



Effect of chromium on seed germination, seedling growth and its remediation with zinc in cucumber (*Cucumis sativus*)

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Abstract: High amount of heavy metals can cause harmful effects to the environment. Chromium (Cr) is the highly toxic pollutant, which constitute a risk for aquatic and terrestrial life. Increased chromium concentration in soil resulting from industrial activities is critical to crop production. Chromium containing tannery effluent inhibits the overall growth and production of the crop plants. The aim of this investigation was to study the effect of different chromium concentrations (0.05, 0.10, 0.50 and 1.00 mM) and its combinations with zinc (0.20 and 0.50 mM) treatment on germination percentage, biomass yield, catalase and peroxidase activities in 15 days old cucumber (*Cucumis sativus*) seedlings grown *in vitro*. The obtained result showed that the increasing concentration of the chromium (0.05, 0.10, 0.50 and 1.00 mM) caused reduction in germination percentage, seedling growth (plumule length, radicle length and number of lateral roots), total biomass production, pigments (chlorophyll, phaeophytin and carotenoids), total protein and total sugar while the activity of antioxidants (catalase and peroxidase) increased. Application of zinc (0.20 and 0.50 mM) with high concentrations of chromium (0.20 and 0.50 mM) shows increased germination percentage, plant growth and reduces level of enzymes (catalase and peroxidase) in comparison alone concentrations of chromium.

Key words: Chromium, *Cucumis sativus*, Biomass, Pigments, Catalase, Peroxidase

Introduction

Chromium is found in abundant amount in earth crust. Beside natural source, chromium enters in ecosystem in various other ways such as major industrial sources including tanning and chromeplating industries (Sharma *et al.*, 2003). Discharges of these industrial residues that contains chromium, constitute a potential risk to aquatic life, animals, humans and plants due to the contamination of air, soil sediments, vegetation and water (Yang *et al.*, 2005). Sewage and fertilizers are also sources of chromium (Pillay *et al.*, 2003). Chromium has not been recognized as an essential element for plant growth however some stimulant effects have been reported (Samantaray *et al.*, 1998) with no specific mechanism for its sorption (Shanker *et al.*, 2005). At higher concentration chromium shows definitive retarding effects in plants (Samantaray *et al.*, 1998). In biological system, Cr occurs as Cr (III) and Cr (VI) but dichromate of Cr (VI) appears to be more toxic and its uptake is an active process. The transport of chromium in the root is very slow, accounting for the low levels of Cr in the tops of plants. Evidently, the element enters the vascular tissue with difficulty but once it enters, it can be rapidly transported which was studied by Skeffington *et al.* (1976). When chromium is present in excess amount in leaves (5-30 mg g⁻¹) it can cause chlorosis of new leaves and injure root growth also. Chromium stress can create metabolic modifications in plants, such as alterations in photosynthesis (Barbosa *et al.*, 2007), defradation of photosynthetic pigments and induction of oxidative stress (Panda and Choudhary, 2005). Chromium stress can also causes leaf chlorosis and necrosis and disturbances in mineral nutrition (Barbosa *et al.*, 2007). Chromium toxicity creates oxidative damages in biomolecules such as lipid and proteins (Vajpayee *et al.*, 2002) disturbance in superoxide dismutase, catalase (Shanker *et al.*, 2003), interference in peroxidase activity (Samantary *et al.*, 2001) and eventually, plant growth reduction which can leads to death of plants. According to Dube *et al.* (2003), higher

concentrations of chromium decrease the level of iron, copper, zinc and sulphur in most of the plants whereas phosphorus and manganese were increased in *Citrullus*.

The study was performed to know the toxic potential of chromium on seedling germination and plant growth and possible remediation through micronutrient application of zinc.

Materials and Methods

Cucumber (*Cucumis sativus*, variety- long green) seeds were taken for the petridish experiment. Petridishes and other glassware such as conical flasks, test tubes, pipettes, beakers *etc.* were washed with the detergent and tap water followed by distilled water and then autoclaved for one hour. Cucumber seeds were sterilized with 0.1% HgCl₂ for 2 minutes with frequent shaking and then thoroughly washed with distilled water.

0.01, 0.05, 0.10, 0.50 and 1.00 mM chromium solution of CrO₃ salt and 0.20 mM and 0.50 mM solution of zinc sulphate (ZnSO₄) were prepared separately in laboratory using double distilled water. Sixty seeds were placed in each treatment separately for overnight soaking in sterilized beakers. Next morning completely sterilized petridishes were lined with Whatman number-1 filter paper in triplicate and 20-20 seeds were placed and then moistened with 3 ml of test solution with respect to their treatments. The petridishes were washed out and fresh test solutions were applied every alternate day for the prevention from contamination and for maintenance of concentration and experiment was under observation for 15 days. After 72 hr germination percentage was recorded. Growth parameters like plumule length, radicle length, number of lateral roots, fresh and dry matter yield were measured after 15 days of inhibition with three seedlings in each petridish. Dry weights were taken after placing samples at 80°C in hot air oven for 48 hr.

Pigment estimation (chlorophyll, pheophytin and carotenoid) was done by using the method of Arnon (1949) as amended by Lichtenthaler (1987). Amylase activity was measured as mg starch hydrolysed g⁻¹ fresh weight of tissue, catalase activity was measured as ml H₂O₂ hydrolysed g⁻¹ fresh weight of tissue, and peroxidase activity as Δ O.D. g⁻¹ fresh weight of tissue by the methods of Katsuni and Fekuhara (1969), Bisht and Mehrotra (1989) and Luck (1963) respectively. The total proteins (μg mg⁻¹ fresh weight tissue) were estimated by the method of Lowry *et al.* (1951), while total sugars (μg mg⁻¹ fresh weight) were determined by the method of Dubais *et al.* (1956). The data observed in the experiment were statistically analyzed for the calculation of standard error (S.E.). Students 't' test was administered for testing the hypothesis with the help of computer software Sigma Stat 2.0 programme.

Results

Results of the experiment are based on mean of three replicates shown in Table 1 - 3, Fig. 1. There was reduction in germination percentage in different concentrations (0.05, 0.10 and 0.50 mM) of chromium but in 1.00 mM concentration there was not any germination found. Increased germination percentage was found in zinc treatments (0.20 and 0.50 mM) which combined with chromium (0.10 and 0.50 mM) concentrations (Fig. 1).

Plant growth in terms of plumule length, radicle length and number of lateral roots was gradually decreased with increasing concentration of chromium. In recovery treatment of 0.20 and 0.50 mM zinc in combination with higher concentration of chromium, growth parameters (plumule length, radicle length and number of lateral roots) were showed good results. Fresh and dry matter yield were decreased in alone concentration 0.05, 0.10 and 0.50 mM of chromium in comparison to control and recovery treatments of zinc (Table 1).

Table 2 shows the effect of chromium on pigments content of cucumber. At 15th day of chlorophyll content (total, 'a' and 'b') decreased as the chromium concentration was increased. Chlorophyll (total, 'a' and 'b') were found to be increased with lower concentration of chromium and zinc. Pheophytin and total carotenoid contents were also decreases gradually from control to 1 mM chromium. In recovery treatments pheophytin (total, 'a' and 'b') and total carotenoid were observed higher with lower concentration of chromium and zinc.

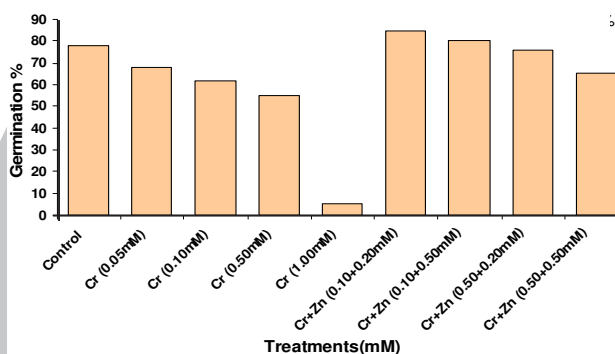


Fig. 1: Germination percentage in different concentrations of chromium and in combination with zinca

The activity of amylase was decreased with increasing concentration of chromium and significantly increases in lower concentration of recovery treatments of zinc. Catalase and peroxidase activity were increases with increasing concentration of chromium upto 0.05 mM and reduced down in 0.10 and 0.50 mM. Catalase and peroxidase activity were also higher in recovery treatment of zinc as compared to control. Peroxidase activity increased upto 0.05 mM concentration of chromium and it was also increased in recovery treatments of zinc in comparison to control (Table 3).

Discussion

The growth and metabolism of cucumber was adversely affected when seedlings were exposed to excess supply of chromium. This might be due to inhibited nutrient uptake into seedlings. The decrease biomass may be due to altered carbohydrate and nitrogen metabolism and reduction in protein synthesis or low photosynthetic reaction under excess chromium conditions as reported by Sharma *et al.* (1993, 2003). Decreased fresh weight of seedlings might be due to heavy loss of moisture contents in leaves. Decrease in dry weight was due to reduction in photosynthesis and chlorophyll a synthesis as suggested in cow-pea by Joshi *et al.* (1999). The decrease in fresh and dry weight biomass in our experiments also resembles to the results of Garg and Chandra (1990), which have shown decrease in biomass at 1 and 2 ppm of chromium (VI) after 18 hr of experiment in *Ceratophyllum demersum*. In the study of Corradi *et al.* (1993), Just 48 hour's exposure resulted in to chlorosis and reduced contents

Table - 1: Effect of chromium and recovery treatments (Cr+Zn) on fresh weight, dry weight, plumule, radicle length and number of lateral roots

Concentrations	Fresh weight (g)	Dry weight (g)	Moisture (%)	Plumule length (cm)	Radicle length (cm)	No. of lateral roots
Control	0.169±0.040	0.019±0.001	88.75±1.85	8.000±0.577*	2.267±0.120	10.333±0.333
0.05 mM Cr	0.147±0.024	0.013±0.002	91.15±1.65	4.133±0.376*	2.167±0.338	4.000±0.577*
0.10 mM Cr	0.126±0.008	0.014±0.034*	88.88±1.58	3.767±0.498*	1.000±0.289*	3.333±0.333*
0.50 mM Cr	0.111±0.005	0.010±0.000*	90.99±1.35	1.733±0.393*	0.400±0.115*	0.000±0.000*
1.00 mM Cr	0.000±0.000*	0.000±0.000*	0.00±0.00	0.000±0.000*	0.000±0.000*	0.000±0.000*
Cr+Zn (0.10+0.20 mM)	0.151±0.007	0.010±0.000*	93.37±1.11	4.167±0.441	1.933±0.233	12.333±2.333*
Cr+Zn (0.10+0.50 mM)	0.217±0.006*	0.017±0.003	92.16±1.25	4.500±0.289	1.300±0.473	6.667±0.667
Cr+Zn (0.50+0.20 mM)	0.178±0.018*	0.011±0.001	93.82±1.64	2.500±0.289	0.500±0.057	3.000±0.528
Cr+Zn (0.50+0.50 mM)	0.142±0.034	0.010±0.000	92.95±1.56	1.830±0.410	0.300±0.115	2.333±0.333

Values are mean of three replicates ± SE, * = Significant at p<0.05 level

Table - 2: Effect of chromium and recovery treatments (Cr+Zn) on pigments (mg g⁻¹ fresh weight of tissue)

Treatments	Chlorophyll			a / b ratio	Pheophytin			Total carotenoid
	Total	a	b		Total	a	b	
Control	0.699±0.025	0.461±0.026	0.230±0.019	2.00	0.988±0.010	0.726±0.005	0.261±0.013	0.340±0.016
0.05 mM Cr	0.657±0.008	0.394±0.015	0.204±0.015	1.93	0.892±0.004	0.625±0.005	0.286±0.016	0.326±0.019
0.10 mM Cr	0.487±0.020*	0.305±0.016*	0.149±0.009*	2.04	0.743±0.025*	0.541±0.028*	0.212±0.015*	0.245±0.017*
0.50 mM Cr	0.217±0.024*	0.107±0.016*	0.059±0.017*	1.81	0.382±0.017*	0.257±0.012*	0.105±0.013*	0.128±0.030*
1.00 mM Cr	0.000±0.000*	0.000±0.000*	0.000±0.000*	0.00	0.000±0.000*	0.000±0.000*	0.000±0.000*	0.000±0.000*
Cr+Zn (0.10+0.20 mM)	0.767±0.021	0.458±0.019	0.252±0.016	1.81	1.037±0.034	0.715±0.019	0.322±0.035	0.396±0.015
Cr+Zn (0.10+0.50 mM)	0.781±0.025	0.469±0.009	0.236±0.011	1.98	1.286±0.011*	0.816±0.011*	0.369±0.005*	0.348±0.011
Cr+Zn (0.50+0.20 mM)	0.495±0.028*	0.345±0.018*	0.110±0.014*	2.22	0.806±0.014*	0.631±0.012*	0.175±0.004*	0.124±0.016*
Cr+Zn (0.50+0.50 mM)	0.275±0.026*	0.131±0.015*	0.110±0.011*	1.19	0.585±0.011*	0.477±0.019*	0.108±0.008*	0.104±0.012*

Values are mean of three replicates ± SE, * = Significant at p<0.05 level

Table - 3: Effect of chromium and recovery treatments (Cr+Zn) on amylase, catalase, peroxidase, total protein and total sugar

Treatments	Amylase (starch hydrolyzed mg g ⁻¹)			Catalase (ml H ₂ O ₂ hydrolysed g ⁻¹)	Peroxidase (Δ O.D. g ⁻¹)	Total protein (μg g ⁻¹)	Total sugar (μg g ⁻¹)
	Total	α	β				
Control	12.60±0.46	8.33±1.14	4.26±0.69	175±20.46	35±3.64	107±6.11	2.31±0.05
0.05 mM Cr	11.36±0.41	8.16±0.35	3.20±0.76	186±10.62	40±2.21	124±15.92	2.25±0.08
0.10 mM Cr	7.50±0.23*	5.40±0.16*	2.10±0.15*	230±15.27*	39±2.95*	130±11.43*	2.00±0.13
0.50 mM Cr	3.61±0.11*	2.61±0.10*	1.00±0.08*	162±6.51	38±1.50	101±6.14	1.16±0.10*
1.00 mM Cr	0.00±0.00*	0.00±0.00*	0.00±0.00*	00±0.00*	00±0.00*	00±0.00*	0.00±0.00*
Cr+Zn (0.10+0.20 mM)	10.20±0.34	8.20±0.75	2.00±0.94	210±12.30	41±6.19	119±5.71	2.17±0.04
Cr+Zn (0.10+0.50 mM)	10.40±0.72	7.35±0.31	3.05±0.53	186±44.51	33±5.14	139±13.26	2.50±0.05
Cr+Zn (0.50+0.20 mM)	5.40±0.34*	4.28±0.17*	1.11±0.17*	174±15.77	48±8.64	145±20.12*	2.43±0.03
Cr+Zn (0.50+0.50 mM)	4.70±0.34*	3.15±0.20*	1.55±0.25*	193±14.04	51±9.10*	121±7.77*	1.46±0.03*

Values are mean of three replicates ± SE, * = Significant at p<0.05 level

of chlorophyll and carotenoid although ultra structure of chloroplast remain unchanged and 10 ppm concentration of chromium exposure resulted in to complete loss of growth in lateral roots while lesser concentration like 5 ppm chromium started damaging root cap, stomata and cotyledonary hair seemed to be collapsed and plasma membrane appeared to be detached from the cell wall under cytological studies.

Total inhibition of germination was observed at high concentration of metals, which might be due to poor growth in shoots and root, that may be correlated to the poor development of pigments as shown by chlorosis and reduced content of chlorophyll and carotenoid, although ultra structure of chloroplast remain unchanged. The growth of shoot and root were decreased with respect to the increase in metal concentration. It is known that biological and ecological consequences of excess of heavy metals, depend on the chemical forms in which these metals occur in the environment (Kabata-Pendias and Pendias, 1999). The chromium accumulation in plants affect their metabolic process in several ways including reduction in growth, photosynthesis, chlorophyll content, inhibition of certain enzyme activities and degradation of chloroplast damage through of thylakoid and mitochondria (Guilizzoni, 1991). With the maturity of the plant leaf senescence, chlorophyll degradation, lipid peroxidation and membrane permeability have also been found to be increased by Trippi (1990) in oat leaves. The chlorophyll content decreased significantly in higher doses of chromium and in both the dilutions of treated effluent in our experiments, can be directly correlated with the previous work of Guilizzoni (1991) and Trippi (1990).

The amylase activity (total amylase, α-amylase and β-amylase) was found to be significantly inhibited in higher doses of the treatments. The poor germination rate and seedling growth in treatments seem due to the poor break down of starch by amylase activity. Amylase and its important role during seed germination through hydrolysis of reserve starch and release in the energy has been worked out by Thevenot *et al.* (1992). Inhibitory effect of excess chromium on germination, growth, cell division and metabolic parameters at early stages of growth was also studied by Jain *et al.* (1999), who correlated their results the same way.

Antioxidant systems enzymes are activated and produced against heavy metal stress, damage of plasma membrane and generation of ROS, H₂O₂. Several cellular functions have also been described for plant catalase. According to Panda and Patra (2000) chromium ions increased the catalase activity in younger leaves while the activity decreased in older ones. The chromium in early stage enhances the catalase levels but in latter stage it causes adverse effect. The catalase activity which may be because of either feed back inhibition due to accumulation (because of poor growth) or some inhibitory effect of chromium on protein synthesis pathway in latter stage of seedling growth. The increase in catalase activity might be due to the increasing toxic effect of H₂O₂ and ROS produced as a result of membrane damage at higher level of heavy metals. Dixit *et al.* (2001) and Tondon and Gupta (2002), also found significant increase in catalase activity. According to Machold (1968) catalase was found to be more responsive to chromium stress than peroxidase. The reduced activity of catalase and

peroxidase were recovered completely in almost all recovery treatments. The decrease in catalase might be due to the direct effect of chromium induced reactive oxygen species on the enzyme protein or the decrease in catalase activity due to increase in H₂O₂ concentration, causing oxidative stress.

Sugar, an important constituent of metabolism is synthesized during photosynthesis and broken down during respiration by plants (Hemlata, 1997). The low sugar levels may be due to the lowered sugar synthesis or diversion of the metabolites to other alternate pathways. In chromium treated plants the activity of another H₂O₂ scavenging enzyme *i.e.* peroxidase increased pronouncedly as has been observed earlier in several species (Tewari *et al.*, 2002). Which might be attributed to rapid diffusivity of H₂O₂ produced in the cytosol or might be due to accumulation of high phenols and low protein formation in such conditions. Plants cope with excess heavy-metals ions by relying on two main mechanisms for detoxifying metals taken up into the cell complexation with organic compounds and partitioning within the vacuole. The vacuoles of metal treated plants often contain high concentration of metals complexed with organic acids. Amino acids can also form complexes with metal ions.

The objective of work was to find out the toxicity level of chromium on germination, amylase, pigment contents, proteins, sugar contents and certain antioxidative enzymes like catalase, peroxidase, and minimization of its toxicity using micronutrient zinc. Thus the toxicity level of chromium can be minimized by using micronutrients. In the present study it was concluded that the soil or water containing chromium toxicity, if used for plant growth, the use of certain micro-nutrients like boron and copper can minimized the heavy metal toxicity.

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