) also reported severe chlorosis and necrosis of leaf in *E. colona* at high concentrations of Cr in solution culture.

Bera *et al.*, (1999) studied the effect of Cr present in tannery effluent on chloroplast pigment content in mung bean 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.

Excess amount of cobalt, chromium and copper had an adverse effect on biomass, concentration of iron, chlorophyll “a” and “b”, protein and catalase activity in cauliflower (Chatterjee and Chatterjee, 2000).

Reductions in contents of chlorophyll (Chl) and δ -aminolevulinic acid and nitrate reductase activity in *Nymphaea alba* were reported by Vajpayee *et al.*, (2000).

The total chlorophyll and protein content of *B. monnieri* decreased with increase in Cd concentration. (Singh *et al.*, 2006).

Gautam *et al.*, (2014) suggested that application of Cr contaminated irrigation water decreased plant height, leaf area/plant, number of fruits/ plant, fresh fruit weight/plant, fruit yield/plant, dry weight/plant, above plant biomass/plant in tomato and eggplant. The decrease summarized for various parameters across treatments ranged from 62% to 84% and 56% to 77% in tomato and eggplant, respectively.

Mamta Gautam *et al.*, (2014) found that the inhibitory effects on photosynthetic pigments gradually increased with increase in concentration. 7.5 Cr ppm concentration resulted in the lowest pigment level (mg/g FW) and maximum inhibition rate compared to control in tomato and eggplant in an experiment.

2.4 Leaf growth

Vazques *et al.*, (1987) stated that the variation in size of areoles is another feature which was markedly affected by different concentrations of chromium in bush bean plant (*Phaseolus vulgaris* L.).. The size of the areoles decreased in plants treated with chromium and this may be due to the decrease in the leaf area.

Leaf numbers/plant was reduced by 50% in wheat when 0.5 mM Cr was added in nutrient solution (Sharma and Sharma, 1993).

Karunyal *et al.*, (1994) studied the effect of tannery effluent on leaf area and biomass and reported that all the concentrations tested decreased leaf area and leaf dry weight in *Oryza sativa*, *Acacia holosericea* and *Leucaena leucocephala*.

The number of leaves in Cr (VI) treated plants were reduced at higher concentration, mainly due to poor and stunted growth of plants and due to senescence of leaves (Mehta *et al.*, 1996).

In a study with several heavy metals, Pedreno *et al.*, (1997) found that Cr had a pronounced effect on leaf growth and preferentially affected young leaves in tomato plants.

Tripathi *et al.*, (1999) found that leaf area and biomass of *Albizia lebbek* seedlings was severely affected by a high concentration (200 ppm) of Cr⁶⁺.

Singh (2001) reported that Cr applied at 60 mg/kg and higher levels reduced the leaf size, caused burning of leaf tips or margins and slowed leaf growth rate in spinach.

Purohoit *et al.*, (2003) stated that there was a decrease in total leaf area under chromium irrigation in tomato and brinjal. This may be due to reduction in cell size.

2.5 Root growth

Under high concentrations of both the Cr species combination, the reduction in root growth 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 (Barcelo *et al.*, 1986).

A long term exposure of chromium caused reduction in growth and decreased the root/shoot ratio (Breckle 1989 and Clark 1982).

Bishnoi *et al.*, (1993a,b) reported that hexavalent chromium was much more toxic and suppressed growth of radicle and plumule of *Pisum sativum* significantly.

Garg *et al.*, (1994) observed the production of stunted, brown-coloured roots by chromium treated excised leaves in contrast to control in which thin, elongated green-coloured roots were formed.

Root length and dry weight of the important arid tree *Ceasalpinia pulcherrima* was inhibited by 100 ppm Cr (Iqbal *et al.*, 2000).

Panda and Patra (2000) found that 1 mM of Cr increased root length in seedling growing under nitrogen (N) nutrition levels while higher Cr concentration decreased root length in all the N treatments.

Chromium significantly inhibited the root growth both in terms of length of primary root and in number of lateral roots in plants of *Brassica oleracea.*, *Lycopersicon esculentum* and *Zea mays* . Furthermore, the overall growth of maize and tomato seedlings was strongly reduced in the presence of Cr(VI), particularly at a concentration of 10 mg/l (Sanitadi Toppi *et al.*, 2000).

Grant *et al.*, (2001) suggested that the root growth was a more sensitive indicator of metal toxicity than shoot growth.

Prasad *et al.*, (2001) reported that the order of metal toxicity to new root primordia in *Salix viminalis* is $Cd < Ni < Cr < Pb$, whereas root length was more affected by Cr than by other heavy metals studied.

Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Tang *et al.*, 2001).

Shankar *et al.*, (2004) in sorghum reported that heavy metal Cr (III) did not affect shoot growth, though root growth was affected significantly. However, with Cr (IV) both root and shoot growth were significantly affected.

Rodriguez *et al.*, (2011) found that chromium exposure affected plant morphology mostly starting from 1,000 mg/L and at that situation roots became progressively thin, brittle and brownish. It was also found that roots exposure to 2,000 mg/L resulted in cell cycle arrest at the G2/M checkpoint.

Varun *et al.*, (2011) suggested that Cr gathered in roots, unlike most organic contaminants, was non-biodegradable and accumulated in living tissues, so that root growth inhibition was the initial effect of the heavy metal's presence in plants.

Duan *et al.*, (2013) investigated that the *Vicia faba* seeds maintained a good growing state when the CrO_3 concentration was below 100 $\mu\text{mol/L}$, while the root growth was suppressed when they were kept in 250 $\mu\text{mol/L}$ of CrO_3 for 72 h or cultured with CrO_3 concentrations above 500 $\mu\text{mol/L}$.

Singh (2015) investigated the genotoxicity and cytotoxicity of chromium in *Allium cepa* L. She observed significant decrease in root length as concentration increased. Also the mitotic index decreased as concentration increased.

2.6 Total Soluble Proteins

Cellular protein patterns change in response to environmental influences like temperature stress (heat shock proteins), water supply (desiccation proteins), pathogen attack (pathogenesis related PR proteins) and heavy metal stress (heavy metal stress induced proteins). Developmentally and environmentally induced changes in the cellular proteins is manifested at qualitative as well as quantitative levels, for example, increase or decrease in enzyme activity due to change in the amount of that particular protein.

At the onset of germination of seeds, proteins are hydrolyzed/ and the products are transported to the growing parts of the plant for synthesis of new proteins (Mikkonen 1986).

Anita (1992) reported that Cr (VI) exerted a deleterious effect on total proteins of embryonic axis and cotyledons in germinating pea seeds.

Mohapatra (1995) reported/that chromium caused a positive phytotoxic effect on the protein content of green gram seeds. The hydrolysis of proteins decreased with higher concentration of chromium because of reduced growth and differentiation.

Seed proteins whether enzymatic or storage reserves are synthesized during seed development and maturation and are deposited within membrane bound protein bodies (Duarte *et al.*, 1996).

No information is available on the effect of chromium toxicity on protein concentration in plant leaves. Chaoui *et al.*, (1997) observed that concentration of protein was not affected by any of metal in upper parts, neither in stem nor in leaves, while it significantly declined in roots of *Phaseolus vulgaris* when subjected to Cd²⁺ and Zn²⁺ treatments.

Shintinawy and El-Ansary (2000) also observed reduction in total protein content in the roots of soybean treated with Cd²⁺ and Ni²⁺.

Cells under oxidative stress display various disfunctions due to lesions caused by ROS to lipids, proteins and DNA (Ercal *et al.*, 2001).

A maximum decrease of protein was observed at 100 mM concentration in *Ocimum tenuiflorum* (Rai *et al.*, 2004).

The protein content was decreased in *Spinacea oleracea* when treated with chromium (Sinha *et al.*, 2005). He found that the protein content of both roots and leaves decreased with increase in Cr concentration (0, 10, 40, 80 and 160 mM). The protein contents of the chromium treated plants were found to be affected by chromium levels in nutrient medium.

In Cr treated plants, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for leaves chlorophyll and protein contents

were 40 and 80 mM Cr, respectively after 48 h exposure while NOEC and LOEC for root protein content were 10 and 40 mM, respectively after 48 h (Sinha *et al.*, 2005).

Cr, Fe, Cu and Co undergo redox cycling reactions, for a second group of metals, Mg, Cd and Ni, the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins (Valko *et al.*, 2005).

The protein content showed induction at lower concentration of Pb and declined with increase in concentration (Mishra *et al.*, 2006).

2.7 Proline

Bisht *et al.*, (1989) reported in green gram that increase in chromium is correlated with more proline accumulation as compared to the control. The accumulation of proline under excess chromium may be due to the induction of water deficiencies in leaves.

Free amino acid content gradually increased in chromium treated seedlings of *Phaseolus mungo*. However, there was no significant change in free amino acid content in cotyledons (Swamy and Theresa, 1992).

Mohapatra (1995) reported that low concentrations of chromium (0.1 mM) stimulate proline accumulation, but further increase in treatment level inhibited proline biosynthesis.

Accumulation of some amino acids especially proline content in plants is suggested as a biochemical marker for heavy metal stress. Costa and Spitz (1997) reported an increase in total free amino acids and hydroxyproline levels with increasing cadmium treatment in *Lupinus albus*.

Rout *et al.* (1998), observed the higher accumulation of proline in chromium-treated plants of *O. tenuiflorum* has been observed which might be attributed to the strategies adapted by plants to cope up with chromium toxicity as proline has multiple functions, such as, osmoticum, scavenger of free radicals, protector role of cytoplasmic enzymes, source of nitrogen and carbon for post stress growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential.

Both in roots and shoots of cadmium treated wheat seedlings, the higher concentrations caused the highest accumulation in total amino acid content. Among the free amino acids, proline serine, glycine, alanine, valine, lysine, histidine, arginine, glutamic acid, isoleucine, leucine and threonine concentrations showed significant rise after treatment (Lesko *et al.*, 2000).

Chromium also induces phytotoxic symptoms in plants like morphological changes, proline accumulation and alterations in antioxidant metabolism (Panda *et al.*, 2003).

Rai *et al.*,(2004) found that the chromium treated *O. tenuiflorum* leaves showed increased proline content. Hyperactivity of the superoxide dismutase (SOD), guaiacol peroxidase (GPX) and catalase indicated that antioxidant enzymes played an important role in protecting the plant from chromium toxicity. The study concludes that plant could grow under chromium stress and protect themselves from phytotoxicity of Cr by altering various metabolic processes.

2.8 Antioxidant enzymes

The term antioxidant be considered to describe any compound capable of quenching active oxygen species (AOS) without itself undergoing conversion to a destructive radical (Nishilnimi-Yagi, 1996). Antioxidant enzymes are those that either catalyze such reactions or are involved in the direct processing. Their function is to interrupt the cascades of uncontrolled oxidation. They play multiple roles in defence reactions, and are major assimilate sinks, present in many tissues at millimolar concentrations (Noctor *et al.*, 1997).

Samantary *et al.*, (2002) stated that the activity of antioxidant enzymes, namely, peroxidase, catalase (CAT), glucose-6-phosphate dehydrogenase and superoxide dismutase (SOD) increased in case of Cr-sensitive of mung bean exposed to different Cr concentrations. However, the level of antioxidant enzymes decreased in Cr-tolerant cultivars.

The main defence mechanism against the over accumulation of ROS is an extensive network of enzymes and low molecular weight antioxidants inside the cell (Mittler, 2002). The level of antioxidants was found to increase in plants to combat metal stress (Sinha and Gupta, 2005).

The defense mechanism consisted of various antioxidant enzymes such as peroxidase (POX) and ascorbate peroxidase (APX); and non-enzymatic antioxidants such as hydrogen peroxide and ascorbic acid (Apel and Hirt, 2004).

Induction of phytochelatins and enzymatic and non-enzymatic antioxidants were monitored as plant primary and secondary metal detoxifying responses (Mishra *et al.*, 2006). The synchronous action of antioxidant enzymes of the ascorbate glutathione pathway viz. catalase (CAT) EC 1.11.1.6, superoxide dismutase (SOD) EC 1.15.1.1 and ascorbate peroxidase (APX) EC 1.11.1.11 with the thiol regulated enzymes dehydroascorbate reductase (DHAR) EC 1.8.5.1, monodehydroascorbate reductase (MDAR) EC 1.6.5.4 and glutathione reductase (GR) EC 1.6.4.2 is a predominant mechanism of ROS quenching under heavy metal stress (Clijsters *et al.*, 1999). Low molecular weight antioxidant metabolites like ascorbic acid (AA) and reduced glutathione (GSH) apart from being electron source to these enzymes the metabolites themselves play an important role in protecting plants from oxidative stress damage. There is dearth of literature with regard to the role of ascorbate glutathione pathway in cellular defense against chromium in general and chromium speciation in particular in plant parts (Shanker and Pathmanabhan, 2004).

Induction and activation of SOD and of CAT are some of the major metal detoxification mechanism in plants (Shankar 2003).

Chromium affects photosynthesis in terms of CO₂ fixation, electron transport, photophosphorylation, and enzyme activities in green alga (Hörcsik *et al.*, 2007).

Zaimoglu *et al.*, (2011) studied the antioxidant responses of *Brassica juncea* and *Brassica oleracea* to soils enriched with Cr (VI) and found that total enzymatic activity was higher in *B. oleracea* than in *B. juncea*. Cr (VI) and also a decrease in CAT activity in both species. Cellular antioxidants play an important role in protecting Brassica sp. to Cr-induced oxidative stress. This high activity of antioxidant enzymes and consequent detoxification of ROS contributes to relative tolerance of these species to Cr (VI).

Surekha *et al.*, (2012) investigated the chromium (Cr) toxicity on enzymes like peroxidase, ascorbate peroxidase and acid invertase was studied in pea seedling to evaluate the relative tolerance of three pea varieties (Arkel, Rachna and HFP-8712).

Acid invertase activity in cotyledons of Arkel was not affected significantly by chromium but increased in Rachna and HFP-8712. Chromium decreased acid invertase activity in radicle and plumule. Chromium treatment increased peroxidase activity in all component of seedling and found to be highest in untreated cotyledons of Arkel. Ascorbate peroxidase activity in the radicle and plumule was higher in Rachna. Enzyme activity decreased significantly with the increasing chromium treatment in cotyledons of Arkel while converse was true with regard to HFP-8712. These results indicate that Rachna is more tolerant followed by HFP-8712 and Arkel.

An experiment conducted by Tang *et al.*, (2012) resulted that the indicators of photosynthesis were all suppressed with increasing Cr stress, and antioxidation and protection systems were destroyed. They also discovered that the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) decreased, while the content of proline (Pro) and malondialdehyde (MDA) and relative conductivities increased. The accumulating Cr in all organs had a positive correlation with Cr concentration. Deposition of Cr in organs was in the following order: stubble > root > leave > stem > branch $\approx$ new stem (a bud and two leaves). We conclude that high concentration of Cr stress had strong inhibition and damage to the normal metabolism and cell implement structure, as well as on the function of tea plant.

Zeng *et al.*, (2014) suggested that rice plants employ two strategies to cope with Cr toxicity: immobilizing Cr ions into cell walls to reduce its translocation and activating antioxidant defense to mitigate Cr-induced oxidative stress.

Chatterjee *et al.*, (2015) studied the effect of chromium (both Cr III and Cr VI) on the oxidative damages and the induction of antioxidant defence in turnip. Exposure of plants to Cr led to increase in Cr concentration, more in the roots than in shoots. Excess supply of Cr (chromium sulphate, potassium chromate and potassium dichromate), though inhibited peroxidase activity (at 4 and 168 h exposure), stimulated catalase, ascorbate peroxidase and superoxide dismutase activities. Localization of different isoforms of APX and SOD on native gels revealed activation of certain isoform in the Cr-treated plants.

Sihag *et al.*, (2016) observed a significant decrease in the peroxidase activity and increase in ascorbate peroxidase activity was observed with increase in chromium concentration in the soil. The increase in activity of peroxidase consumed hydrogen

peroxide and thus it resulted in decrease of hydrogen peroxide content, while due to decrease in activity of ascorbate peroxidase, ascorbate content get increased by Cr (VI) application.

2.8.1 Catalase (CAT) :

Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. Since H_2O_2 is strong oxidant that rapidly oxidizes thiol groups, it can not be allowed to accumulate in excess in organelles such as chloroplasts where photosynthesis depends on thiol-regulated enzymes. The most important function of catalase in leaves is the removal of H_2O_2 during photorespiration.

Sen *et al.*, (1994) observed a decrease in catalase activity and increase in peroxidase activity at concentrations above 10 mM l-1 Cr(VI) whereas the enzyme activities were least affected by Cr(VI) at lower concentrations.

Gwozdz *et al.*, (1997) found that at lower heavy metal concentrations, activity of antioxidant enzymes increased whereas , at higher concentrations SOD activity did not increase further and catalase activity decreased.

The calli derived from *L. leucocephala* growing on contaminated soil when supplemented with 15 mM Cr exhibited higher catalase and peroxidase activities than those from uncontaminated soil (Rout *et al.*, 1999).

Samantary *et al.*, (1999) used peroxidase and catalase activities as enzyme markers for identifying Cr tolerant mung bean cultivars.

Excess of Cr (0.5 mM) restricted the activity of catalase in leaves of cauliflower (Chatterjee and Chatterjee, 2000).

Dixit (2000) reported increased activity of CAT at both 4 and 4 mM Cd concentrations in pea.

A decline in the specific activity of catalase with increase in Cr concentrations from 20 to 80 ppm was observed (Jain *et al.*, 2000).

In *E. colona* plants supplemented with Cr at 1.5 mg l⁻¹ activities of peroxidase and catalase were higher in tolerant calluses than in non-tolerant ones (Samantary *et al.*, 2001).

CAT activity decreased with increase of metal concentrations and the order of its sensitivity to the three metals was $\text{Cd}^{2+} > \text{Cr}^{6+} > \text{Pb}^{2+}$ (Ren *et al.*, 2002).

The high concentration of CAT of upto 10% to 25% of the organelle's protein guarantees rapid detoxification of H₂O₂ at the sets of production and prevents, under normal conditions, leakage of membrane – permeable molecule out of peroxisomes and oxidation of extra peroxisomal proteins (Reumann *et al.*, 2004).

No significant increase in CAT activity and MDHAR was observed under Cr(III) (Shanker *et al.*, 2004).

There is decline in the catalase activity under Cd stress in *Bocopa monnieri* L. (Mishra *et al.*, 2006).

Liu *et al.*, (2005) found that the CAT activity decreased in *A. viridis* L. exposed to Cr (VI) but an increase in SOD and guaiacol peroxidase (POX) activity was observed with increase of Cr(VI) concentration.

Lipid peroxidation was increased by Cr (VI) and the activities of superoxide dismutase and catalase (CAT) were increased. However, the CAT activity was reduced by high Cr(VI) concentration. The activities of ascorbate peroxidase and glutathione reductase were significantly reduced by Cr(VI) treatment (D. Subrahmanyam, 2008).

2.8.2 Superoxide Dismutase (SOD) :

SOD is a group of metalloenzymes that catalyze the dismutation of O₂⁻ to H₂O₂ and O₂. Thus, it plays an important role for protection against superoxide derived oxidative stress in plant cells.

Gwozdz *et al.*, (1997) reported that at lower heavy metal concentrations the activity of antioxidant enzymes increased, whereas at higher concentrations the SOD activity did not increase further and CAT activity decreased.

Dixit *et al.*, (2001) reported initial increase in SOD activity in *Pisum sativum*, however it declined by days 5 and 7 with 4 and 40 mM Cd treatment, respectively, but remained higher than control.

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

In *Brassica chinensis*, SOD activity followed a rise-fall pattern along metal concentration gradient (Cr^{6+} , Cd^{2+} , Pb^{2+}) but its sensitivity increased with the duration of metal treatment (Ren *et al.*, 2002).

The maximum stimulation of SOD activity (3.29 fold) was observed in *Ocimum tenuiflorum* when plants were exposed to 20 mM for 48 h and minimum at 100 mM chromium exposure for 72 h duration (Rai *et al.*, 2004).

In *Vigna radiata*, there was significant increase in activity of SOD as early as 12 h after treatment with Cr (VI) and continued to be significant at 120 h. In contrast, Cr (III) addition to the nutrient medium although did not increase the SOD activity, significant increase was seen only at 24 h (Shanker *et al.*, 2004).

In roots of *Sorghum bicolor* Cr (III) induced significant increase SOD activity. 50 mM Cr (VI) treated roots exhibited highly significant increase in the activity of SOD (Shanker and Pathmanabhan, 2004).

A steep rise in SOD activity (60-85%) was observed in *Brassica juncea* when exposed to moderate to high salt stress (100-250 m mol/l NaCl) (Verma and Mishra, 2004).

The oxidative stress of SOD increased with the increase of Cr (VI) concentration in *Oxya chinensis* (Li L.J. *et al.*, 2005).

Panda and Choudhury (2005) reported decline in SOD activity under chromium stress in plants and animals.

SOD activity at lower levels of Cr (VI) supply remained higher all through the treatments with 0.2, 2 and 20 mM in *Brassica juncea* (Pandey *et al.*, 2005).

In *Pistia stratiotes* the SOD activity increased at all concentrations (0, 10, 40, 80 and 160 mM) of Cr at 144 h than their controls (Sinha *et al.*, 2005).

SOD showed induction at lower concentration and duration followed by decline in *Ceratophyllum demersum* when exposed to various concentrations of Pb (1-100 mM) (Mishra *et al.*, 2006).

Mishra *et al.*, (2006) reported increased SOD activity in *Bacopa monnieri* upto 4 d (maximum 25 mM Cd) in leaves, while upto 2 d (maximum 10 mM Cd) in roots. After 7 days activity decline sharply showing maximum of 53-60% decrease at 100 mM Cd in leaves and root, respectively.

Sinha *et al.*, (2006) reported increased in activity in roots of *Bacopa monnieri* under Fe stress except 80 and 160 mM at 72 h, whereas, it decreased in leaves except 10 and 40 mM after 48 h as compared to their respective controls.

Leaves of *Tagetes erecta* showed decreased in SOD activity by both Cd treatments (1mg/l or 5 mg/l), however, *Avena strigosa* leaves showed higher activities of SOD (Uraguchi *et al.*, 2006).

Dey *et al.*, (2009) suggested that the SOD and CAT activities decreased in *T. aestivum* L. grown in the presence of K₂Cr₂O₇ in roots and shoots.

2.9 Accumulation of Chromium(Cr)

Huffman and Allaway (1973) found that bean seeds accumulated about 0.1% Cr, while roots accumulated 98%.

Among many vegetable crops such as cauliflower, cabbage, celery, garden pea, lettuce, onion and spinach, highest Cr concentrations were detected in members of Brassicaceae family such as cauliflower and cabbage and accumulation of Cr by roots was 100 fold higher than that by shoots (Zayed *et al.*, 1998).

Chatterjee and Chatterjee (2000) also found low levels of translocation of Cr from roots to the shoots in cauliflower (*Brassica oleracea*) grown on sand with 0.5mM Cr (III).

The maximum quantity of element contaminant was always contained in roots and the minimum in the vegetative and reproductive organs (Pulford *et al.*, 2001).

Vernay *et al.*, (2007) in *Lolium perenne* grown in the presence of 500 μ M of Cr(VI) showed that roots accumulated 10 times more Cr than leaves .

Liu *et al.*, (2008) studied hydroponically grown *A. viridis* L. under different concentrations of Cr (VI) and found that Cr was accumulated primarily in roots.

Spinach(*Spinacia oleracea* L. cv. “Banarasi”) grown in the presence of Cr (VI) showed more accumulation of Cr in the roots than in leaves and stem showed the least accumulation.(Gopal *et al.*, 2009).

Tiwari *et al.*, (2009) found that the pea plants when exposed to Cr there was an increase in concentration of Cr in different parts of the plant with the increase of Cr supply. Accumulation of Cr in the different parts of the plant was in the following order roots >stem>leaves>seed.

Paiva *et al.*, (2009) resulted that Cr accumulates mainly in roots and shoots; however roots accumulate the major part, being usually only a small part translocated to the shoots.

L’opez-Luna *et al.*, (2009) found that roots of wheat, oat, and sorghum accumulated more Cr than shoots; however in spite of that, wheat, oat, and sorghum showed Cr translocation from roots to shoots.

Sundaramoorthy *et al.*, (2010) stated that the Cr accumulation increased with the increase concentration of Cr.

Nematshahin *et al.*, (2012) observed in *Allium cepa* cv. Hybrid that the concentrations more than 150 mg/L chromium cause the reduction of morphology parameters in the treatment plants rather than control plant and Cr ³⁺ addition in the cultures caused enhancement of chromium content in roots and shoots of plant seedlings. It was also noted that accumulation of chromium in the roots were much higher than the shoots of the seedlings under treatment.

Chatterjee *et al.*, (2015) found that the exposure of plants to Cr led to increase in Cr concentration, more in the roots than in shoots in turnip.

2.10 Effect of chromium on other nutrients

Dube *et al.*, (2003) suggested that the watermelon plants grown in the presence of Cr, an increase in concentrations of P and Mn and decrease in Fe, Cu, Zn, and S contents in leaves was observed.

Gardea Torresdey *et al.*, (2004) found that Fe was significantly concentrated in the root of Cr (VI) treated plants of *Convolvulus arvensis* as in genotype SV36.

Chromium, due to its structural similarity with some essential elements, can affect mineral nutrition of plants in a complex way. Competition mechanisms for transport bindings in the plant resulting in a decrease in micronutrient uptake and translocation have also been described (Shanker *et al.*, 2005).

Decreased uptake of the micronutrients Mn, Fe, Cu, and Zn was detected by Liu *et al.*, (2008) in *A. viridis* L. exposed to Cr (VI). High content of Cr may displace the nutrients from physiological binding sites and consequently decrease uptake and translocation of essential elements.

Gopal *et al.*, (2009) that reported decreases of Mn and Fe contents in stems and leaves of cauliflower and spinach respectively. He also stated that the decrease in Fe concentration in leaf tissue in response to Cr toxicity is suggestive of Cr (VI) interference in the availability of Fe, leading to impairment of Fe metabolism.

Mallick *et al.*, (2010) found that Cr exposure decreased Cu absorption in *Zea mays* roots, while leaves were not affected.

Sundaramoorthy *et al.*, (2010) suggested that with increase in Cr concentration the uptake of macronutrient (N, P, K) and the micronutrients were gradually decreased.

Gautam *et al.*, (2014) observed that the N, P and K content in fruits were significantly affected by the Cr treatments in tomato and eggplant.

2.11 Economic yield

Bishnoi *et al.*, (1993a,b) reported that hexavalent chromium was much more toxic and suppressed growth of radicle and plumule of *Pisum sativum* significantly.

In chromium-treated plants a larger proportion of pods failed to set seeds and the average number of seeds per pod was lower.

Sharma *et al.*, (1995) observed that in response to chromium supply, wheat grain yield was severely affected and no seed formation was observed at 1.0 mM Cr(VI).

Shanker *et al.*, (2005) found a gradual decline in all the yield parameters were observed at higher chromium concentrations.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS METHOD

An experiment was conducted in pots collaborately by AICRP on micronutrient, Department of Soil Science and Agricultural Chemistry and Department of Plant Physiology, College of Agriculture, OUAT, Bhubaneswar Odisha (20°16' and 20°17' latitude and 85°48' and 85°49' East longitude) during Rabi 2018 to study “**Effect of chromium on phytotoxicity in Okra (*Abelmoschus esculentus* L. Moench)**”. The experiment was laid out in a completely randomized design (CRD) and were replicated thrice. The details of the experiment, materials used and methods followed were briefly explained in this chapter.

3.1 Soil characteristics

The composite soil collected from the farmer's field was analyzed for different physiochemical properties before the start of the experiment. The soil test was done in the laboratory of AICRP on Micronutrients. The details of soil test report is given in the table-1.

Table-1

S.N	SOIL PROPERTIES	VALUE
	Soil-Acid Lateritic Soil Order- <i>Alfisols</i>	
1	pH(1:2)	5.50
2	EC(dsm ⁻¹)	0.19
3	Organic carbon(%)	1.22
4	Nitrogen (kg/ha)	183
5	Phosphorus (kg/ha)	26.4
6	Potassium (kg/ha)	378.81
7	Chromium (mg/kg soil)	0
8	Zinc (mg/kg soil)	2.64
9	Iron (mg/kg soil)	166.28
10	Copper (mg/kg soil)	7.4
11	Manganese (mg/kg soil)	21

3.2 Climatic condition during the experiment

The climate during the crop growth and development was relatively warm and humid in nature. The weather data recorded and given in the Table- 2.

Table-2 Weather data from the month of February 2018 to April 2018

Year	Month	Tmax (°C)	Tmin (°C)	Rainfal l (mm)	Max. RH (%)	Min. RH (%)	BSH
2018	FEBRUARY	33.7	15.9	0.00	91	29	8.4
2018	MARCH	36.9	22.2	0.00	92	33	6.6
2018	APRIL	37.3	24.7	43.9	87	47	8.4

3.3 Experimental details

Season: *Rabi* 2018

Crop : Okra

Variety: Kajari

Design : CRD(Complete Randomized Design)

Methodology: Pot experiment in the net house of AICRP on Micronutrients

Okra cultivar ‘Kajari’ was sown in pots in three sets as three replications containing untreated soil: vermicompost mixture of 3kg in the ratio of 2:1. Ten seeds of Kajari were sown initially in each pot and different concentration of chromium (K_2CrO_4), available form of Cr (VI), was treated as chemical formulation of Cr (0, 2.5, 5, 10, 15, 25, 50, 75, 100, 200, 300 mg/kg soil) of 100ml as basal application along with recommended dose of fertilizer(N:P:K::80:40:40 kg/ha and N on split doses as 20%, 40%, 40%). Thereafter, all the pots were watered regularly with distilled water. The plants were thinned, as necessary, after a week of seedling establishment and a single plant per pot was maintained. Two splits of nitrogen were top dressed and the need based plant protection measures were applied to raise a normal crop.

3.3.1 Experimental layout and design-

The experiment was carried out in completely randomized design with 11 treatments and replicated thrice. The details of the experiment are given below:

Design Completely Randomized Design (CRD)

No. of treatments 11

No. of replications 03

No. of Pots 33

Details of treatments

- T0 : Control (Without Cr)
- T1 : 2.5 mg of Cr (chromium (K_2CrO_4), available form of Cr-VI)
- T2 : 5.0 mg of Cr
- T3 : 10 mg of Cr
- T4 : 15 mg of Cr
- T5 : 25 mg of Cr
- T6 : 50 mg of Cr
- T7 : 75 mg of Cr
- T8 : 100 mg of Cr
- T9 : 200 mg of Cr
- T10: 300 mg of Cr

3.3.2 Calender of operation

DOS (Date of sowing)- 05/02/2018

DOT (Date of treatment)- 05/02/2018

Date of N Top dressing- 20/02/2018(First top dressing)

07/03/2018(Second top dressing)

Date of harvest (first picking)-20/03/2018

3.4 Observation recorded

3.4.1 Germination test -

Twenty five no. of okra seeds were put under germination test in two sets at different concentrations of Cr (0, 2.5, 5.0, 10, 15, 25, 50, 75, 100, 200 and 300ppm as

K₂CrO₄) and germination percentage was determined as (No. of seeds germinated/total no. of seeds)*100. The shoot and root length of germinated were not clearly prominent at 7days in the treatments.

3.4.2 Growth parameters-

The growth parameters including plant height, no. of leaves/plant, leaf area and biomass were studied and listed below;

3.4.2.1 Plant height:

Height from the base of the plant to the top most leaf of the top branch of different treatments measured in centimeter (cm). Observations were recorded from 25 days of sowing at 15days of interval till harvest.

3.4.2.2 Number of leaves:

Total number of leaves per plant was counted from each treatment and each replication and leaf number was averaged out. Observations were recorded from 25 days of sowing at 15days of interval till harvest.

3.4.2.3 Leaf area:

Length and breadth of 2nd from the top leaf was taken of every treatment and each replication. Leaf area was estimated by multiplying the length and breadth of 2nd leaf from the top with the correction factor (0.725) and measured in cm². Observations were recorded from 25 days of sowing at 15days of interval till harvest.

3.4.2.4 Total biomass:

After harvestings plants of each treatment and replication were uprooted and the fresh weight and dry weight of plants (both shoots and roots) were recorded in g (gram).

3.4.2.5 Root length:

After harvesting, the root length of each treatment and replications were recorded in centimeter (cm).

3.5 Collection of plant samples for biochemical analysis:

The plant samples were collected at 50DAS and were kept in the deep freezer for further biochemical and enzymatic studies.

3.6 Biochemical parameters:

3.6.1 Plant Analysis-

3.6.1.1 Estimation of chlorophyll by Chlorophyll meter(Hansatech,Model CL-01)

It is simple and non- destructive method of chlorophyll estimation. It can be used in laboratory as well as in the field of standing crop. SPAD (Soil and Plant Analytical Development) values that indicates relative chlorophyll content. It is based upon a unique relationship between SPAD values and leaf area based nitrogen concentration in plants (Peng *et al.* 1993).

3.6.1.2 Estimation of protein content by Lowry's method

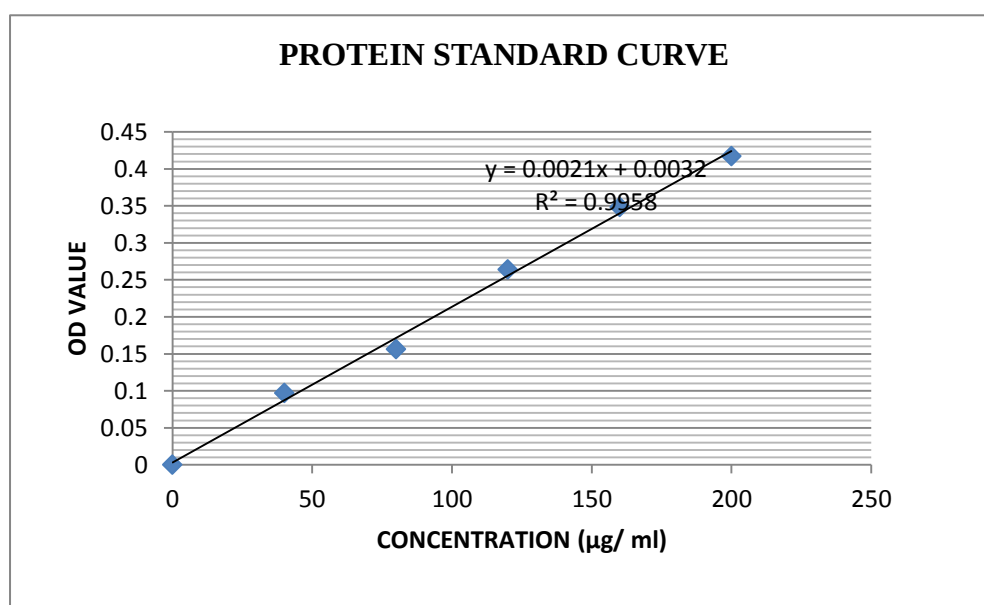


Figure-1: Protein standard curve

Macerate 1g fresh Or 0.2g dry sample by pestle and mortar in 10 ml TCA (10%) solution. Transfer to centrifuge tube and centrifuge at 5000 rpm for 10minutes.

Discard the supernatant of centrifuge tube. Add 10 ml of NaOH and mix well with the help of glass rod. Again centrifuge for 10000 rpm for 10 minutes. Collect the supernatant for true protein estimation. Pipette out 0.2 ml of sample extract in to the test tube. Make up the volume to 2ml in all the test tube with water. Add 10 ml of reagent C to each tube including the blank and stand for 10 minutes. Again add 1ml reagent D, mix well and incubate at room temperature and rest in the dark for 30 minutes. Blue colour is developed. Take the OD value at 660 nm. Express the amount of protein mg/g or 100g sample.

3.6.1.3 Estimation of proline content (Bates *et al.*1973)

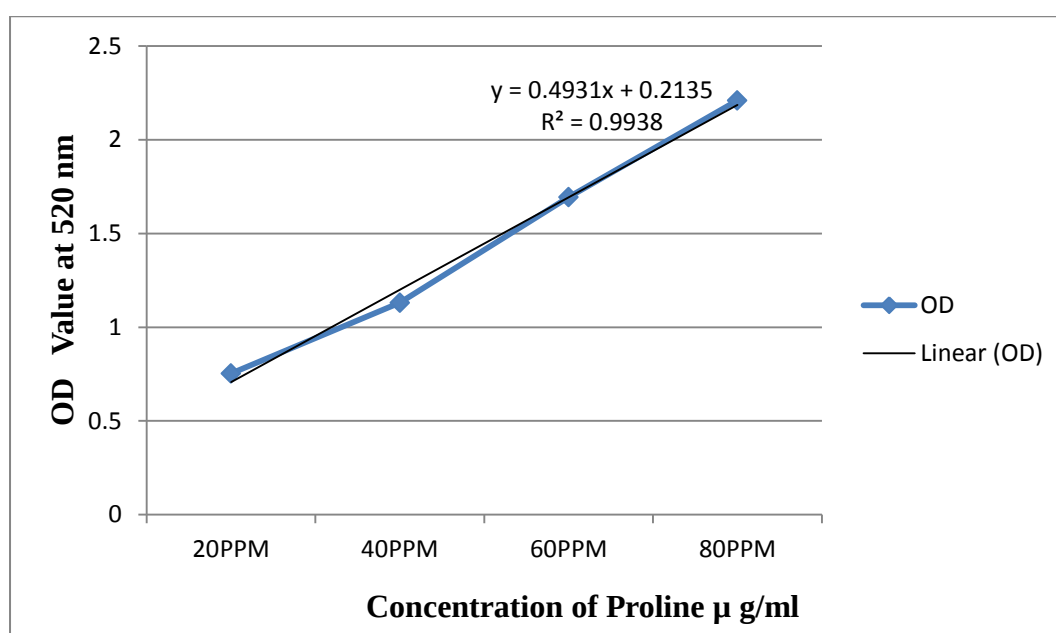


Figure 2: Proline standard curve

Leaf sample of 100mg was taken in a mortar and homogenized with 10 ml of 3% aqueous sulfosalicylic acid and filtered through whatmann No 2 filter paper. The extract filtrates were added 2 ml each of glacial acetic acid and acid-ninhydrin and mixed. Then test tubes are incubated in boiling water bath at 100°C for 1 hour and test tubes were kept in ice bath to terminate the reaction and 4 ml toluene added and mixed vigorously. The chromatophore is aspired and OD is measured at 540 nm

$$\mu \text{ moles of proline/g tissue} = X/2 \times 10/100 \times 1000$$

3.6.1.4 Catalase: (Hydrogen Peroxide: Hydrogen peroxide oxidoreductase EC 1.11.1.6)

Final Assay concentrations – In a 3.00 ml reaction mix, the final concentrations are 50mM potassium phosphate, 0.036%(w/w) hydrogen peroxide.

1. Using a suitable thermostatted spectrophotometer set at A_{242} and 25°C , blank the instrument versus a quartz cuvette containing phosphate buffer.
2. Pipette 2.90 ml of hydrogen peroxide solution into a quartz cuvette.

Note : Run only one test at a time

3. Place the cuvette in the spectrophotometer and allow the substrate to equilibrate 25°C .
4. Record the time required for the A_{240} to decrease from 0.45 to 0.40 absorbance unit.

Calculation:

$$\text{Catalase (U/min/g FW)} = \frac{(\text{Change in abs./min}) \times \text{Total volume (ml)}}{\text{Extinction coefficient} \times \text{Volume of sample taken (ml)}}$$

$$\text{Extinction coefficient} = 6.93 \times 10^{-3} \text{ mM}^{-1} \cdot \text{cm}^{-1}$$

$$\text{Specific activity (mol UA/mg protein)} = \frac{\text{Unit/min/g FW of catalase}}{\text{Protein content (mg/g FW)}}$$

3.6.1.5 SOD (Superoxide dismutase)(EC 1.15.1.1)-

Fresh leaves (0.5g) were homogenized with 3ml of ice-cold 0.15M potassium phosphate buffers (pH-7.8) containing 1mm EDTA in an ice bath. The homogenized slurry was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected. Protein concentration in the supernatant was determined according to Lowry *et al.*,(1951).

Superoxide dismutase (SOD; EC 1.15.1.1) was assayed by its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) at 560nm (Beauchamp and Fridovich, 1971). The assay was carried out at 25°C in a reaction mixture (3ml) containing 50 µM NBT, 10 mM L-methionine, 0.5 M EDTA and 5 mM riboflavin in 50 mM sodium phosphate buffer (pH 7.8). Riboflavin was added last and the test tubes containing the reaction mixture were incubated at 25°C for 10 min under 300mol m⁻² s⁻¹ irradiance.

The reaction mixture with no enzyme developed maximum purple colour due to the maximum rate of reduction of NBT. The non-irradiated reaction mixture did not develop colour and was used as control. One unit of SOD activity was defined as the quantity of SOD required to produce a 50% inhibition of NBT, and the specific enzyme activity was expressed as units per mg FW.

3.6.1.6 Estimation of chromium content (Atomic Absorption Spectrophotometry) (Lindsay and Norvell, 1978)

The Cr content was determined by atomic absorption spectrophotometer (PHCS17042501, PinAAcle 300H PerkinElmer Atomic absorption spectrometer). Dried powdered plant samples (leaves, fruits, seeds, roots) of 0.5g were digested overnight in 5ml of HNO₃. Then with adding of a di-acid mixture of HNO₃:HClO₄ in the ratio of 2:1 (v:v), these were kept on digestion chamber at 500°C till a transparent solution was obtained. After that 10ml of distilled water was added and allowed to stand for 1hr and filtered and final volume of 50ml was done for Cr estimation. The Cr concentration was measured in wavelength of 357.8 nm. The unit of metal uptake was expressed in ng per g dry weight.

3.6.1.7 Estimation of micronutrient (Fe, Cu, Zn, Mn) contents: (Atomic Absorption Spectrophotometry) (Lindsay and Norvell, 1978)

The micronutrient contents were determined by atomic absorption spectrophotometer (PHCS17042501, PinAAcle 300H PerkinElmer Atomic absorption spectrometer). Dried powdered plant samples (leaves, fruits, seeds, roots) of 0.5g were digested overnight in 5ml of HNO₃. Then with adding of a di-acid mixture of HNO₃:HClO₄ in the ratio of 2:1 (v:v), these were kept on digestion chamber at 500°C till a transparent solution was obtained. After that 10ml of distilled water was added

and allowed to stand for 1hr and filtered and final volume of 50ml was done for further estimation. The concentration of Fe, Cu, Zn and Mn were measured in wavelengths of 248.3nm, 324.7nm, 213.8nm and 279.48nm respectively. The unit of nutrients were expressed in ng per g dry weight.

3.6.1.8 Estimation of N, P, K content of fruits of okra

Nitrogen

Nitrogen in the processed sample was determined by Kjeldahl digestion method as described in AOAC (1960).

Phosphorus (P) and Potassium (K)

The samples were predigested in diacid mixture [HNO_3 : HClO_4 (3:2)]. Aliquot was collected, dilution was done. Phosphorus was estimated by help of spectrophotometer, K by flame photometer.

3.6.2 SOIL ANALYSIS

The residual chromium content and micronutrients in soil of each treatment has been analysed as per previous estimation. Cr and micronutrients were extracted from soil with DTPA (0.005M + 0.01M CaCl_2 + 0.1M TEA, pH 7.3) solution by shaking the mixture in mechanical shaker for 2hrs. After filtration the concentration of extract was estimated using atomic absorption spectrophotometer (PHCS17042501, PinAAcle300H PerkinElmer Atomic absorption spectrometer) following the method described by Lindsay and Norvell (1978).

3.7 Yield and yield attributes

3.7.1 Number of fruits

Total number of fruits were counted at the time of harvesting of each replication from each treatment.

3.7.2 No. of seeds/fruits

Total number of seeds per fruit were counted at the time of harvesting of each replication from each treatment.

3.7.3 Test weight

It is the average weight of 100 dried seeds of okra for each treatment and replication.

3.7.4 Harvest Index

$$\text{Economic yield} / \text{Total Biomass} \times 100$$

3.8 Statistical analysis

The data collected from the experiment on various aspects of germination, growth, biochemical parameters, yield and yield attributing characters of okra under Cr stress were arranged in appropriate tables according to the treatment and were subjected to statistical analysis appropriate to the CRD design with the help of the “Statistical procedures for agricultural research” by Gomez, K.A. and A.A. Gomez, (1984).

CHAPTER 4

RESULTS

RESULT

The present experiment was carried out in *Rabi* 2018 to find the effect of chromium on phytotoxicity in okra (*Abelmoschus esculentus* L. Moench.) to different concentrations of chromium (K_2CrO_4), soluble form of Cr VI, at the rate of 0, 2.5, 5.0, 10, 15, 25, 50, 75, 100, 200 and 300 ppm as chemical formulations. Thirty three numbers of pots were arranged in three rows to fit in a CRD design with three replications. Cr was applied as basal application at the time of sowing of seeds cv. Kajari and was watered regularly. Cr translocation and accumulation in okra plant parts were analyzed and its phytotoxicity to different growth parameters, alteration of metabolites and yield components were also recorded. The data generated was statistically analyzed and the results obtained are presented with tables and figures as and where necessary in this chapter.

4.1 Germination Test

Phytotoxicity of different concentrations of Cr on okra seed germination were determined before the start of experiment and presented in fig-3 revealed that germination was not occurred at 300ppm of Cr VI. However, the germination percentage decreased with a range of 100% to 40.8% between control and treated seeds. As the concentration of Cr increased from 2.5ppm to 200ppm, the rate of germination decreased linearly. However, germination percentage was significantly ($p<0.05$) affected at 25ppm to 200ppm by 40.7% to 59.2%. In a similar manner the seedling root and shoot length were also decreased.

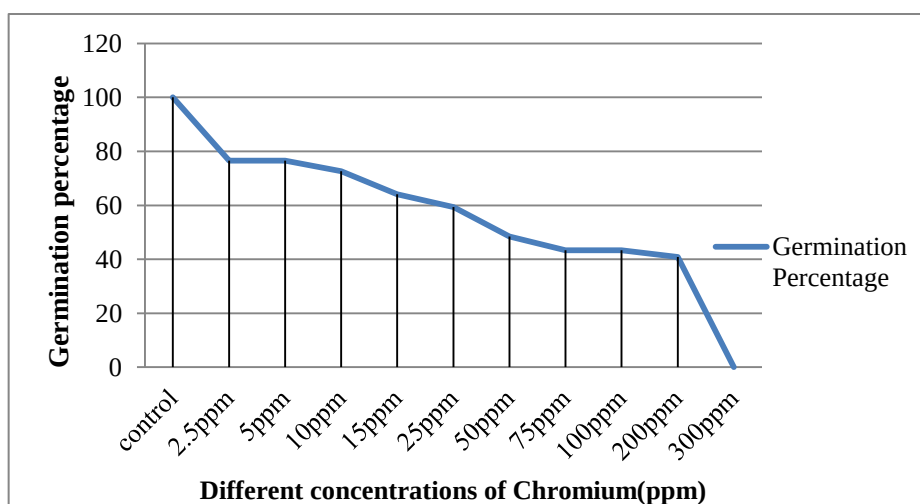


Fig 3: Effect of different concentrations of Cr on germination of okra seeds

4.2 Growth parameters

Cr concentration of 300ppm was found extremely toxic to okra seed germination and no seedling was established.

4.2.1 Shoot elongation

The results after 25 days of exposure of Cr to *Abelmoschus esculentus* showed considerable reduction in shoot elongation (3.2% to 63.5%). The length of the plant was adversely affected due to chromium (table-3). In the subsequent observations on plant height at an interval of 15days from the previous observations (up to 70 DAS) was also found similar trend of decreased plant height to high level of Cr concentration. Decrease in plant height among the treatments was found statistically significant.

Table 3:Effect of Different concentrations of Cr on plant height at 25 DAS, 40 DAS, 55 DAS AND 70 DAS. Data presented as mean of three replications.

Treatments	Plant height at 25 DAS(cm)	Plant height at 40 DAS(cm)	Plant height at 55 DAS(cm)	Plant height at 70 DAS(cm)
Control	22.43	29.97	38.60	44.10
2.5ppm	21.70 (-3.25)	29.27 (-2.33)	37.90 (-1.81)	39.73 (-9.90)
5ppm	18.87 (-15.87)	25.60 (-14.58)	33.70 (-12.69)	38.87 (-11.85)
10ppm	18.43 (-17.83)	24.33 (-18.81)	32.97(-14.58)	36.40 (-17.46)
15ppm	18.33 (-18.27)	24.93 (-16.81)	33.73 (-12.61)	37.77 (-14.35)
25ppm	16.47 (-26.57)	23.43 (-21.82)	32.27 (-16.39)	36.30 (-17.68)
50ppm	15.87 (-29.24)	22.67 (-24.35)	29.23 (-24.27)	34.17 (-22.51)
75ppm	13.00 (-42.04)	18.40 (-38.60)	22.37 (-42.04)	29.00 (-34.24)
100ppm	8.97 (-60.00)	16.10(-46.27)	22.40 (-41.96)	27.07 (-38.61)
200ppm	8.17 (-63.57)	15.30 (-48.94)	20.93 (-45.77)	23.47 (-46.78)
SE(m)±	0.403	0.649	1.011	1.28
CD 5%	1.18	1.90	2.97	3.75
CV %	8.54	7.34	8.37	9.45

The (-) parentheses in the bracket indicate the percentage of decrease of the data as compared to control.

4.2.2 Root length

Root length recorded at the time of harvest was presented in fig-4 indicated that root length decreased significantly as the Cr concentration increased from 2.5 to 200ppm from 27.3cm to 13.3cm respectively.

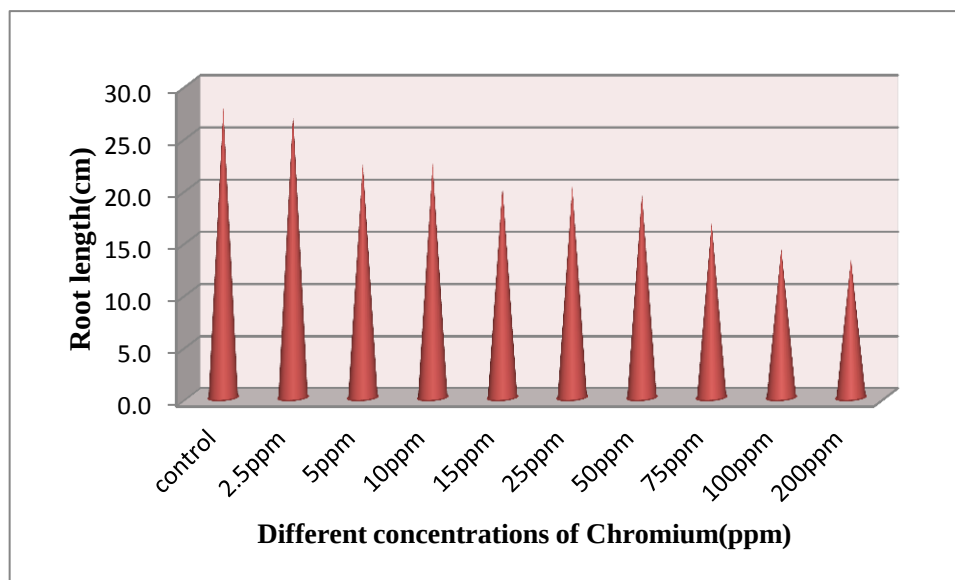


Fig 4: Effect of different concentrations of Cr on root length of okra plant. Data presented as mean of three replications.

4.2.3 No. of leaves per plant

The leaves retention in the treated and untreated plants was recorded after 25 DAS(Days after sowing) as presented in table-4 which indicated that the Cr phytotoxic effect was visible among treatments and significantly decreased as the concentration of Cr increased. The reduction in the leaf number varied from 8.5% to 62.8% at 25DAS. Though the leaf number increased during the growth of the crop but similar trend of decrease in leaf number than control plant was observed and found statistically significant among the treatments.

Table 4: Effect of different concentrations of chromium on number of leaves of okra at 25 DAS, 40 DAS, 55 DAS and 70 DAS. Data presented as mean of three replications.

Treatments	No. of leaves (25 DAS)	No. of leaves (40 DAS)	No. of leaves (55 DAS)	No. of leaves (70 DAS)
Control	11.67	15.33	15.33	15.67
2.5ppm	10.67 (-8.56)	14.67 (-4.30)	15.00 (-2.15)	15.00 (-4.27)
5ppm	10.33 (-11.48)	14.00 (-8.67)	14.33 (-6.52)	14.33 (-8.55)
10ppm	9.67 (-17.13)	12.00 (-21.72)	12.33 (-19.56)	12.67(-19.14)
15ppm	9.33 (-20.05)	11.33 (-26.09)	12.00 (-21.72)	12.33(-21.31)
25ppm	9.00 (-22.87)	11.67 (-23.87)	11.67 (-23.87)	11.67(-25.52)
50ppm	8.67 (-25.70)	10.67 (-30.39)	11.33 (-26.09)	11.00(-29.80)
75ppm	7.67 (-34.27)	10.67 (-30.39)	11.00 (-28.24)	10.67(-31.90)
100ppm	6.33 (-45.75)	9.67 (-36.92)	10.00 (-34.76)	9.67 (-38.28)
200ppm	4.33 (-62.89)	9.00 (-41.29)	9.33 (-39.13)	9.00 (-42.56)
SE(m)±	0.302	0.560	0.550	0.492
CD 5%	0.88	1.64	1.61	1.44
CV %	12.05	10.03	10.22	9.48

The (-) parentheses in the bracket indicate the percentage decrease of the data as compared to control.

4.2.4 Leaf area

The leaf area calculated in cm² per plant was depicted in table-5 which revealed that the photosynthesizing leaf area was decreased within the treatments in a trend of T0>T1.....>T9 at 25DAS. The similar pattern of decrease in leaf area was observed in the subsequent date of observations with an exception of 25 DAS to 40 DAS in 2.5ppm and 5ppm treated plants where the leaf area increased than the control plants but afterwards decreased. The leaf area was found statistically significant among the treatments.

Table 5: Effect of different concentrations of Cr on leaf area per plant of okra at 25 DAS, 40 DAS, 55 DAS and 70 DAS. Data presented as mean of three replications.

Treatments	Leaf area (25 DAS)	Leaf area (40 DAS)	Leaf area (55 DAS)	Leaf area (70DAS)
Control	75.11	105.09	198.38	206.26
2.5ppm	72.34 (-3.68)	106.21 (+1.06)	196.69 (-0.85)	197.16 (-4.41)
5ppm	69.17 (-7.90)	105.75 (+0.62)	193.65 (-2.38)	196.91 (-4.53)
10ppm	65.23 (-13.15)	104.91 (-0.17)	172.15 (-13.22)	179.12 (-13.15)
15ppm	60.35 (-19.65)	100.99 (-3.90)	167.24 (-15.69)	170.17 (-17.49)
25ppm	57.19 (-23.85)	99.63 (-5.19)	164.20 (-17.22)	169.25 (-17.94)
50ppm	53.76 (-28.42)	96.87 (-7.82)	147.99 (-25.40)	153.66 (-25.50)
75ppm	45.61 (-39.27)	64.76 (-38.37)	134.77 (-32.06)	145.21 (-29.59)
100ppm	36.66 (-51.19)	65.83 (-37.35)	123.33 (-37.83)	134.63 (-34.72)
200ppm	29.19 (-61.13)	52.33 (-50.20)	114.65 (-42.20)	115.02 (-44.23)
SE(m)±	0.849	2.136	4.224	3.702
CD 5%	2.49	6.26	12.39	10.86
CV %	5.04	7.07	6.38	5.57

The (-) parentheses in the bracket indicate the percentage decrease and (+) parentheses in the bracket indicate the percentage increase of the data as compared to control.

4.2.5 Total biomass

The total biomass estimated after harvest of the crop on both fresh weight and dry weight basis were presented on table-6 which showed significant decrease in total biomass as compared to the untreated plant with a range of 5.5% to 47.8% and 11.3% to 50.8% respectively. The heavy metal stress effect visible on dry weight was found statistically significant.

Table 6: Effect of different concentrations of Cr on total fresh weight and total dry weight of okra plant. Data presented as mean of three replications.

Treatments	Total fresh weight(g)	Total dry weight(g)
Control	28.07	7.80
2.5ppm	29.62 (+5.52)	6.92 (-11.28)
5ppm	23.58 (-15.99)	6.02 (-22.82)
10ppm	23.75 (-15.39)	5.56 (-28.71)
15ppm	24.20 (-13.78)	5.11 (-34.48)
25ppm	24.00 (-14.49)	4.75 (-39.10)
50ppm	23.19 (-17.38)	4.46 (-42.82)
75ppm	21.48 (-23.47)	4.45 (-42.94)
100ppm	20.87 (-25.65)	4.16 (-46.66)
200ppm	14.66 (-47.77)	3.84 (-50.76)
SE(m)±	0.829	0.242
CD 5%	2.43	0.71
CV %	9.80	10.93

The (-) parentheses in the bracket indicate the percentage of decrease and (+) parentheses indicate the percentage of increase of the data as compared to control.

4.3 Biochemical parameters

4.3.1 Plant analysis

4.3.1.1 Proline content

Proline accumulation in leaf because of heavy metal (Cr) stress was determined on fresh weight basis and presented in fig-5 which indicated that proline content considerably increased at high concentration of chromium and found 0.44mg/g FW to 2.6mg/g FW among the different treatments. The proline content among the treatments was found significant.

4.3.1.2 Total soluble protein content

Total soluble protein contents of leaves estimated for different treatments was presented in fig-6. The data obtained indicated that the protein content decreased up to 62.7% with the increased level of Cr concentration applied in the plants. The protein content was also found statistically significant among the treatments.

4.3.1.3 Catalase

Unit change in the catalase activity per unit protein per minute was determined among the treatments and was shown in fig-7 which revealed that the catalase activity increased up to a tune of 0.854 to 3.592 UA/mg protein/minute and as high as 320.6%. The catalase activity was found significant among the treatments.

4.3.1.4 Superoxide dismutase

The superoxide dismutase enzyme activity was calculated on fresh weight basis among the treatments and presented in fig-8 which indicated that the activity of SOD gradually increased from 0.52units/mg FW to 1.12units/mg FW as the concentration of Cr treatment increased in okra plants and showed the stress activities up to 115.4%. The SOD activity was found significant among the treatments.

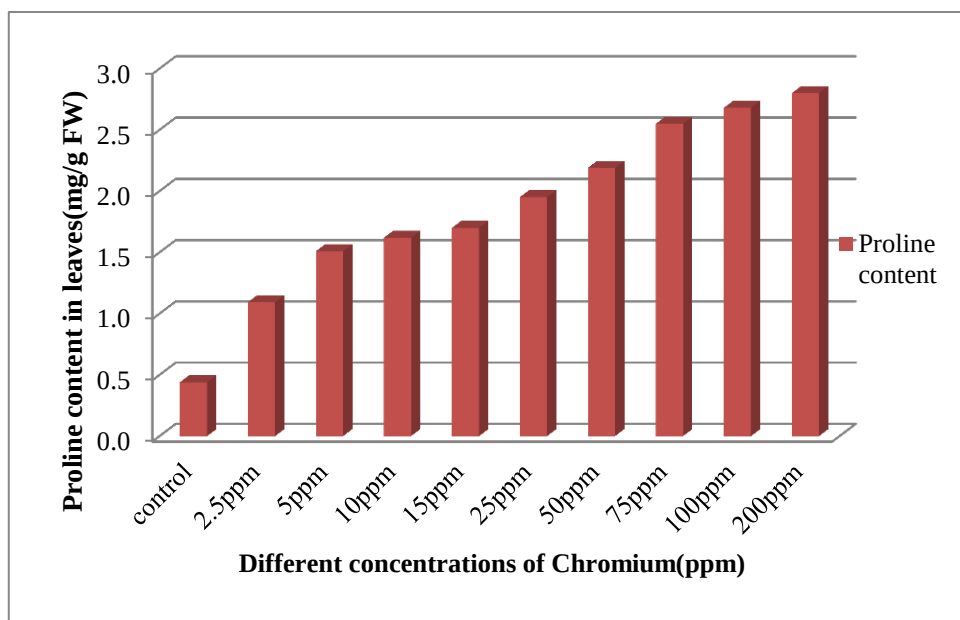


Fig 5: Effect of different concentrations of Cr on proline content (mg/g FW) in leaves of okra. Data presented as mean of three replications.

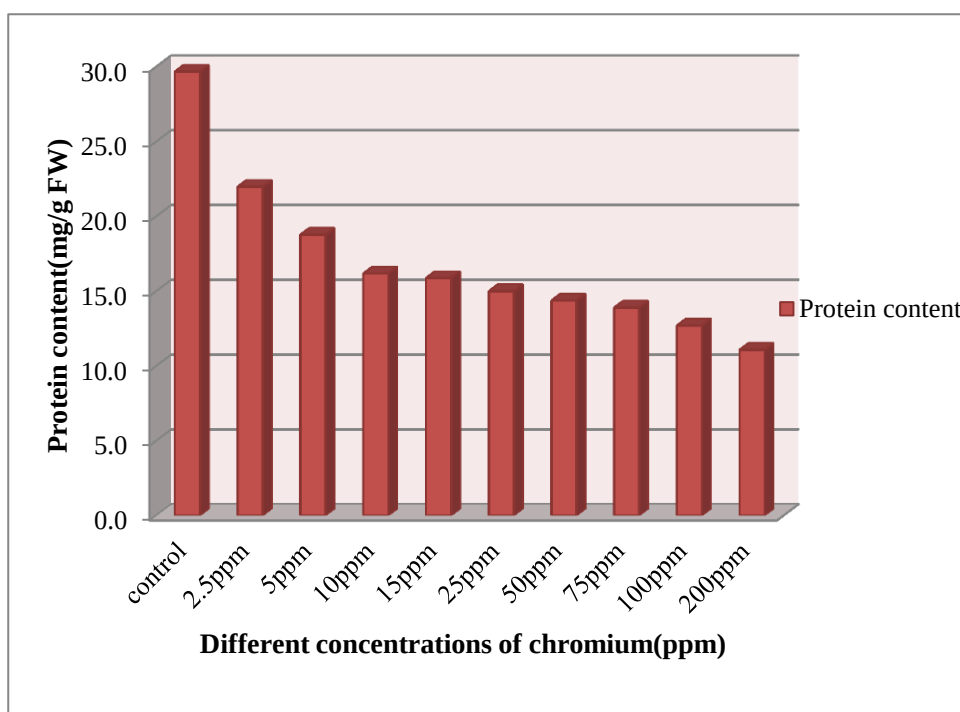


Fig 6: Effect of different concentrations of Cr on protein content (mg/g FW) in leaves of okra. Data presented as mean of three replications.

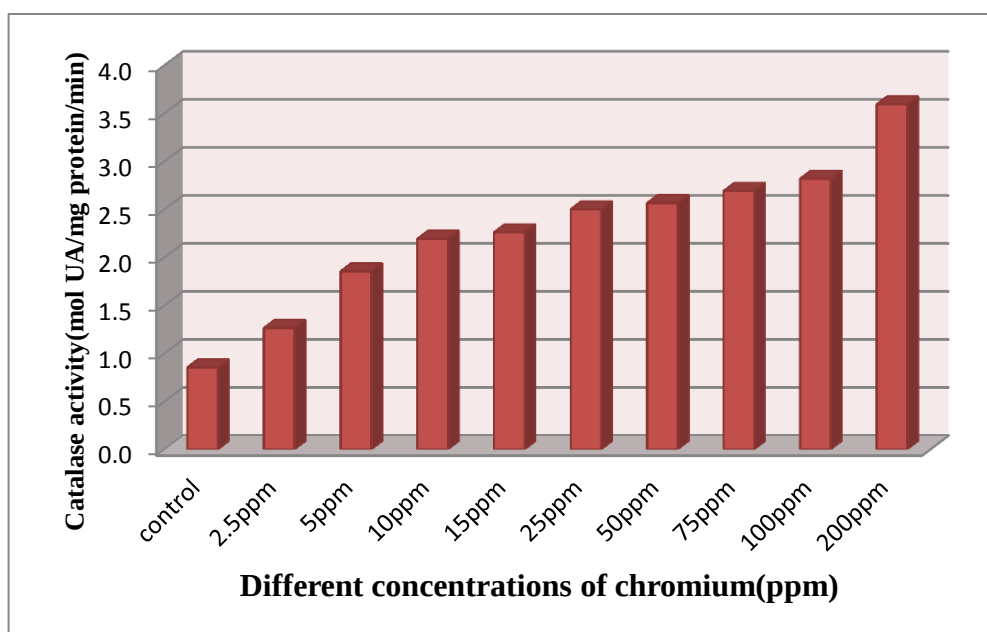


Fig 7: Effect of different concentrations of Cr on catalase activity (mol UA/mg protein/min) in leaves of okra. Data presented as mean of three replications.

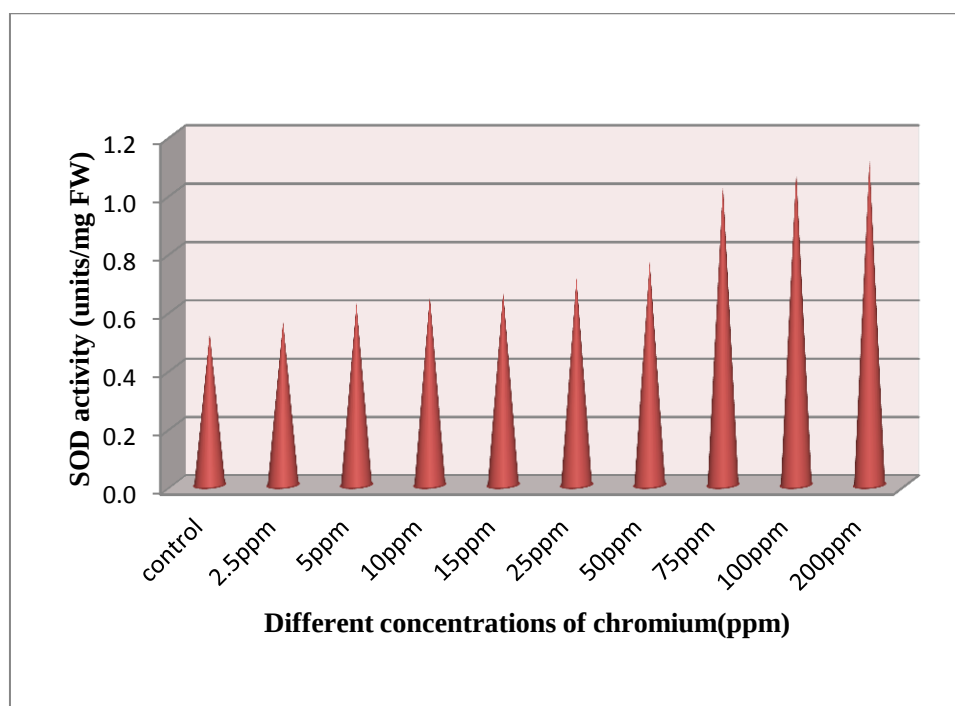


Fig 8: Effect of different concentrations of Cr on SOD activity (Units/mg FW) in leaves of okra. Data presented as mean of three replications.

4.3.1.5 Chromium translocation and accumulation in okra plant

Chromium was taken up in the okra plant by the roots and found at different parts of the plants viz. roots, leaves, fruits and seeds and got accumulated which was estimated on dry weight basis of the above parts and depicted in Fig-9. The data revealed that the rate of Cr accumulation was in order of root> leaf> fruit> seed with a tune of 39.6%, 27.5%, 21.1% and 11.8% respectively at 25ppm Cr application. But as the concentration increases the accumulation in the roots was the highest followed by leaf, fruit and seeds. The Cr accumulated in roots was highest in plants treated with 200ppm (98.7ng/g DW).

The Cr accumulation was detected in leaf tissues of plants grown in different concentration of Cr. The deposition of Cr in leaf increased with the increase in Cr concentration. The Cr accumulation was in order of rate of increase of Cr concentration treated in the plants and was found highest in 200ppm (23.60ng/g DW).

Fruits harvested from plants, grown in different graded doses of Cr were analyzed and showed that Cr got accumulated in higher rate while we increase its concentration. The highest Cr accumulation was found in the plants grown in 200ppm (22.47ng/g DW).

The Cr accumulation in okra seeds was also detected. The increase in the chromium concentration was related to rate of Cr applied to okra plants. The highest Cr accumulated in seeds of the plant treated with 200ppm (8.67ng/g DW). The Cr accumulation in seeds was in a range of 2.03ng/g DW to 8.67ng/g DW at 2.5ppm to 200ppm respectively.

The accumulation of Cr in different plant parts (roots> leaves> fruits> seeds) was found statistically significant among the treatments.

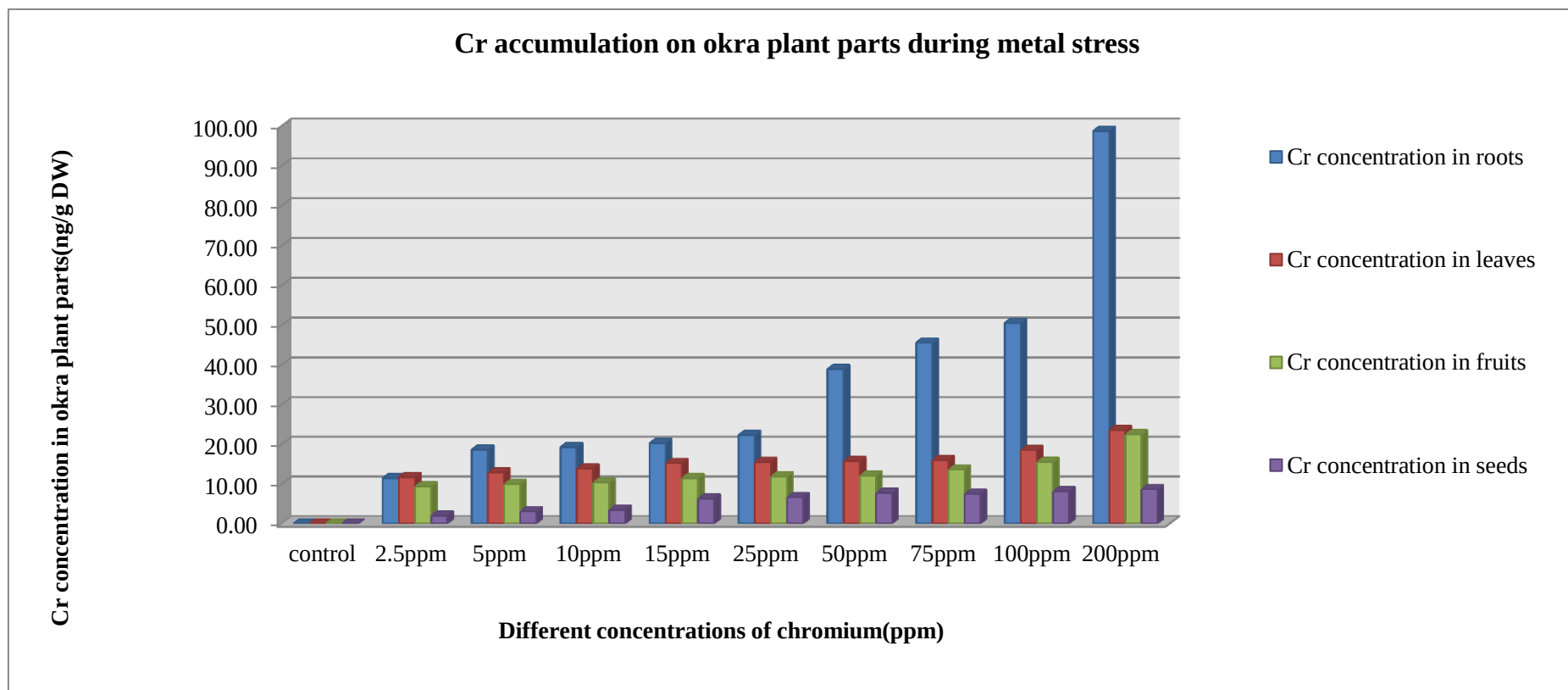


Fig 9: Accumulation of Cr (ng/g DW) in different plant parts of okra.

Data presented as mean of three replications.

4.3.1.6 Mineral nutrition

(A)Concentration of other micronutrients (Zn, Fe, Mn and Cu)

The concentration of other micronutrients (Zn, Fe, Mn and Cu) in relation to synergistic and antagonistic effect of Cr was estimated in different plant parts like roots, leaves, fruits and seeds was determined and presented in fig 10 to fig 13. The data on concentration of different micronutrients analyzed was found significant among the treatments.

The data depicted in the fig-10 on Zn accumulation to different plant parts in relation to increased concentration of Cr application to okra plant showed that an antagonistic effect to Zn uptake and accumulation as compared to Cr uptake by plants. As the Cr concentration increased the Zn accumulation in the plant parts gradually decreased than their controls. The Zn accumulation in plant parts was in the order of leaf> seeds> root> fruit indicated that proper translocation of Zn from root to sink was occurred in okra plants, although Zn level decreased at high concentrations of Cr. Zn concentration was 38.5ng/g in roots, 394.67ng/g in leaves, 34.83ng/g in fruits and 94.67ng/g in seeds in control and 26.23ng/g in roots, 56.67ng/g in leaves, 22.40ng/g in fruits and 14.67ng/g in seeds in 200ppm Cr respectively.

Fe translocation and accumulation in different parts of okra plant was presented in fig-11 which revealed that the Fe accumulation was gradually increased in roots to the high rate of Cr application and decreased in fruits and seeds. However, a very irregularly disorder accumulation was recorded in leaf Fe loading.

Manganese loading in different plant parts analyzed in respect to graded doses of Cr application (fig-12) indicated that Mn concentration was in order of seed< root< fruit< leaf. Mn was accumulated highest in leaf but its accumulation gradually increases from 2.5 to 25ppm and thereafter a decreased accumulation of Mn in leaf was observed, but the Mn accumulation in leaf in all the treatments was higher than control. Mn accumulated in seed significantly like other micronutrients.

Cu accumulation was highest in leaves and in order of leaves> roots> seeds> fruits. The data was presented in fig-13 which indicated that the uptake and translocation of Cu in presence of Cr was not restricted and found prominently in seeds (5.50 to 2.80ng/kg) and was found statistically significant.

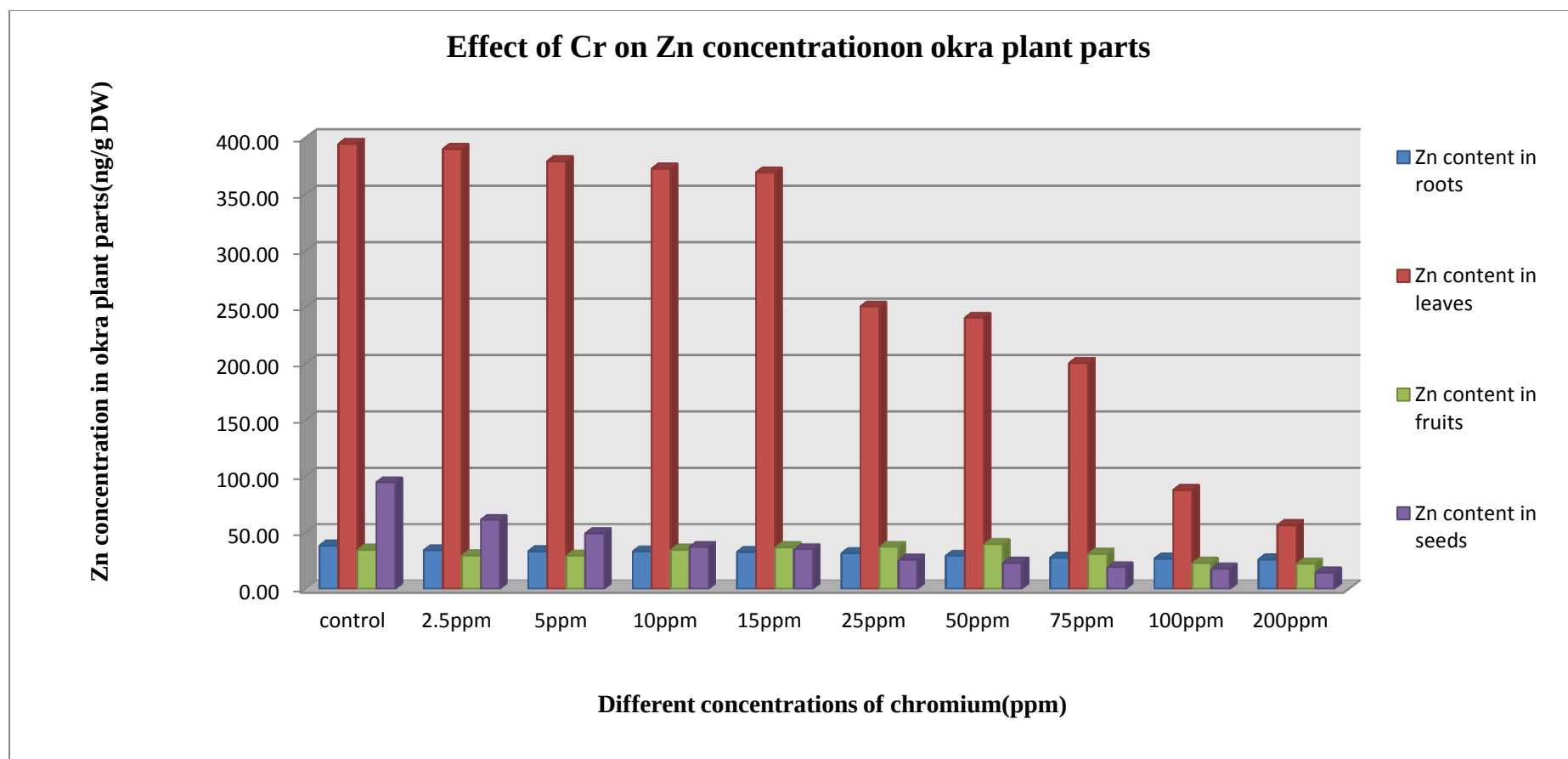


Fig10: Concentration of Zn in different plant parts of okra during metal stress.

Data presented as mean of three replications.

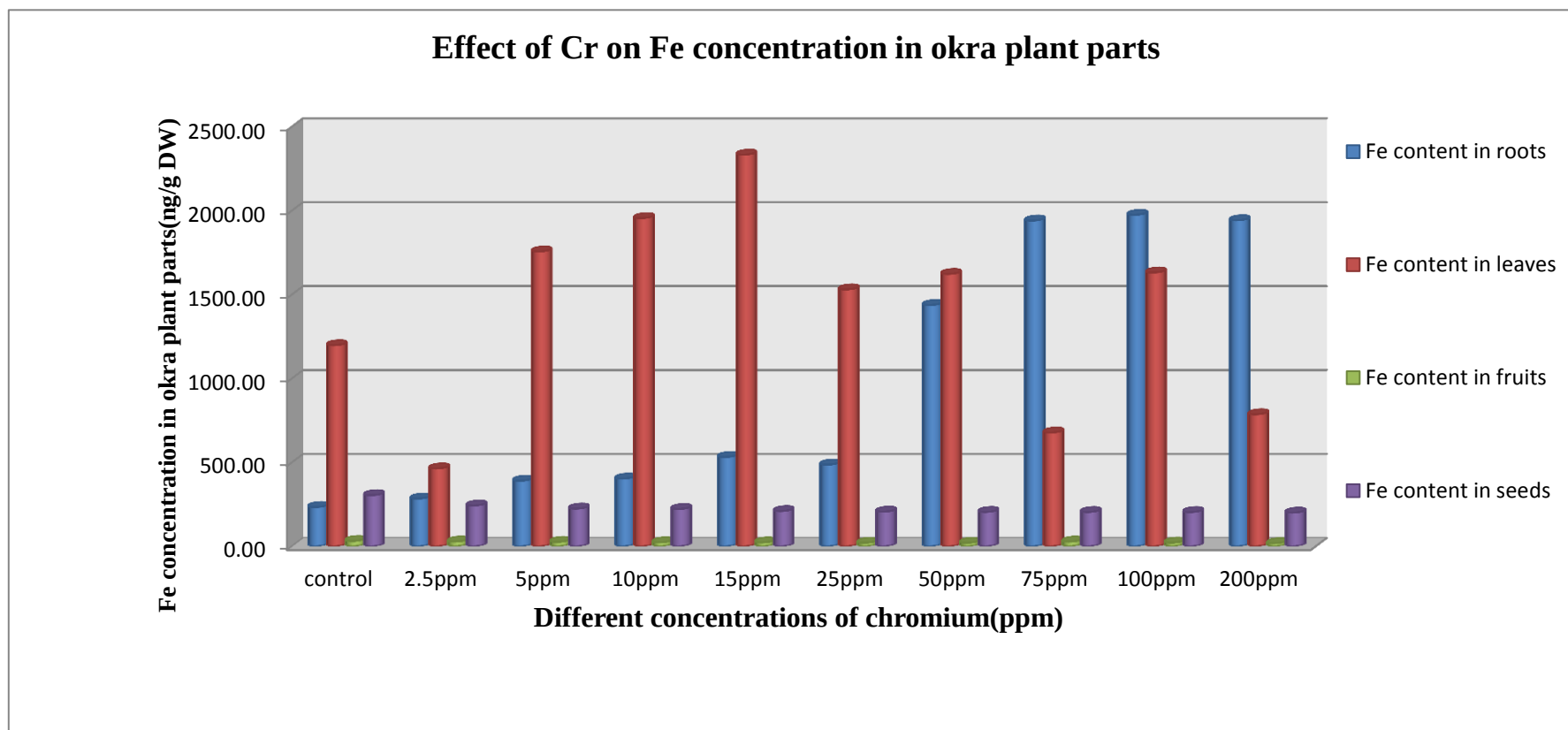


Fig 11: Concentration of Fe in different plant parts of okra during metal stress. Data presented as mean of three replications.

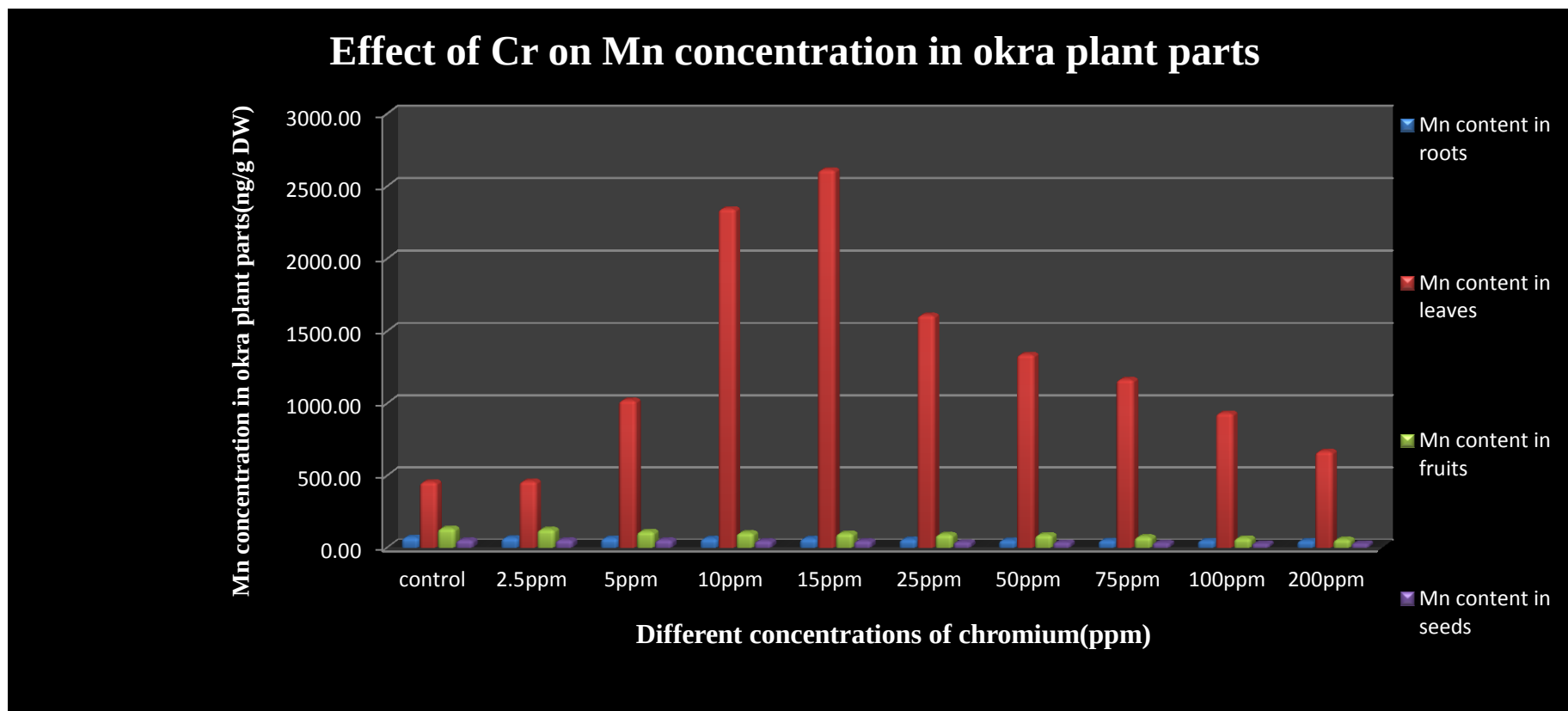


Fig 12: Concentration of Mn in different plant parts of okra during metal stress.

Data presented as mean of three replications.

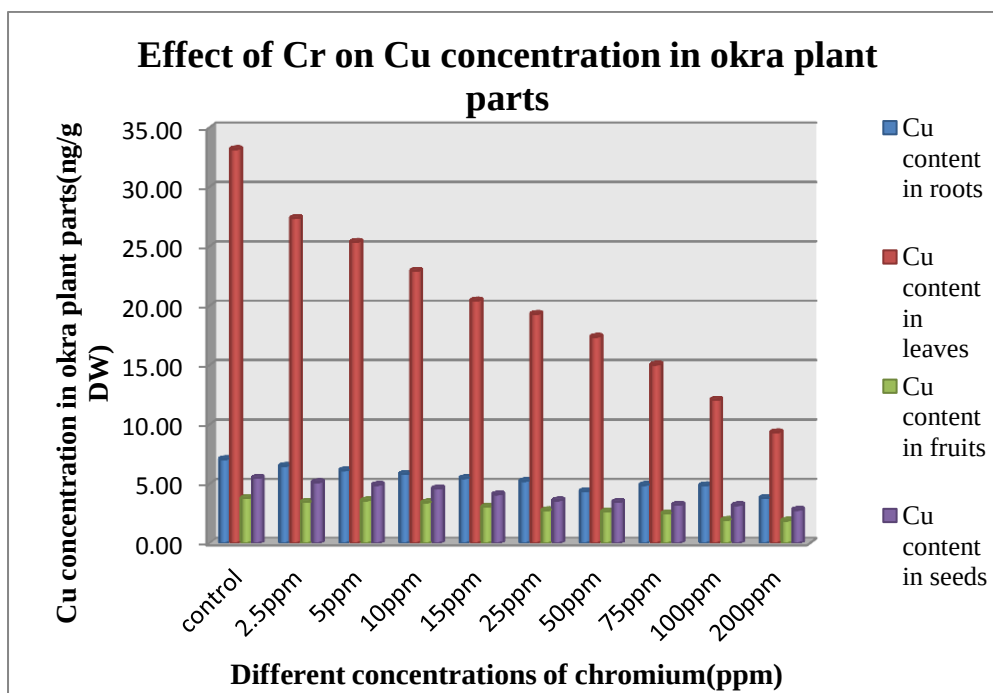


Fig 13: Concentration of Cu in different plant parts of okra during metal stress. Data presented as mean of three replications.

(B) Concentration of macronutrient content in okra fruits

The macronutrients like N, P, K content of okra fruits were estimated and presented in table-7 which revealed that all the three elements were available in a decreased trend to the graded doses of Cr used in okra plants.

The concentration of N decreased in a tune of 1.39% to 0.82% and as high as 41% than control.

The P content was also decreased in the Cr treated plants as compared to untreated plants and varied in a range of 0.55% to 0.14% than control. K content in okra fruits showed the similar decrease trend as compared to untreated plants that ranges 0.45% to 0.19% and as high as 57.8% than control.

The effect of heavy metal (Cr) on N, P, K content in fruits of okra was found statistically significant.

Table 7: Effect of different concentrations of Cr on N, P, K content of fruits of okra. Data presented as mean of three replications.

Treatments	N%	P%	K%
Control	1.39	0.55	0.45
2.5ppm	1.35 (-2.88)	0.51 (-7.27)	0.44 (-2.22)
5ppm	1.31 (-5.76)	0.49 (-10.91)	0.42 (-6.67)
10ppm	1.28 (-7.91)	0.43 (-21.82)	0.40 (-11.11)
15ppm	1.27 (-8.63)	0.41 (-25.45)	0.33 (-26.67)
25ppm	1.22 (-12.23)	0.34 (-38.18)	0.30 (-33.33)
50ppm	1.20 (-13.67)	0.30 (-45.45)	0.29 (-35.56)
75ppm	1.17 (-15.83)	0.23 (-58.18)	0.28 (-37.78)
100ppm	1.10 (-20.86)	0.20 (-63.64)	0.20 (-55.56)
200ppm	0.82 (-41.01)	0.14 (-74.55)	0.19 (-57.78)
SE(m)±	0.015	0.006	0.028
CD 5%	0.05	0.02	0.08
CV%	3.26	7.68	2.48

The (-) parentheses indicate the percentage decrease of the data as compared to control.

4.3.1.7 Chlorophyll index by SPAD

The Chlorophyll index by SPAD was recorded after 25 DAS(Days after sowing) as presented in table-8 which indicated that the Cr phytotoxic effect was visible among treatments and significantly decreased as the concentration of Cr increased. The reduction in the chlorophyll index varied from 7.27% to 19.19% at 25DAS. Though the leaf number increased during the growth of the crop but similar trend of decrease in leaf number than control plant was observed and found statistically significant among the treatments.

Table 8: Effect of different concentrations of Cr on Chlorophyll index (by SPAD) in leaves of okra at 25 DAS, 40 DAS, 55 DAS and 70 DAS. Data presented as mean of three replications.

Treatments	SPAD value (25 DAS)	SPAD value (40 DAS)	SPAD value (55 DAS)	SPAD value (70 DAS)
Control	21.57	18.36	27.07	27.79
2.5ppm	20.00 (-7.27)	17.96 (-2.18)	25.24 (-6.76)	27.51 (-1.01)
5ppm	16.00 (-25.82)	17.87 (-2.67)	24.98 (-7.72)	26.69 (-3.95)
10ppm	19.40 (-10.06)	17.53 (-4.52)	25.00 (-7.64)	25.51 (-8.20)
15ppm	16.97 (-21.32)	17.43 (-5.07)	24.74 (-8.60)	25.11 (-9.64)
25ppm	18.0 (-16.55)	16.64 (-9.36)	23.20 (-14.29)	23.85 (-14.17)
50ppm	12.47 (-42.18)	16.41 (-10.62)	21.85 (-19.28)	24.14(-13.13)
75ppm	19.33 (-10.38)	16.11 (-12.25)	20.72 (-23.45)	23.35 (-15.97)
100ppm	19.39 (-10.10)	15.69 (-14.54)	21.66 (-19.98)	21.58 (-22.34)
200ppm	17.43 (-19.19)	15.48 (-15.68)	17.57 (-35.09)	16.87 (-39.29)
SE(m)±	0.703	0.724	1.007	0.926
CD 5%	2.06	2.12	2.95	2.72
CV%	6.98	8.11	9.93	9.51

The (-) parentheses indicate the percentage decrease of data as compared to control.

4.3.2 Soil analysis

The residual Cr content in the soil of the experiment was analyzed and presented in fig-14 which revealed that the residual Cr in soil was more in the treated plants from 2.5-25ppm and thereafter, as the concentration of Cr increased in the soil the residual Cr left in soil gradually decreased. The Cr available in soil was 0.75mg/kg in the treated soil of 200ppm of Cr and 0.94mg/kg in 25ppm of Cr treated soils.

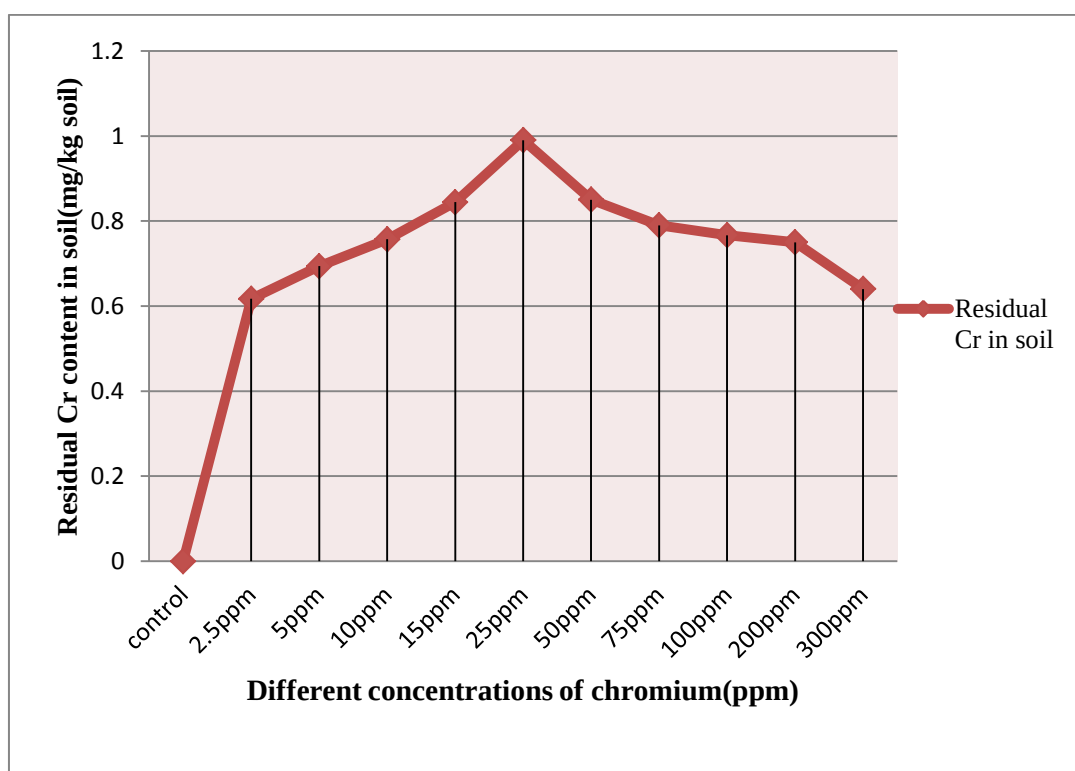


Fig 14: Residual Cr content in the postharvest soil. Data presented as mean of three replications.

4.4 Yield parameters

4.4.1 Number of fruits per plant

Total number of fruits per plant harvested after maturity was recorded and presented in table-9 which indicated that the Cr phytotoxic effect was clearly visible among treatments because of gradual reduction in fruit number as the concentration of Cr increased in the okra plants. The highest fruit no. was 7 in control plant and decreased up to 3 in the treatment of 200ppm Cr. The reduction in the number of fruits varied from 9.5% to 52.4% and was found statistically significant.

4.4.2 Total number of seeds per pod

Number of seeds formed per pod was counted in all the treatments and was shown in table-9 which indicated that the Cr effect reduced the seed number per pod significantly among the treatments. Alike, reduction in the fruit number the graded doses of Cr had also impact on reduction in seed number per pod and the range among the treatments varied from 37 to 15 corresponding to 10.9% to 59.9% respectively. The reduction in number of seeds per fruit among the treatments was found statistically significant.

4.4.3 Test weight

Average weight of 100 numbers of seeds was measured and showed in table-9 which indicated that the 100 seed weight of treated plants was decreased as compared to the untreated plant within a range of 5.46g to 4.33g. The heavy metal stress effect on test weight of 100 seeds was found statistically significant.

4.4.4 Harvest index (HI %)

The harvest index of the plants in all the treatments was calculated taking economic yield and biological yield taken into consideration and was presented in table-9 which indicated that HI% decreased significantly as the Cr concentration increased and varied between of 26 to 16.

Table 9: Effect of Cr on yield and yield attributes in okra plants. Data presented as the mean of three replications.

Treatments	Fruits/plant	Seeds/fruit	Test weight(g)	Harvest index
T1	7.00	36.67	5.64	26.64
T2	6.33 (-9.57)	32.67 (-10.90)	5.46 (-3.19)	25.99 (-2.43)
T3	6.00 (-14.28)	29.33 (-20.01)	5.36 (-4.96)	25.54 (-4.12)
T4	5.67 (-19.00)	27.33 (-25.47)	5.23 (-7.26)	24.94 (-6.38)
T5	5.33 (-23.85)	24.33 (-33.65)	5.17 (-8.33)	23.42 (-12.08)
T6	5.00 (-28.57)	23.33(-36.37)	4.83 (-14.36)	23.23 (-12.80)
T7	4.67 (-33.28)	21.33 (-41.83)	4.82 (-14.53)	22.42 (-15.84)
T8	4.33 (-38.14)	21.00 (-42.73)	4.72 (-16.31)	21.90 (-17.79)
T9	4.00 (-42.85)	19.00 (-48.18)	4.50 (-20.21)	20.55 (-22.86)
T10	3.33 (-52.42)	14.67 (-59.9)	4.33 (-23.22)	15.58 (-41.51)
SE(m)±	0.246	0.449	0.137	0.696
CD 5%	0.72	1.32	0.40	2.04
CV%	12.79	5.31	5.47	7.73

The parentheses indicate the percentage decrease of data as compared to control.

CHAPTER 5

DISCUSSION

DISCUSSION

Chromium (Cr) being a non-essential element for plants does not have any specific mechanism for its uptake. Cr uptake is a passive process and is also performed by carriers of essential elements as it is extremely soluble under all pH conditions. Cr VI is more toxic to plants and Cr toxicity in plants has been related to the interaction between uptake and translocation of mineral nutrients. Therefore, the present experiment on “Effect of Chromium on phytotoxicity in okra (*Abelmoschus esculentus*)” was conducted during *rabi* 2018 in the net house of AICRP on micronutrient in pots and the results obtained on the phytotoxic effect of Cr in plants, Cr accumulation and its translocation, growth and development, mineral nutrition, enzymes and other compounds and yield attributing parameters during the experimentation and the findings are discussed under different subheadings in this chapter.

5.1 Effect of Cr toxicity on seed germination

The results of the present study reveals that higher chromium concentration adversely influences the germination process of *Abelmoschus esculentus* seeds. The Cr treatment at 300ppm has no seed germination indicating that higher level of Cr produced toxic effects in seed germination. Similar results were also reported by Hira *et al.*, 2013. Increasing concentration of heavy metal significantly reduce the germination process as compared to lowest concentration of heavy metals which have the least harmful influence on the germination (Peralta *et al.*, 2001).

5.2 Chromium in plants

At high concentration of K_2CrO_4 , available form of Cr-VI, at 300ppm was found fatal and no seedling was established. So the treatments ranging from 2.5 to 200ppm is analyzed and discussed thoroughly below.

5.2.1 Growth and yield performance

Besides germination, root growth is also affected by heavy metals (Peralta and coworkers 2001) and the present finding showed that at higher doses of Cr-VI, there was a dose inhibition effect. Cr-VI in concentration up to 200ppm decreased root

growth of okra. But in lower concentrations cv. Kajari showed root elongations similar to the control. Decrease in root growth in presence of Cr might be occurred as a result of tissue collapse and in capacity of the roots to absorb water and nutrients from the soil.

Stem growth was affected by Cr exposure and the shoot length of okra decreased gradually to different doses of Cr-VI. These decrease in plant height could be due to reduced root growth and consequently decrease the nutrients and water transport to the higher parts of the plant. Moreover, Cr transport to aerial part of the plant can have direct impact on cellular metabolism of shoots contributing to the reduction in the plant height (Helena 2012).

Okra plants grown on soil with different graded doses of Cr-VI showed suppression of leaf growth, leaf area and leaf number in response to increase of Cr concentration. Reduction of leaf area might be a consequence of reduction of number of leaves and reduction in leaf size. The findings corroborate with the work of Dube *et al.*, 2003.

Plant yield is dependent on leaf growth, leaf area and leaf number as Cr affects most of the biochemical and physiological processes in plants, productivity and yields are also affected as revealed in the present findings. The increase in Cr supplied from 2.5 to 200ppm markedly decrease the grain weight and total biomass when compared to control plants. The reason might be reduction in nitrogen content as nitrogen, the primary essential element, involved as a constituent of biomolecules such as nucleic acids, nitrogen bases, coenzymes and proteins. Any deviation in nitrogen content would therefore severely inhibit the growth and yield of plants (Sundaramoorthy *et al.*, 2010).

5.3 Biochemical response

5.3.1 Chromium translocation and accumulation in okra plant

The pathway of Cr uptake in plants is not yet clearly elucidated. Plant uptake of Cr a passive process, no energy expenditure is required by the plants. However, Cr VI is an active mechanism performed by carriers. As Cr-VI has higher solubility, thus Cr is more toxic at lower concentrations and form stable complexes in soil. Cr accumulates mainly in roots and different parts of shoots like leaves, stems, fruits,

seeds and roots accumulate a major part and usually a small part is translocated the shoots. The accumulation of Cr in the different parts of okra was observed in the following order of roots> leaves> fruits> seeds corresponds to root (64%), leaf (15.4%), fruit (14.6%) and seed (5.6%).Corroborating these results are the findings of several workers, Tiwari *et al.*,2009 found that seeds accumulated about 1% Cr, while roots accumulated 98%.

5.3.2 Mineral nutrition

(A) Macronutrient (N, P, K)

Cr is structurally similar to other essential elements may affect plant mineral nutrition as evident in the present findings. Cr exposure decreases the uptake of macronutrients (N, P, K) and micronutrients (Zn, Fe, Mn, Cu) with the increase in Cr concentration. The reduction in N, P, K might be because of its displacement from physiological binding sites as high content of Cr displace the nutrients and consequently decrease uptake and translocation of essential elements.Similar differences in macronutrient accumulation due to metal stress was reported by Sundaramoorthy *et al.*, 2010 and Helena 2012. The toxic effects of Cr impaired P absorption processes and decrease the availability of P with the addition of Cr in soil as Cr can complexatively inhibit either the uptake and transport. The content of K in root and shoot is gradually decrease with the increase in the concentration of Cr might be because of changes in conducting tissues and blockage of xylem elements and contribute to a limited transportation of K from the root. It may be due to competition of Cr ions with K that in turn regulate the K uptake.

(B) Micronutrients (Zn, Fe, Mn, Cu)

A decreased uptake of micronutrients Zn, Mn and Cu was detected in the present study corroborated with the work of Liu *et al.*,2008 in *Amaranthus viridis*. But the Fe translocation and accumulation within the plant parts deviate from the other three micronutrients.Fe accumulation in root increases and decreases in fruit and seeds to the higher Cr concentration. But Fe accumulation in the leaf showed very irregular pattern. These findings agreed with the result of Sundaramoorthy *et al.*,2010.The reduction in Fe content in fruits and seeds because of the Cr competitiveness of the active sites of Fe. The increase concentration of root iron

might be (1) due to initial high Fe availability in the soil. (2) Fe may be competing with Cr due to the chemical similarity of these ions which leads to higher uptake of Fe by the roots. However the enhancement in the Fe concentration in this study contradicts the earlier result of Sundaramoorthy *et al.*,2010.

Zn content of the roots and shoots of the plants decrease gradually with concomitant increase in Cr concentration. The highest Zn content was recorded in control and the lowest was recorded in 200ppm. The reduction in the Zn content might be due to metal stress. Zn concentration in shoot was found higher when compared with the root which indicated that Zn uptake was affected by Cr treatments but not the translocation of Zn within the plant. The finding is in agreement with Jinhua *et al.*, 2006 and Sundaramoorthy *et al.*,2010.

Manganese (Mn) functions as enzyme activator and nitrogen metabolism. Mn content decreased significantly as per the increase in Cr concentration in roots, fruits and seeds. However, an initial increase in Mn content was observed in leaves from 2.5 to 25 ppm and later on the content of Mn decreased. The increase in the Cr concentration might have displaced Mn from the absorption sites. The findings corroborates with the work of Vartika *et al.*,2007.

Copper(Cu) content of roots and shoots decreased with a steady increase of Cr concentration and the Cu accumulation in the different plant parts are in the order of leaf > root > seed > fruit. The results are in conformity of the work of Sundaramoorthy *et al.*, 2010 and the decrease in Cu content may be due to the close association of Cu with N ligands.

5.3.3 Enzymes and other compounds

The activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) increased in case of Cr treated in okra plant to different Cr concentrations. Increase activities of CAT and SOD in Cr exposed plants are in accordance with earlier studies and other plant species (Chatterjee *et al.*, 2015).The results appeared might be initial antioxidant defense against Cr induced oxidative stress was provided by increased activities of SOD which is responsible for dismutation of superoxide(O_2^-) and hydrogen peroxide(H_2O_2) molecules. These observations suggest that these enzymes might have provided sufficient antioxidant

defense against H₂O₂ generation. Similar results have also been reported by Shankar and Pathmanabham (2004) and Panda and Choudhury (2005).

The metabolic responses of okra plant to Cr-VI exposure increased the concentration of osmolytes like proline and decreased in the proteins (osmoprotectant) as Cr induced oxidative stress. The result corroborates with the work of Sinha *et al.*, 2009.

5.3.4 Chlorophyll index by SPAD

The chlorophyll index recorded at different stages of crop growth treated with differential doses of Cr concentration revealed that the chlorophyll index was decreased as the concentration of Cr increased from 2.5 to 200 ppm than the control with %. SPAD (Soil and Plant Analytical Development) get readings in SPAD values that indicate relative chlorophyll content and is based upon a unique linearship between SPAD values and leaf area based N concentration in plants (Peng *et al.*,1993). As the nitrogen uptake is limiting under Cr heavy metal stress the chlorophyll is lower.

5.4 Soil analysis

Analysis of the residual Chromium availability in the soil after harvest revealed that more Cr was remain unutilized in the treated plants of 2.5, 5, 10,15, 25 ppm gradually increase in its concentration and thereafter the concentration decreased minimum to a level of 0.23mg/kg. The result indicated that up to 2.5 to 25 ppm concentration the Cr uptake by the plant root was in a restricted manner because of the competitiveness with the other essential elements, but as the Cr concentration increased from 50 to 200 ppm the uptake of Cr was more in the plant system might be because of mass passive uptake rather than energy dependent process. It suggests that plant will utilize more of Cr and the phytotoxicity is visible at high concentration of Cr available at soil root zone.

CHAPTER 6

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Okra (*Abelmoschus esculentus* L. Moench) is one of the most popular, tasty and gelatinous vegetables and it plays a major role in human diet. It contains protein, carbohydrates and vitamin C. It also has anti-oxidant and antidiabetic properties. It is one of the most significant vegetable crops having wider adaptation and economic importance in the horticultural industry.

Even though vegetables are an important component of our daily diet, there is little information available as to its contamination by heavy metals. Heavy metal like Cr causes detrimental effect, reduction in the productivity and yield. Odisha, being a state surrounded by many mining areas, the chances of stress due to heavy metals are much high. Okra being a hardy crop and suitable for various climatic conditions and soil types it is used in the present experiment. Thus, the aim of this work was to investigate the “**Effect of chromium on phytotoxicity in Okra (*Abelmoschus esculentus* L. Moench)**”, the effect of different concentrations of Cr on the growth, biochemical constituents and enzyme antioxidant system in a pot culture of okra as an approach to understand the plant’s response to heavy metals and the effects of pH on solubility and its interaction with other transition metals(Fe and Cu) available in soil.

Keeping the above fact view an experiment was conducted in the net house of AICRP on micronutrient, Department of Soil Science and Agricultural Chemistry during *Rabi* season 2018. Experiment was laid out in completely randomized design (CRD), 11 treatments and replicated thrice where the plants stressed with different concentrations of Cr (0, 2.5, 5, 10, 15, 25, 50, 75, 100, 200 and 300ppm Cr) at basal dose. K_2CrO_4 , available form of Cr-VI used in this experiment in chemical formulations. Observations were recorded and the result obtained in the investigation are summarized below,

Phytotoxicity of different concentrations of Cr in okra seed germination were determined before the start of experiment and revealed that germination was not occurred at 300ppm of Cr VI. However, the germination percentage decreased with a range of 100% to 40.8% between control and treated seeds. As the concentration of Cr increased from 2.5ppm to 200ppm, the rate of germination decreased linearly. However, germination percentage was significantly ($p<0.05$) affected at 25ppm to

200ppm by 40.7% to 59.2%. In a similar manner the seedling root and shoot length were also decreased.

Cr concentration of 300ppm was found extremely toxic to okra seed germination and no seedling was established.

The results after 25 days of exposure of Cr to *Abelmoschus esculentus* showed considerable reduction in shoot elongation (3.2% to 63.5%). The length of the plant was adversely affected due to chromium stress. In the subsequent observations on plant height at an interval of 15days from the previous observations (up to 70DAS) was also found similar trend of decreased plant height to high level of Cr concentration.

Root length recorded at the time of harvest decreased significantly as the Cr concentration increased from 2.5 to 200ppm from 27.3cm to 13.3cm respectively.

Effect of Cr on number of leaves was visible among treatments and it decreased as the concentration of Cr increased. The reduction in the leaf number varies from 8.5% to 62.8% at 25 DAS. Though the leaf number increased during the growth of the crop but similar trend of decrease in leaf number than control plant was observed.

The leaf area per plant (cm²) decreased within the treatments in a trend of T0>T1.....>T9 at 25 DAS. The similar pattern of decrease in leaf area was observed in the subsequent date of observations with an exception of 25 DAS to 40 DAS in 2.5ppm and 5ppm treated plants where the leaf area increased than the control plants but afterwards decreased.

The total biomass of treated plants decreased significantly as compared to the untreated plant with a range of 5.5% to 47.8% and 11.3% to 50.8% respectively.

Proline accumulation in leaf because of heavy metal (Cr) stress increased at high concentration of chromium and found 0.44mg/g FW to 2.6mg/g FW among the different treatments up to 490.1% at 200ppm.

Degradation of Total soluble protein contents of leaves were noticed in different treatments. The protein content decreased up to 62.7% with the increased level of Cr concentration applied in the plants.

Increase in the catalase activity was observed up to a tune of 0.854 to 3.592 UA/mg protein/minute and as high as 320.6%.

The superoxide dismutase enzyme activity of fresh leaves indicated that the activity of SOD gradually increased from 0.52units/mg FW to 1.12units/mg FW as the concentration of Cr treatment increased in okra plants and showed the stress activities up to 115.4%.

The rate of Cr accumulation was in order of root> leaf> fruit> seed. Accumulation of Cr increased with the increased in Cr concentration. The highest Cr accumulation was found in 200ppm.

The concentration of other micronutrients (Zn, Fe, Mn and Cu) in relation to synergistic and antagonistic effect of Cr in different plant parts like roots, leaves, fruits and seeds was determined. As the Cr concentration increased the Zinc (Zn) accumulation in the plant parts gradually deceased than their controls. The Zn accumulation in plant parts was in the order of leaf> seeds> root> fruit.

The iron (Fe) accumulation was gradually increased in roots to the high rate of Cr application and decreased in fruits and seeds. However, a very irregularly disorder accumulation was recorded in leaf Fe loading.

Manganese (Mn) concentration was in order of seed<root<fruit<leaf. Mn was accumulated highest in leaf but its accumulation gradually increases from 2.5 to 25ppm and thereafter a decreased accumulation of Mn in leaf was observed. In roots, fruits and seeds, Mn concentration decreased with the increase in Cr concentration.

Cu accumulation was highest in leaves and in order of leaves> roots> seeds> fruits. Similar decrease trend in Cu accumulation with the increase in the Cr concentration was also observed.

The macronutrients like N, P, K content of okra fruits were available in a decreased trend to the graded doses of Cr used in okra plants.

The reduction in the chlorophyll index varied from 7.27% to 19.19% at 25 DAS. Though the leaf number increased during the growth of the crop but similar trend of decrease in leaf number than control plant was observed.

The residual Cr in soil was more in the soils treated from 2.5-25 ppm and thereafter, as the concentration of Cr increased in the soil the residual Cr left in soil gradually decreased. The Cr available in soil was 0.75mg/kg in the treated soil of 200 ppm of Cr and 0.94mg/kg in 25ppm of Cr treated soils.

There was remarkable reduction in yields and yield attributing parameters due to metal stress. The Cr phytotoxic effect was clearly visible among treatments because of gradual reduction in fruit number as the concentration of Cr increased in the okra plants. The highest fruit no. was 7 in control plant and decreased up to 3 in the treatment of 200ppm Cr. The reduction in the number of fruits varied from 9.5% to 52.4%.

Alike, reduction in the fruit number the graded doses of Cr had also impact on reduction in seed number per pod and the range among the treatments varied from 37 to 15 corresponding to 10.9% to 59.9% respectively.

The 100 seed weight of treated plants was decreased as compared to the untreated plant within a range of 5.46g to 4.33g.

The harvest index of the plants in all the treatments was calculated taking economic yield and biological yield taken into consideration indicated that HI% decreased significantly as the Cr concentration increased and varied between of 26 to 16.

CONCLUSION

In the present study it was concluded that heavy metal chromium (Cr) has a very detrimental effect on growth, development and productivity of okra. There was a great reduction in metabolic activity of plant during Cr stress. Reduction in plant height, leaf area, numbers of leaves and fruits were also remarkable and also accompanied with various biochemical changes like degradation in chlorophyll, protein content increase in catalase activity and SOD (superoxide dismutase) activity. At higher treatment of Cr proline content increased. Under Cr stress yield is greatly hampered. Cr caused reduction in number of fruits up to 52%, HI% decreased up to 42%. Dry matter production, fruit weight and quality were also adversely affected by heavy metal (Cr). As the uptake was not hindered at root region, thereby, the translocation and accumulation of Cr in different plant parts increased with the increase in metal concentration at root zone. In order of passive movement of Cr, as Cr is not an essential element its accumulation was in order of roots> leaves> fruits> seeds. The amount of Cr uptake by different parts of plant caused improper nutrient mobilization as evident from the study. Consequently, this study will help to understand the phytoremediation technology and molecular mechanism in okra crop growth and productivity under heavy metal stress.

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