International Research Journal of Natural Sciences

Vol.4, No.3, pp.45-63, September 2016

Published by European Centre for Research Training and Development UK (www.eajournals.org)

ANTIOXIDANT SYSTEM RESPONSES OF THE CORIANDER (*CORIANDRUM SATIVUM*) PLANT TO CHROMIUM TOXICITY

Ameera Amen Aytah, Dina Zein ElAbdin Abdel Kader[1],Farida lafi Elharbi

Biology department, Taibah University, Al Madinah Al Mounawarrah, KSA.

ABSTRACT :*The aim of this study was to compare the effect of different doses of chromium on antioxidant system of the coriander. A total of the coriander seeds were divided among 3 groups. The cultivation of plants was occurred and treated with different concentrations of sulfate (0.4, 0.8 &1.6 mM magnesium sulfate) for 6 weeks until completely growth and ensure for healthy. Each treatment group was divided into three subgroups to handle treatment concentration of 0 µM, 50 µM and 100 µM chromium. Antioxidants (peroxidase, GSH, Ascorbic acid, ASPX, SOD and Catalase*) *and lipid peroxidation were recorded. All level of Cr treatments lead to numerically decreased in peroxidase, GSH, Ascorbic acid, ASPX, SOD of the coriander than the control. Furthermore, 100 µM of Cr treatments lead to numerically decreased in catalase of the coriander than the control. However, all level of Cr treatments led to numerically increase in lipid peroxidation of the coriander plant than the control. These adverse effects of chromium were encountered with increasing sulfur supply in the irrigation solution. Sulfur decreases the uptake of chromium in coriander plant. Sulfur also alleviates most harmful effect of chromium by enhancement of the antioxidative system (especially GSH content) in the coriander plant. The effect of sulfur was more effective at 50 µM Cr than 100 µM Cr. The two concentration of sulfur were both effective*.

KEYWORDS: *Chromium toxicity, sulfur, antioxidants, coriander.*

INTRODUCTION

Heavy metal contamination of soil is one of the world's major environmental problems, posing significant risks to ecosystems; therefore, development of a remediation strategy for metal contaminated soil is necessary for environmental conservation and human health.

Chromium (Cr) is a naturally occurring element and it is one of the components of the earth's crust. Chromium compounds are among the most dangerous environmental pollutants due to their high toxicity and wide use in numerous industrial processes. They are extensively employed in leather processing and finishing **(Nriagu and pacyna, 1988)** in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture, in the production of chromic acid and specialty chemicals. Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and, until recently, wood preservation.

It is assumed that chromium plays an important role in the metabolism of all living cells. However, at high concentrations it is toxic, mutagenic, and carcinogenic, especially in Cr (VI) form, which causes oxidative stress, DNA damage, and modulates the activity of regulatory apoptotic gene *p53*. The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species, although there are various other valence states which are unstable and short-lived in biological systems. Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments **(Becquer *et al*., 2003).**

Being a heavy metal Cr is a potential agent of oxidative stress inducing oxidative damage to lipids, proteins and DNA-like biomolecules **(Shanker and pathmanabahn, 2004).** Cr changes in activity of antioxidant enzymes as well as of other enzymes in order to disrupt the nutrients and water balance **(Pandey and Sharma, 2003)**. It is also reported that Cr could stimulate the formation of free radicals and reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). Furthermore, under natural growth conditions, plants are adapted to minimize the damages induced by ROS **(Jinhua *et al.*, 2009).**

Chromium is easily absorbed by the plants from the soil and atmosphere, accumulate in the organs of the plants and show their cytotoxic and phytotoxic effects **(Parmar and Chanda, 2005).**

An important response to Cr stress by aerobic cells is the production of reactive oxygen species (ROS), like superoxide radical , (O_2^-)hydroxyl radical ($OH^{\cdot}$), alkoxy radical ($RO^{\cdot}$), singlet oxygen ($^1O_2$), and toxic hydrogen peroxide (H_2O_2) molecules **(Breusegem *et al*.,2001).**

Many methods have been tried for the removal of Cr (VI) from wastewaters, including ion-exchange resins, filtration and chemical treatment **(Singh and Tiwari, 1997).** However, none of these methods are completely satisfactory and all feature the following disadvantages: (a) generation of a large amount of secondary waste products due to various reagents used in a series of treatments such as reduction of Cr (VI), neutralization of acidic solution and precipitation, and (b) instability of ion-exchange resins due to serious oxidation by hexavalent chromium. Thus, the development of new, cost-effective, more environmentally friendly methods is needed **(Bijay et al,.2010)**.

Due to Cr application the activity of iron porphyrin enzyme catalase was improved in bean plants **(Samantary, 2003)** The Cr exposure decreased the activity of catalase which coincides with the findings of **(Panda *et al.*, 2003)** who reported depression in catalase activity due to borderline elements.**(Panda *et al.*, 2003)** found improved activity of catalase due to Cr in leaves of wheat

In plants, glutathione (GSH) is involved in cellular processes, including defense against reactive oxygen species (ROS). Glutathione exists in two form reduced glutathione

(GSH) and oxidized glutathione (GSSG). The reduction potential of glutathione depends on the intracellular GSH/GSSG ratio **(Pekker *et al.* , 2002).** Change in the redox ratio of glutathione mainly depends on the pH, total GSH concentration, GSH biosynthesis and GSH catabolism. Ammonium ion, derived either from nitrogen assimilation or from photorespiration, is incorporated into glutamine by a reaction catalyzed by glutamine synthase (GS), and glutamine is further converted into glutamate catalyzed by glutamate synthase (GOGAT). The conjugation of GSH with such molecules is governed by glutathione S-transferase (GST). GST catalyze the conjugation of GSH with metal ions and help them to sequester into vacuole **(Shanker *et al.* , 2004; Hossein *et al.* , 2006).**

The enzymatic mechanism of detoxification involves GPX, GR and other enzymes. The oxidized glutathione (GSSG) formed is converted back to GSH by NAD(P)Hdependent glutathione reductase (GR) glutathione peroxidation (GPX) and its regenerating cycle **(Shanker and pathmanabhan. , 2004).** Moreover, **Sies (1999)** provided evidence that GR is the rate limiting enzyme in the ascorbate dependen H_2O_2t destruction.

One of the most deleterious effects induced by heavy metal exposure in plants is lipid peroxidation, which can directly cause biomembrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane, is regarded as a reliable indicator of oxidative stress **(Dey *et al.* , 2007).**

Chromate ion is accumulated intra-cellularly by the sulfate transport system. The dual role of chromium in cell metabolism foresees the presence of effective mechanisms of controlling its entry into the cells, accumulation, and detoxification.

It has been suggested that the entrance of Cr (VI) into the yeast cells in an oxy-anionic form occurs *via* sulphate- specific transport systems. Genes involved in sulphate and chromate transport has been identified. Uptake of Cr (VI) by intact barley seedlings was inhibited by sulphate **(Cherest *et al*., 1997 and Smith *et al*., 1995).** Increasing sulphate concentration in the medium may compete with chromium and decrease its entrance to the cell.

Apart from the role of sulfate transporters in Cr uptake, S metabolism may affect Cr tolerance and accumulation due to the capacity of certain reduced S compounds to bind and detoxify metals and metalloids **(Freeman *et al.*, 2004; Pilon-Smits et al., 1999; Zhu *et al.*, 1999a, 1999b).**To investigate the potential role of plant S metabolism in Cr accumulation and tolerance, the interactions between sulfur (S) nutrition and Cr tolerance and accumulation were considered in this study.

MATERIALS AND METHODS

The coriander seeds were obtained from market. Seeds were surface sterilized with 70% ethyl alcohol then washed with dist water several times. The seeds were tested for germination percentage and it was 98%. Seeds were cultivated in pot 20 cm diameter half filled with beatmoos. Cultivated seeds were divided into 3 groups depending on sulfur

(Mg sulfate) concentration (0.4 mM (control), 0.8 mM and 1.6 mM). Each group consists of 45 pots and each pot contains 20 seeds. Seeds were irrigated with Hoagland' solution containing the different concentrations of sulfur for two weeks until suitable growth and ensure for healthy and safety. Each group was subdivided into 3 subgroups and supplied with 0.0, 50 and 100 µM Cr (potassium dichromate $K_2Cr_2O_7$). Plants were harvested 2, 4 and 6 weeks post chromium treatments.

Estimation of antioxidants

- ***Preparation of enzyme extract***
- Extract of fresh plant tissue in 0.1M phosphate buffer pH 7 by grinding with a pre-cooled mortar and pestle. Centrifuge the homogenate at 18000g at 5 C for 15mim. Use the supernatant as enzyme source within 2-4hr. Store on ice till the assay is carried out.
- **Peroxidase activity (POD)**
- Peroxidase (POD) activity was determined by following the dehydrogenation of guaiacol at 436 nm (**Malik and Singh, 1980**).
- **Catalase activity (CAT)**
- Catalase activity was assayed in a method following (**Aebi, 1983**).
- **Determination of Glutathione (GSH) content**

Total glutathione content was determined spectrophotometrically following the method described by **Griffith (1980).** Two milliliters of the leaf extract were added to 8 ml of phosphate solution. One milliliters of DTNB reagent was added. The optical density of the solution was measured at 412 nm. Blank was prepared using 2 ml of phosphate buffer solution, and 1 ml DTNB reagent. Standard curve was used to calculate the glutathione level.

- **Ascorbic acid content (Free Ascorbate)**

Free ascorbate was assayed photometrically following **Frank (1955)** through the reduction of 2, 4-dichlorophenolindophenol (DCPIP). Leaf segments were homogenized and extracted in 5mL ice cold 2% (w/v) metaphosphoric acid. The homogenate was filtered through a paper filter. An aliquot of 0.3mL of the filtrate was mixed with 0.2mL of 45% (w/v) K_2HPO_4 and 0.1mL distilled water. After incubation for 15 minutes at 25°C, 1mL of 2kmol m^{-3} citrate buffer (pH 2.3) and 1mL of 0.003% (w/v) DCPIP were added. The absorbance was measured at 524nm immediately. The content of ascorbate was calculated by referring to a standard curve.

- **Ascorbic acid peroxidase (ASPX) activity**

ASPX was determined using the method of **Nakano & Asada (1987)**. Activity was determined by following the H_2O_2- dependent decomposition of ascorbate at 290 nm. One milliliter of the reaction mixture contained 50 mM potassium phosphate (pH=7), 0.5 mM ascorbate, 0.1 mM EDTA and 0.1 mM hydrogen peroxide. The reaction was initiated by addition of hydrogen peroxide, and oxidation of ascorbate was followed by the decrease in absorbance at 290 nm at 30 seconds interval for 5 min. One unit of APX activity is defined as the amount of enzyme that oxidizes 1 µmol of ascorbate per min at room temperature.

- One enzyme unit = 0.358 absorbance change/min.
- **Superoxide dismutase (SOD) activity**
- SOD was measured by the photochemical method as described by **Winter *et al.* (1975).** Assays were carried out under illumination. One unit SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reaction at 560 nm.

Cu/Zn-SOD was measured by the photochemical method as described by **Giannopolitis and Ries (1977)**. Assays were carried out under illumination. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reduction at 560 nm.

Lipid peroxidation measurement as malondialdehyde (MDA) accumulation

Estimation of lipid peroxidation was assayed spectrophotometrically using TBA-MDA assay. Lipid peroxides were extracted using 0.5g fresh tissue with 0.3 ml of 1 % meta-phosphoric acid (PH = 2) and 1 ml of 0.6 % thiobarbituric acid (TBA) in air tight tube and kept in aboiling water bath for 45 min . The samples were cooled room temperature and 5ml butanol was added along with stirring of mixture. The butanol phase was separated by centrifugation at 1000 rpm for10min and transferred to glass cuvettes. The color of TBA chromogen was measured at 532 nm **(Hodges *et al*, 1999).**

- MDA level = (A 532 * 100) / 1.56. st.coffienient

RESULTS

- **Peroxidase:**
- The results in fig. (1) showed that peroxidase content declined with increasing Cr concentration. The result also showed that increasing sulfate induced decreases in peroxidase content when it is applied alone.
- The combination between S and Cr significantly increased peroxidase content after 6 weeks of treatment. The concentration 0.8 mM S was the most effective.
- **Catalase (fig. 2):**
- Increasing Cr concentration induced a sharp decline in catalase content after 2 and 4 weeks when compared to control. While after 6 weeks there was a slight decline in catalase content.
- Increasing S concentration encountered the adverse effect of Cr and increased catalase content all over the experimental period. The effect was dose dependent.
- GSH (table 1)**:**
- The results showed that GSH increased with increasing plant age. Increasing sulfur concentration significantly increased GSH concentration in both Cr treated or untreated plants. It was also shown that increasing Cr concentration significantly decreased glutathione concentration when compared to control.
- Increasing S concentration significantly increased GSH levels in Cr treated plants. The most effective concentration was 1.6 mM S. the highest value was at 1.6 mM S with 50 µM Cr.

- **Ascorbic acid:**
- The data in figure (3) showed that ascorbic acid increased with increasing plant age. The data showed also a non-significant difference in ascorbic acid concentration with increasing either Cr or S concentration.
- Superoxide dismutase (SOD):
- Increasing Cr decreased SOD content in coriander leaves. On the other hand, increasing sulfur concentration induced a highly significant increase in SOD content when compared to control.(figure 4)
- Application of sulfur to Cr treated plants significantly increased SOD content.
- ASPX**:**
- Increasing Cr induced a significant decrease in ASPX content when compared to control. The most effective concentration was 100 µM Cr.(figure 5)
- Treatment with sulfur alleviates the adverse effect of Cr by increasing ASPX content. The effect was more pronounced at 50 µM Cr.
- **Lipid peroxidation (table 2):**
- Lipid peroxidation significantly increased with increasing Cr concentration. The highest value was after 2 weeks at 100 µM Cr. It was shown that increasing sulfur concentration alleviated the adverse effect of Cr by decreasing lipid peroxidation.
- The lowest values were obtained at 1.6 mM S with 50 µM Cr and 0.8 mM S with 100 µM Cr after 6 weeks of treatment.

DISCUSSION

Heavy metals like Zn, Fe, Cu and Mn are essential for plant growth and important constituent of many enzymes of metabolic importance. Other metals like Pb, Cd, As, Se Al and Cr are biologically non-essential and toxic above certain threshold levels. Cr is toxic to plants and does not play any role in plant metabolism **(Dixit *et al*., 2002).** Accumulation of Cr by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultrastructural modifications of the chloroplast and cell membrane **(Panda, 2003; Choudhury and Panda, 2004 and Hu *et al*., 2004).**

The results in the present investigation indicated that all level of Cr treatments lead to numerically decreased in peroxidase, GSH, ASPX, SOD of the Coriander than the control group. Furthermore, the results indicated that 100 µM of Cr treatments lead to numerically decreased in catalase of the Coriander than the control group. However, the results indicated that all level of Cr treatments lead to numerically increased in lipid peroxidation of the Coriander than the control group. These results are in agreement with those reported by **Maiti *et al.* (2012)** reported that the antioxidative responses of enzymes like SOD, guaiacol peroxidase (GPX), and APX showed significant variation to Cr. However, lipid peroxidation and protein oxidation were increased under Cr stress. This indicates that Cr-induced oxidative damage in green gram leaves are linked to lipid peroxidation. The increase in lipid peroxidation in Cr-treated green gram leaves may be a reflection of the decline of antioxidative enzymes. Similar results were earlier obtained in

our laboratory with Al and Cr **(Karuppanapandian *et al*., 2000). Verkleij and Schat (1990) and Gallengo *et al*. (1996)** reported a similar increase in lipid peroxidation when plants were treated with Cr. It has been shown that Cr can react with $O2^{\cdot -}$ radical to form more reactive $OH^{\cdot}$ radical. Since lipid peroxidation is generally considered to be induced by free radicals, it is possible that the effect of Cr on the oxidative damage in green gram leaves is mediated through earlier, especially $OH^{\cdot}$ radical. If this suggestion is correct, then the promotive effect of Cr on the oxidative damage in green gram leaves should be prevented by the addition of free radical scavengers.

Plant tolerance mechanisms require the coordination of complex physiological and biochemical processes, including changes in global gene expression **(Dalcorso *et al*., 2010).** Plants employ various strategies to cope with the toxic effects of metals or metalloids. Resistance to HM stress can be achieved by "avoidance" when plants are able to restrict metal uptake or by "tolerance" when plants survive in the presence of high internal metal concentration. Avoidance involves reducing the concentration of metal entering the cell by extracellular precipitation, biosorption to cell walls, reduced uptake or increased efflux. In a second type of situation, HMs are intracellularly chelated through the synthesis of amino acids, organic acids, GSH, or HM-binding ligands such as metallothioneins (MTs), phytochelatins (PCs), compartmentation within vacuoles and up regulation of the antioxidant defense and glyoxalase systems to counter the deleterious effects caused by ROS and MG **(Leyval *et al*., 1997 and Seth *et al*., 2012).**

The antioxidative enzymes tolerant to Cr induced oxidative stress recorded a similar response. Peroxidases in plants are commonly referred as monomeric heme containing enzymes which are able to catalyze the reactions with phenolic moieties as electron donor to reduce H_2O_2 into H_2O. The hyperactivity of peroxidase under Cr contaminated waste water in many aquatic plants has been implicated as constant detoxification of H_2O_2. Our results are in conformity with earlier studies on upregulation of antioxidative enzymes under heavy metal **(Shanker *et al*., 2005).** Peroxidase is attributed to lignifications by the cross linking of phenolic residues in the cell wall matrix. This is probably forwarded for the rigidity of cell wall or shielding the membrane from peroxidation under the condition of metal stress **(Bhattacharjee, 2005).**

Likewise, ASPX enzyme is displayed with its variable isozymic forms for antioxidation processes. In H_2O_2 most cases, ASPX is responsible for such activities with cytosolic, chloroplastic, mitochondrial and other organelle under various metal stresses **(Hu *et al*., 2004).** To be more specific, ASPX is supposed to be less involved in H_2O_2 detoxification rather than the regulation of H_2O_2 as a signaling molecule **(Cuypers *et al*., 2011).** SOD is furnished with first line of defense, where O_2. - is dismuted in H_2O_2 and O_2. The accumulation of $O_2^{\cdot -}$ or other free radicals in general could be detoxified by SOD.

Cr, like other heavy metals, is quite efficient to induce SOD to detoxify the effect of O_2. - . However, a discriminatory pattern is also reported in many studies. Those findings are coordinated with variation of metal concentrations, duration of exposure, tissue types and even the valence of metals **(Tanaka *et al*., 1996).** Whatever the cases might be, the

elevation of SOD activity in general is a clear indication to detoxify the O_2^- and, other ROS in downstream pathways in different cell organelle under metal stress.

- A decrease in enzymatic activity may be due to formation of protein complex with Cr (III) changing the conformation and solubility of the protein.
- The results of present study clearly revealed an induction of the antioxidant enzymes (Peroxidase, ASPX, Catalase and SOD) upon exposure to different Cr concentrations. The increase of POD activity as indicator of heavy metal stress was reported by many studies **(MacFarlance and Burchett, 2001; Markkola *et al.* , 2002; Baycu *et al.* , 2006; Wang *et al.* , 2010).** In this work, the changes in antioxidant enzyme activities in response to heavy metal stress appeared to be dependent on metal concentration. Activities of these enzymes might increase in order to cope with the oxidative stress imposed by heavy metals on plants, as was repeatedly found in other experiments **(Thomas *et al.* , 1999; Radotic *et al.* , 2000; Shah *et al.* , 2001; Iannelli *et al.* , 2002; El-Khatib *et al.* , 2004; Baycu, *et al.* , 2006).**

As a general response of plants to heavy metal stress, activity of antioxidant enzymes, such as PRX, APX, SOD and GR, increase at lower concentrations, whereas their activity decrease at higher concentrations. According to **(Shanker *et al.*,**2004) the increase in antioxidant enzymes observed might have been in direct response to the generation of superoxide radical by Cr-induced blockage of the electron transport chain in the mitochondria. In addition, the decrease in the activity of the enzymes as the concentration of the external Cr increased might be because of the inhibitory effect of Cr ions on the enzyme system itself. On the other hand **Sen *et al.*,(1994)** reported a decrease in CAT activity at higher Cr (VI) concentrations in *Salvinia natans* L. plants; which is parallel to our results. It was also reported by **Dhir *et al.*,(2009)** that the activities of APX and GR were enhanced under Cr stress except CAT in *Salvinia natans* L.

The excess production of ROS by Cr (VI) inactivated CAT activity through inactivating enzyme-bound heme group, which was reported by **Arvind and Prasad** (2005) in cadmium-induced oxidative stress. **Pereira *et al.* (2008)** showed that chromate causes a strong decrease of sulfur assimilation flux and of sulfur metabolite pools including GSH. It is tempting to consider that this sulfur depletion can be a direct consequence of the inhibition of sulfate uptake by chromate. According to literature, three main hypotheses have been proposed for chromate toxicity: (1) its reduction to Cr(V)/ Cr(IV) leading to Fenton reactions and oxidative damages, (2) the formation of Cr(III)-DNA-L adducts, and (3) protein oxidation and aggregation. The three hypotheses are not exclusive.

The addition of methionine relieves chromate toxicity **(Pereira *et al.* ,2008)** Molecular characterization of sulfate transport has been reported for different microorganisms. The sulfate transport in *E. coli* and in a cyanobacterium (synechococcus sp) has been shown to be composed of five polypeptides: the thiosulfate and the sulfate binding proteins, two inner membrane transport proteins and a hydrophilic membrane associated ATP binding protein, probably an energy-couphg component **(Sirko et al., 1990)**. In *N. rrnssn*, two sulfate transporters are encoded by two unlinked genes. The *Cy14* gene encoding permease I1 has been cloned and sequenced. The analysis of the sequence of the *Cy-I4*

gene has shown that sulfate permease I1 is probably a carrier protein with 12 putative helical membrane spanning domains **(Ketter *et al.* 1991).** The two sulfate permeases from A *crnssa* are expressed at different developmental stages of the fungus and are under the control of a complex regulatory system **(Marzlcf 1994).** In S. *crrmisiat',* they have also found two different sulfate transporters, **(Sandai and Marcker 1994).**

High levels of Cr were detected in roots of *Brassica juncea* accumulated more Cr when chromate was offered in the absence of sulfate. This suggests inhibition of chromate uptake and accumulation by sulfate when both anions are present in the growth medium, likely due to competition for the active binding site of the same sulfate transporters. Interaction between sulfate and chromate uptake is also supported by the results obtained from the sulfate uptake experiment, which showed a markedly diminished ability of plants exposed to chromate to take up sulfate from the nutrient solution **(Michela *et al.*, 2008).** Concomitant with the reduction in sulfate uptake rates, a significant decrease of transcript accumulation for the low-affinity sulfate transporter *Bj*ST1 was observed in plants grown in the presence of Cr under –S and +S conditions. Therefore, other than the probable competition with sulfate for the binding to the same carriers, chromate had an additional way to interfere with sulfate absorption (i.e., the inhibition of low affinity sulfate transporters transcription).

Earlier studies reported a down regulation of *BjST1* associated with the activation of the first steps of sulfate reduction and the concomitant enhanced accumulation of thiols in *B. juncea* in response to cadmium stress **(Heiss *et al.*, 1999).** Similar to these results, in *Brassica* plants exposed to chromate, the observed down regulation of *BjST1* was concomitant with an induction of three genes encoding components of the sulfate assimilation pathway and GSH biosynthesis. The expression of *atps6*, *apsr2*, and *gsh2* seemed to be regulated in a coordinated way: all were induced on chromate treatment in –S and +S plants. Similar coordinated changes in transcript amounts of *BjST1*, ATP-sulfurylase, and APS reductase, together with variations in contents of *O*acetylserine (thiol)lyase and thiols observed in *B. juncea* plants in response to Cd, were hypothesized by **Heiss *et al.* (1999)** to represent an essential part of the cellular response in countering Cd toxicity. Consequently, the higher sulfate uptake at root level and the enhanced sulfate assimilation were thought by the authors to be necessary for providing metal-chelating S compounds, such as GSH and phytochelatins (PCs).

A similar explanation could in part be extended to Cr stress. We found the levels of GSH considerably increased in plants of Cr-treated S-supplied plants **(Heiss *et al.* 1999)** found a depression of GSH levels in plants supplied with Cd, which was parallel with the increased synthesis of PCs, in our study GSH was accumulated more in Cr-treated plants, in agreement with observations by **(Sanità di Toppi *et al.* 2002)** in *B. oleracea***.**

- Therefore, there may be a coordinated regulation of the sulfur assimilation enzymes in response to an augmented demand for GSH, as already hypothesized for Cd **Heiss *et al.*, (1999).**

Rather than being a substrate for PCs biosynthesis during Cr stress, GSH may play a major role in the reduction of Cr(VI) to the less toxic trivalent form. In such a reaction, the transfer of three electrons is required, and only few biological compounds in cells function as efficient reductant for Cr(VI), including GSH and ascorbate **(Kaim and Schwedersky, 1994).**

The oxidation of GSH by Cr(VI) has been previously reported by **McAuley and Olatunji (1977).** Furthermore, during Cr(VI) to Cr(III) reduction, reactive oxygen species can be produced, and in this view the ascorbate/glutathione cycle might represent a fundamental mechanism regulating the cellular oxidative balance **(Noctor and Foyer, 1998).**

CONCLUSION

It could be concluded that Cr increased lipid peroxidation and decreased most antioxidants (enzymatic and non-enzymatic). These adverse effects of chromium were encountered with increasing sulfur supply in the irrigation solution. Sulfur decreases the uptake of chromium in coriander plant. Sulfur also alleviates most harmful effect of chromium by enhancement of the antioxidative system (specially GSH content) in the coriander plant.

The effect of sulfur was more effective at 50 µM Cr than 100 µM Cr. The two concentration of sulfur were both effective.

REFERENCES

Aebi, H.E., (1983): Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Verlag Chemie, Weinhem, pp: 273-286.

Aravind P, Prasad M (2005) Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids. Chemosphere 61:1720–1733

Baycu, G., Tolunay, D., Ozden, H., & Gunebakan, S. (2006): Ecophysiological and seasonalvariations in Cd, Pb, Zn, and Ni concentrations in the leaves of urban deciduous trees in Istanbul. *EnvironPollut, 143*, 545-554. http://dx.doi.org/10.1016/j.envpol.2005.10.050

Becquer T, Quantin C, Sicot M, Boudot JP (2003): Chromium availability in ultramafic soils from New Caledonia. The Science of the Total environment 301, 251-261.

Bhattacharjee S. (2005): Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. *Current Science India* 89: 1113–1121.

Bijay Kumar Sethi1, Satyajit Kanungo1,*, Jyoti Ranjan Rout1, Prativa kumari Nanda2, Santi Lata Sahoo1.(2010): Effect of Chromium on *Mucor* species and optimization of growth conditions. Nature and Science;8(4).

Breusegem FV, James F, Dat D, Inze D (2001): The role of active oxygen species in plant signal transduction. Plant Sci. 161: 423-431.

Cherest H, Davidian JC, Thomas D, Benes V, Ansorge W, Surdin-Kerjan Y (1997): Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae, Genetics, 145:*627–635.
Choundhury S, Panda SK (2004): Toxic effects, oxidative stress and ultrastructural changes in moss Taxithelium nepalense (Schwaegr.) Broth. Under chromium and lead phytotoxixity. Water Air Soil Pollut. 167(1-4):73-90.
Cuypers A, Smeets K, Ruytinx J, Opdenakker K, Keunen E, Remans T, Horemans N, Vanhoudt N, Van Sanden S, Van Belleghem F, Guisez Y, Colpaert J,Vangronsveld J (2011): The cellular redox state as a modulator in cadmium and copper responses in Arabidopsis thaliana seedlings. J. Plant Physiol. 168:309-316.
DalCorso G, Farinati S, Furini A (2010): Regulatory networks of cadmium stress in plants. Plant Signal Behav 5:663–667.
Dey, S.K., J. Dey, S. Patra and D. Pothal.(2007): Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Braz. J. Plant Physiol.,* 19(1): 53-60.
Dhir B, Sharmila P, Pardha Saradhi P, Nasim SA (2009) Physiological and antioxidant responses of Salvinia natans exposed to chromium-rich wastewater. Ecotoxicol. Environ. Saf. 72:1790-1797.
Dixit, V.; Pandey, V. and Shyam, R., (2002): Chromium ions inactivate electron transport and enhance superoxide generation in vivo in pea (Pisum sativum L.cv. Azad) root mitochondria. Plant Cell and Environment. 25, 687–693.
El-Khatib, A.A., Faheed, F.A. and Azooz, M.M. (2004): physiological response of *Eucalypuse -rostrata* to heavy metals air pollution. El-Minia Science Bulletin, Egypt, 15: 429-451.
Frank W., (1955): Ascorbinsaure. In Moderne Methoden der pflanzen analyse, vol. II (eds K.K. peach & M.V. Tracey), PP. 95-112. Springer, Berlin.
Freeman, J.L., M.W. Persans, K. Nieman, C. Albrecht, W. Peer, I.J. Pickering, and D.E. Salt. (2004): Increased glutathione biosynthesis plays a role in nickel tolerance in thlaspi nickel hyperaccumulators. Plant Cell 16:2176–2191.
Gallengo SM, Benavides MP, Tomaro ML (1996): Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Sci. 121: 151-159.
Giannopolitis, N. and Ries, S.K. (1977): Superoxide Dismutase. 1. Occurrence in higher plants. Plant Physiol. 59:309-314.
Griffith, O.W.,(1980): Determination of glutathione and glutathione disulfide using glutathione reductase and 2 vinylpyridine. Anal. Biochem. 106, 207–211.
Heiss, S., H.J. Schaefer, A. Haag-Kerwer, and T. Rausch. (1999): Cloning sulfur assimilation genes of *Brassica juncea* L.: Cadmium diff erentially aff ects the expression of a putative low affi nity sulfate transporter and isoforms of ATP sulfurylase and APS reductase. Plant Mol. Biol. 39:847–885.
Hodges DM, DeLong JM, Forney CF, Prange RK.(1999): Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds.Planta207,604–611.

Hossain MZ, Hossain MD, Fujita M (2006): Induction of pumpkin glutathione S-transferases by different stresses and its possible mechanisms, Biologia Plan-tarum, 50(2006): 210-218.
Hu, J., Lo, I.M.C., Chen, G., (2004): Removal of Cr(VI) by magnetite nanoparticle.Water Sci. Technol. 50 (12), 139e146.
Iannelli, M.A., Pietrini,F.,Fiore,F.,Petrilli,L.,Massacacci,A.,(2002): Antioxidant responses tocadmiumin Phragmitesaustralis plants. PlantPhysiol.Biochem. 40, 977–982.
Jinhua Zou, Keli Yu, Zhonggui Zhang, Wusheng Jiang and Donghua Liu. (2009): Antioxidant response system and chlorophyll fluorescence.
Kaim, W., and B. Schwedersky. (1994): Bioinorganic chemistry: Inorganicelements in the chemistry of life. John Wiley & Sons, New York.
Karuppanapandian T, Pritam Bala Sinha, Kamarul Haniya A, Premkumar G, Manoharan K (2000): Aluminium-induced changes in antioxidative enzyme activities, hydrogen peroxide content and cell wall peroxidase activity in green gram (Vigna radiata L. cv. Wilczek) roots. J. Plant Biol. 33(3): 241-246.
Ketter., S., GJ. m1,Y. H. Fu and G. A. Marllu (1991): Nucleotide sequence, messenger RNA stability, and DNA recognition elements of cys-14, the structural gene for sulfate permease I1 in *Neurospora crassa*. Biochemistry 30: 1780- 1787.
Leyval, C.; Turnau, K. and Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects, *Mycorrhiza*.7, 3:139–153.Lytle, C.M.; Lytle, P.W. and Yang, N. (1998). Reduction of Cr (VI) to Cr (III) by wetland plants: potential for in situ heavy metal detoxification, Environmental Science and Technology. 32, 20: 3087–3093.MacFarlane, G.R., Burchett, M.D., (2001): Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the Grey Mangrove Avicennia marina (Forsk.) Veirh. Marine Pollution Bulletin 42, 233–240.
Maiti S, Ghosh N, Mandal CH, Das K, Dey N and Adak M K (2012): Responses of the maize plant to chromium stress with reference to antioxidation activity. Braz. J. Plant Physiol., 24(3): 203-212.
Malik, C.P. and M.B. Singh, (1980): Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi, India, pp: 53.
Markkola, A.M., Tarvainen, O., Ahonen-Jonnarth, U., Stro¨mmer, R., (2002): Urban polluted forest soils induce elevated root peroxidase activity in Scots pine (Pinus sylvestris L.) seedlings. Environ. Pollut. 116, 273–278.
Marzluf, G. A., (1994): Genetics and molecular genetics of sulfur assimilation in fungi. Adv. Genet. 31: 187-206.
McAuley A, Olatunji MA.(1977): Metal ion oxidations in solution. Part XIX. Redox pathways in the oxidation of penicillamine and glutathione bychromium (VI). Can J Chem; 55:3335-40/
Michela S, Elizabeth AH, Pilan S, Marhus W, Ridiger H, Mario M (2008): Interaction between chromium and sulphar metabolism in *B. juncea*. J. Environ. Qual. 37:1536-1545.

Nakano Y, Asada K (1987): Purification of ascorbate peroxidase in spinach chloroplasts: Its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol 28:131–140

Noctor, G., and C. Foyer. (1998): Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:249–279.

Nriagu, JO, Pacyna, JM (1988): Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature (London) 333, 134–139.

Panda SK (2003): Heavy metal phytotoxicity induces oxidative stress in *Taxithelium*sp. Curr. Sci. 84:631-633.

Panda SK, Chaudhury I, Khan MH (2003): Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves. Biol. Plant. 46:289-294.

Pandey, N. and Sharma, C.P. (2003): Chromium interference in iron nutrition and water relations of cabbage.Environment. Exp. Botany. 49:195-200.

Parmar NG, Chanda SV (2005): Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of *Phaseolus vulgaris*. Turk J. Biol. 29: 15-21.

Pekker I, Elisha TO, Mittler R (2002): Reactive oxygen intermediates and glutathione regulate the expression of cytosolic ascorbate peroxidase during iron-mediated oxidative stress in bean. Plant Mol. Biol., 49: 429-438.

Pereira Y, Lagniel G Godat E, Baudouin-Cornu P, Junot C, and Labarre J, (2008): Chromate Causes Sulfur Starvation in Yeast Toxicological sciences 106(2), 400–412.

Pilon-Smits, E.A.H., S.B. Hwang, C.M. Lytle, Y.L. Zhu, J.C. Tai, R.C. Bravo, Y.C. Chen, T. Leustek, and N. Terry. (1999): Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. Plant Physiol. 119:123–132.

Radotic, K., T. Ducic and D. Mutavdzic. (2000): Changes in peroxidase activity and isozymes in spruce needles after exposure to different concentrations of cadmium. *Environ. Exp. Bot.*, 44: 105-113.

Samantary S., (2003): Biochemical responses of Cr-tolerant and Cr-sensitive mung bean cultivars grown on varying levels of chromium. Chemosphere 47: 1065-1072.

Sandai., N. N., and K. A. Marcker (1994): Similarities between a soybean nodulin, *Neurospora crassa* sulphate permease I1 and a putative human tumour suppressor. Trends Biochem. Sci. 19: 19.

Sanità di Toppi, L., F. Fossati, R. Musetti, I. Mikerezi, and M.A. Favali. (2002): Effects of hexavalent chromium on maize, tomato, and caulifl ower plants. J. Plant. Nutr. 25:701–717.

Seth, C.S.; Remans, T. and Keunen, E. (2012): Phytoextraction of toxic metals: a central role for glutathione, Plant, Cell and Environment.35, 2: 334–346.

Sen, AK,Mondal NG,Mandal S.(1994):Toxic effects of chromium (VI) on the plant *salvinia anatans* L.Environ Ecol ,12:279-83

Shah K, Kumar RG, Verma S, Dubey RS (2001): Effect of cadmium on lipid peroxidation superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161:1135.

Shanker AK, and Pathmanabhan G. (2004): Speciation dependant antioxidative response in roots and leaves of sorghum (*Sorghum bicolor* (L.) Moench cv CO 27) under Cr(III) and Cr(VI) stress. *Plant and Soil* 265: 141–151.

Shanker, A.K.; Cervantes, C.; Loza-Tavera, H. and Avudainayagam, S. (2005): Chromium toxicity in plants.Environment International. 31:739-753.

Shanker, A.K.; Djanaguiraman, M.; Sudhagar, R.; Chandrashekar, C.N. and Pathmanabhan, G. (2004): Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (Vigna radiata (L.) R. Wilczek. cv CO_4) roots. Plant Sci. 166:1035-1043.

Sies H (1999): Glutathione and its role in cellular functions, Free Radical Biol. Med., 27: 916-921.

Singh VK and Tiwari PN (1997): removal and recovery of chromium (VI) from industrial waste water. J. Chemical technology and biotechnology, 69(3):376-382.

Sirko A, Hryniewicz M, Hulanicka D, Böck A. (1990): Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cys*TWAM gene cluster. *Journal Bacteriol.* 172: 3351-3357.Smith F W., Hawkesforid M J., Prosserand M., Clarkson D T, (1995): Isolation of a cDNA from Saccharomyces cerevisiae that encodes a high affinity sulphate transporter at thep lasma membrane. Mol. Gen. Genet. 247: 709-715.

Tanaka K, Takio S, Yamamoto I, Satoh T (1996): Purification of the cytosolic CuZn-superoxide dismutase (CuZn-SOD) of Marchantia paleacea var. diptera and its resemblance to CuZn-SOD from chloroplasts. Plant Cell Physiol. 37:523-529.

Thomas, D.J., Thomas, J.B., Prier, S.D., Nasso, N.E., Herbert, S.K. (1999): Iron superoxide dismutase protects against chilling damage in the *cyanobacterium Synechococcus* species PCC7942. Plant Physiology 120: 275-282.

Verkleij JAC, Schat H (1990): Mechanisms of metal tolerance in higher plants. In: Shaw, AJ (ed), Heavy metal tolerance in plants,Evolutionary aspects. CRC Press, Boca Raton, Fl, pp. 179-194.

Wang BJ, Sheu HM, Guo YL, Lee YH, Lai CS, Pan MH, Wang YJ (2010): Hexavalent chromium induced ROS formation, Akt, NF-kappaB, and MAPK activation, and TNF-alpha and IL-1alpha production in keratinocytes. Toxicol. Lett. 198:216-224.

Winter, B., C. Christine, E. Rosemary, M. Brian and R. Carrell, (1975): The Estimation of red cell Superoxide Dismutase activity. J., Lab Clin. Med., 75: 337-341.

Zhu, Y., E.A.H. Pilon-Smits, L. Jouanin, and N. Terry. (1999a). Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. Plant Physiol. 119:73–79.

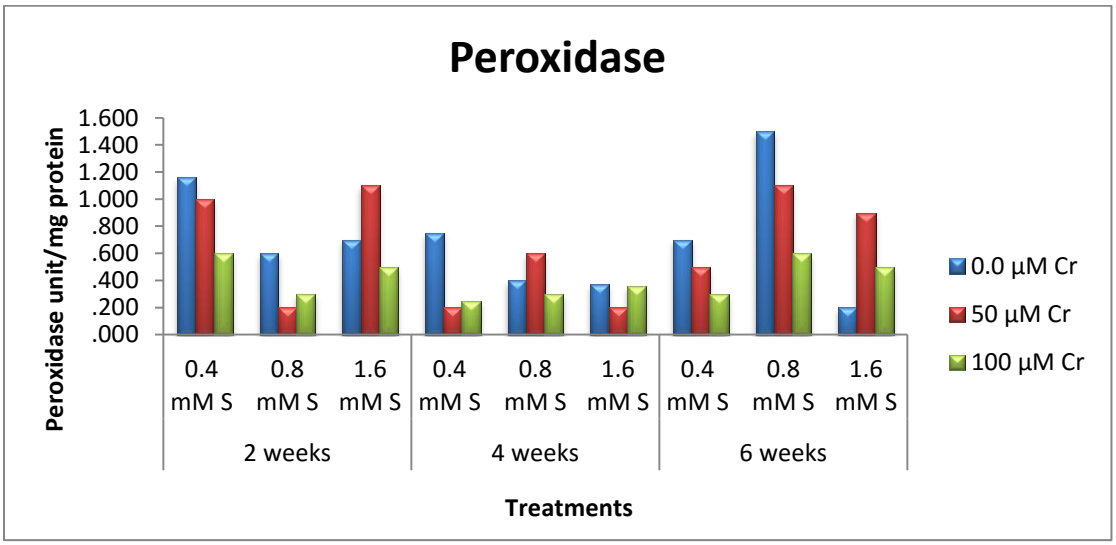

Figure (1) Data of peroxidase of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

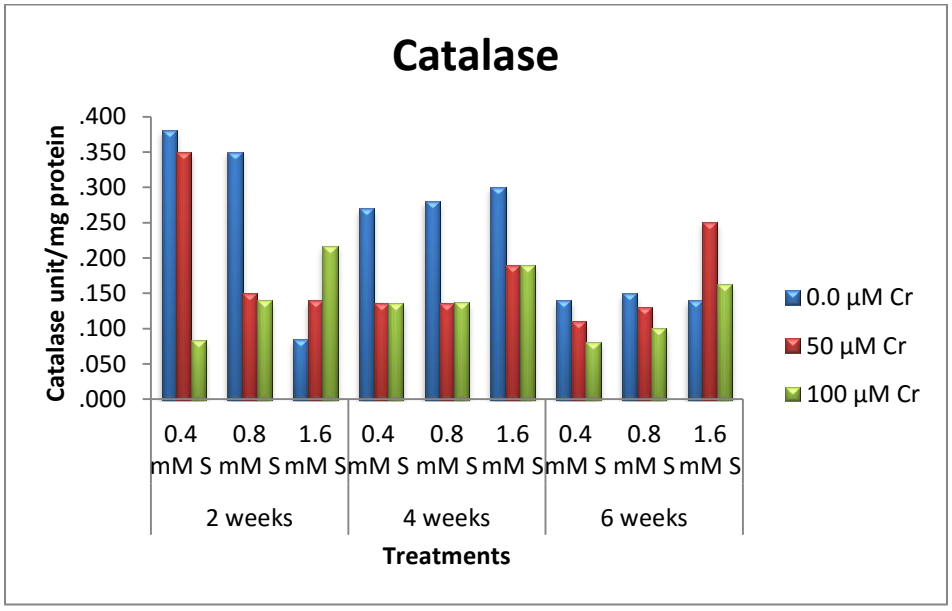

Figure (2) Data of catalase of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±S

Table (1) Data of GSH of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

Plant age	GSH ug/g fresh weight(mean ±SE)			
2 weeks	Treatments	0.0 µM Cr	50 µM Cr	100 µM Cr
	0.4 mM S	1.46±.006	1.27±.009	1.11±.143
	0.8 mM S	2.23±.010	2.31±.011	2.29±.013
	1.6 mM S	2.30±.011	2.38±.014	2.36±.005
F ratio		112.4		
P value		.000		
4 weeks	0.4 mM S	2.46±.011	1.75±.024	1.04±.005
	0.8 mM S	2.81±.015	2.39±.025	2.88±.017
	1.6 mM S	2.97±.046	2.66±.014	2.29±.018
F ratio		613.0		
P value		.000		
6 weeks	0.4 mM S	2.72±.091	1.84±.004	1.34±.078
	0.8 mM S	3.46±.053	2.89±.169	1.78±.026
	1.6 mM S	3.74±.108	2.92±.694	2.62±.057
F ratio		5.7		
P value		.001		

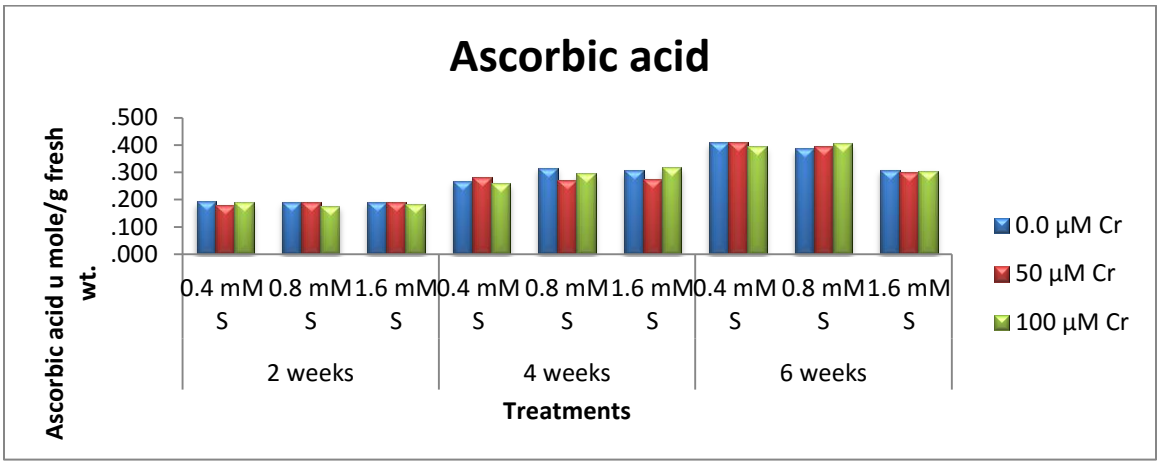

Figure (3) Data of Ascorbic acid of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

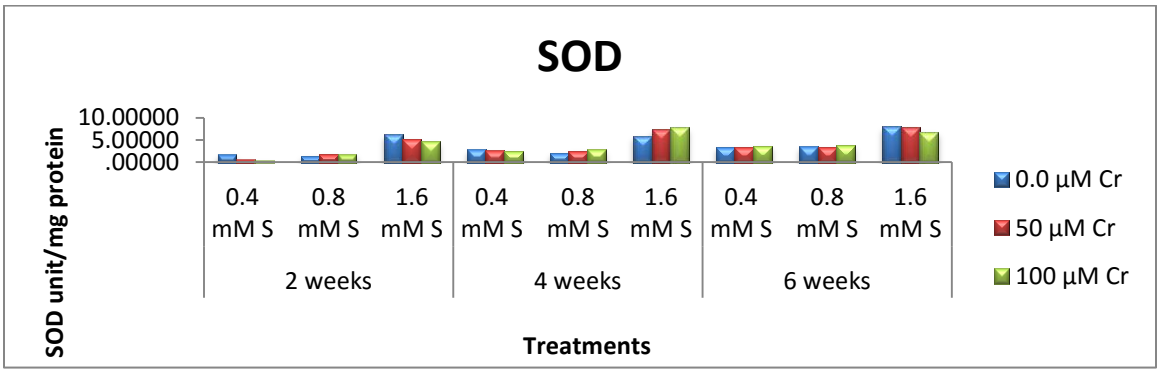

Figure (4) Data of SOD of the Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

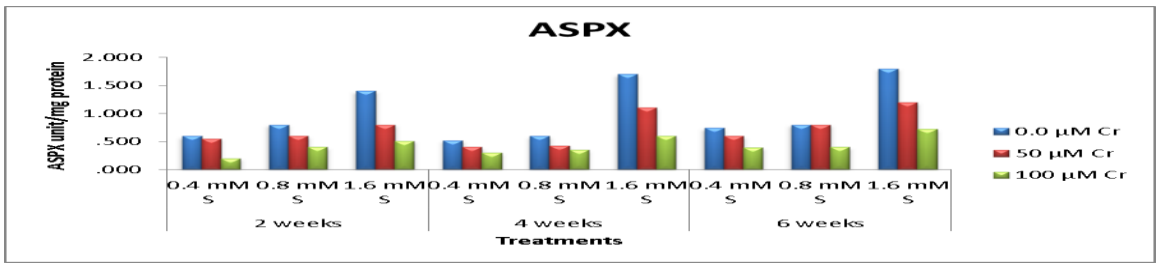

Figure (5) Data of ASPX of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

Table (2) Data of lipid peroxidation of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±S

• Plant age	• Lipid peroxidation (unit mole/g fresh wt) (mean ±SE)			
2 weeks	• Treatments	• 0.0 µM Cr	• 50 µM Cr	• 100 µM Cr
	• 0.4 mM S	• 3.20±.098	• 6.30±.110	• 14.55±.037
	• 0.8 mM S	• 5.17±.057	• 6.05±.043	• 6.15±.064
	• 1.6 mM S	• 4.85±.021	• 6.37±.022	• 7.70±.037
• F ratio		• 1770.4		
• *P value*		• .000		
4 weeks	• 0.4 mM S	• 3.11±.021	• 5.88±.056	• 6.89±.021
	• 0.8 mM S	• 5.02±.021	• 5.40±.037	• 5.75±.064
	• 1.6 mM S	• 4.79±.074	• 7.35±.140	• 6.87±.037
• F ratio		• 265.7		
• *P value*		• .000		
6 weeks	• 0.4 mM S	• 3.18±.082	• 4.40±.043	• 5.93±.056
	• 0.8 mM S	• 5.28±.077	• 5.94±.077	• 4.25±.056
	• 1.6 mM S	• 4.38±.056	• 4.12±.043	• 4.55±.064
• F ratio		• 117.0		
• *P value*		• .000		

Table (2) Data of lipid peroxidation of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

• Plant age	• Lipid peroxidation (unit mole/g fresh wt) (mean ±SE)			
2 weeks	• Treatments	• 0.0 µM Cr	• 50 µM Cr	• 100 µM Cr
	• 0.4 mM S	• 3.20±.098	• 6.30±.110	• 14.55±.037
	• 0.8 mM S	• 5.17±.057	• 6.05±.043	• 6.15±.064
	• 1.6 mM S	• 4.85±.021	• 6.37±.022	• 7.70±.037
• F ratio		• 1770.4		
• *P value*		• .000		
4 weeks	• 0.4 mM S	• 3.11±.021	• 5.88±.056	• 6.89±.021
	• 0.8 mM S	• 5.02±.021	• 5.40±.037	• 5.75±.064
	• 1.6 mM S	• 4.79±.074	• 7.35±.140	• 6.87±.037
• F ratio		• 265.7		
• *P value*		• .000		
6 weeks	• 0.4 mM S	• 3.18±.082	• 4.40±.043	• 5.93±.056
	• 0.8 mM S	• 5.28±.077	• 5.94±.077	• 4.25±.056
	• 1.6 mM S	• 4.38±.056	• 4.12±.043	• 4.55±.064
• F ratio		• 117.0		
• *P value*		• .000		