Journal of Forest Science
Vol. 26, No. 3, pp. 171~179, December 2010

Effect of Chromium Stress on Antioxidative Enzymes and Malondialdehyde Content Activities in Leaves and Roots of Mangrove Seedlings *Kandelia Candel* (L.) Druce

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ABSTRACT : Effect of chromium (Cr) stress on antioxidant enzyme activities and malondialdehyde (MDA) content were investigated in leaves and roots of mangrove (italic (L.) Druce) seedlings. Cr toxicity effects were also assessed on young seedlings. The seedlings were grown in green house condition for three months in nutrient solution with 0, 0.5, 1, 1.5, 2, 2.5, and 3 mg L^{-1} $CrCl_3$. This study showed that Cr led to the change of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and activities at different concentrations. The activity of antioxidant enzymes in leaves of *K. candel* seedlings indicates that enzymes engaged in antioxidant defense in certain level especially in low concentration of Cr treatments. The activities of SOD and POD were activated by Cr in the root level, while CAT activity was inhibited. CAT activity decreased in response to high concentrations of Cr. In the present study indicated that SOD in root was active in scavenging the superoxide produced by Cr. Both in roots and leaves, an increase in malondialdehyde (MDA) content was observed with increase in metal concentration and exposure periods. Our finding indicated that the high concentration of excessive Cr supply may interfere with several metabolic processes of seedlings, causing toxicity to plants as exhibited by chlorosis, necrosis, photosynthetic impairing and finally, plant death.

Keywords : Chromium toxicity, Superoxide dismutase, Peroxidase, Catalase, Antioxidant defense, Lipid peroxidation, Kandelia candel

INTRODUCTION

Mangrove forests are among the world's most productive ecosystems (Kathiresan and Bingham, 2001) in terms of biomass production. Mangrove areas are mainly distributed in the south-eastern part of China, reduced from 5,665 ha in 1950s to less than 1,545 ha in 2002, which means about 73% of mangrove forest areas were removed during last 50 years, and the reduction mainly has occurred in the areas with strong economic expansions and pollution such as Guangdong and Fujian province (Islam et. al., 2009). These forest ecosystems are under stress and became vulnerable to heavy metal pollution in China. Chromium, cadmium, copper, nickel, lead, mercury, and arsenic are the most common heavy metal pollutants. Amongst these heavy metals chromium and cadmium are of greatest concern for living organisms, including mangrove wetland species (Rahman et. al., 2009).

Mangrove ecosystems can act as sinks for heavy metals, which can become pollution sources to plants. Some mangrove plants appear to possess a great tolerance to high levels of heavy metal pollution but different species have different abilities to absorb and enrich heavy metals (Zhang et. al., 2007). However, in excessive heavy metal contamination, mangrove plants may initiate a variety of subcellular responses, i.e. metabolic reactions, which can cause damage

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at the cellular level or lead to wider phytotoxic responses (Zhang et. al., 2007). Toxic levels of heavy metal affect a variety of processes in plants. One of the major consequences is the enhanced production of reactive oxygen species (ROS), which damage cell membranes, nucleic acids and chloroplast pigments (Tewari et. al., 2002). Antioxidant enzymes such as SOD, POD and CAT play a vital role in scavenging reactive oxygen species (ROS) produced under oxidative stress thereby protects potential cell injury against tissue disfunction. The enzyme SOD dismutates superoxide radical oxygen to H_2O_2 and oxygen. POD, which is found in cytosol, vacuole, cell wall as well as in extracellular space and use various substrates as electron donor, utilizes H_2O_2 in the oxidation of various inorganic and organic substrates (Sinha et. al., 2005). POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blikhina et. al., 2003). CAT which is located in peroxisomes, cytosol and mitochondria, dismutates the H_2O_2 in to H_2O and O_2 (Zhang et. al., 2007). Cr has been demonstrated to stimulate formation of free radicals (FR) and ROS such as superoxide radicals $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH) either by direct electron transfer involving metal cations or as a consequence of metal mediated inhibition of metabolic reactions. Their presence cause oxidative damage to the biomolecules such as lipids, proteins and nucleic acids (Kanazawa et. al. 2000). Under high heavy contamination mangrove plants respond in a variety of ways at the sub cellular level, an example is the production of ROS, which causes damage to the cell membrane, nucleic acids and chloroplast pigments etc. The ability of the plant to cope with ROS stress is enhanced by the availability of antioxidant enzymes such as SOD, POD and CAT, which play vital roles in scavenging ROS. Chromium has been demonstrated to stimulate the formation of FR and ROS (Sinha, et. al., 2005). Since plants lack a specific transport system for Cr, it is taken up by carriers of essential ions such as sulfate or iron. Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield (Shanker et. al., 2005). However, very little information is available on mangrove plant physiological and biochemical mechanisms under Cr metal stress. So, it is essential to study mangrove plants and heavy metal for an understanding of physiological and biochemical response of heavy metal to mangroves. Thus, this study was undertaken to address the following objectives:

1. to investigate the effect of Cr stress on antioxidative enzymes and MDA content in *K. candel* leaves and roots;
2. to assess the effects of Cr toxicity on young *K. candel* seedlings.

MATERIALS AND METHODS

Field collection and germination

The propagules of *K. candel* were collected from plants grown at the Jiulongjiang mangrove forest stand (24°24′ N, 117°23′ E), Xiamen, Fujian, China. The region is subtropical with most of the annual rainfall (1284 mm) derived from summer typhoons. The average annual temperature of estuarine waters ranges from 14.8 to 27.8°C, with salinities adjacent to the mangroves ranging from 12 to 26 psu. After removal of the bracts, only complete, undamaged propagules with testa intact and no emergent hypocotyls or radicles were selected for planting. Propagules chosen for germination were those collected in the most abundant weight class, 18.0-19.55 g fresh weights. Propagules were planted in plastic pots filled with washed sand. Four propagules were randomly planted in a plastic pot for germination and growth. Three plastic pots (35 cm diameter × 15 cm height) were placed inside a plastic container (30 cm long × 40 cm wide × 30 cm height). The propagules were kept in a greenhouse under natural lighting, with relative humidity at 85% and a temperature of 28 ± 5°C. Each pot was irrigated twice weekly using two liters of tap water. The water level in each container was adjusted daily with tap water (free-NaCl) to compensate for water lost by evaporation. Propagules started to

germinate within one month and growth continued thereafter. After three weeks of germination, the young seedlings were treated with Hoagland's nutrient medium (pH~6.0) (Table 1). The solutions were changed every seven days to prevent depletion of metals, nutrients and oxygen.

The study design

A one way complete randomized design (CRD) with five replicates per treatment was used in this study. The random samples with five independent replicates were assigned to different treatments of Cr concentrations (viz., 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}).

Two-months old *K. candel* seedlings were put into individual plastic containers holding 1000 ml of Hoagland's solution prepared with the addition of Cr (as $CrCl_3$) treatment in seven levels: 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}. The concentrations of metal ions were maintained at a constant level by adding tap water up to a mark on the plastic containers in order to compensate for evaporation losses. Control (CK) plants were irrigated with 1000 ml of Hoagland's solution without $CrCl_3$. Plants were exposed to Cr under greenhouse conditions for three months with the result that symptoms of heavy metal's toxicity appeared in the seedlings; we assessed the effects of Cr toxicity on the basis of these symptoms. During the Cr treatment period, the growth parameters of all plants were monitored monthly in a pot by pot inspection, recording a leaf count, stem height, and the largest leaf length.

Table 1. Basal nutrient solution used for sand culture of Kandelia candel (L.) Druce seedlings

Solution	Chemical composition	Value (g/L)
A	KNO_3	70.77
	$NH_4H_2PO_4$	23.00
	$MgSO_4.7H_2O$	49.29
B	H_3BO_3	2.86
	$CuSO_4.7H_2O$	0.08
	$ZnSO_4.H_2O$	0.22
	$MnSO_4$	1.55
	$(NH_4)_6MO_7O_{24}.4H_2O$	0.61
C	$FeSO_4.7H_2O$	5.57
	EDTA. Na_2	7.45
D	Ca $(NO_3)_2$	118.07

Note: 1L Hoagland's solution = 10 ml solution A + 1 ml solution B + 1 ml solution C + 10 ml solution D with rest of water.

Antioxidant enzyme extraction and assay

To measure the effects of Cr stress on antioxidative enzymes (SOD, POD and CAT activities) and MDA in leaves and roots of mangrove plants, samples were collected after 24 h of the latest exposure of heavy metal to seedlings. The plants were uprooted from the plastic pots, washed thoroughly with tap water and washed again in distilled water. Then leaves and roots were separated from the plant for the extraction of enzymes. Samples of 0.5 g freshly collected leaves and roots were homogenizaed. The homogenization process occurred in a chilled mortar and pestle of 5 ml 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 4% (v/v) polyvinylpyrrolidone (PVP) and SiO_2 to neutralize the interference effects of phenol in mangrove plant tissues. The homogenate was centrifuged at 19000 × g for 20 minutes at 4°C and the supernatant was used for the enzymatic assays.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to the photo-chemical nitroblue tetrazolium (NBT) method (Beyer and Fridovich, 1987). The assay was based on the ability of SOD to inhibit reduction of NBT to formazan by superoxide. The 3 ml assay mixture containing 50 mM phosphate buffer (pH 7.8), 130 mM methionine, 0.75 mM NBT, 0.1 mM EDTA−Na_2, 0.02 mM riboflavin, and 50 μL enzyme extract was placed under cool fluorescent light at light intensity 175 μmol s^{-1} m^{-2} for 20 min. Moreover, riboflavin was added as the last component. Another two tubes without enzyme extract were used as controls, one incubated in the dark and the other under the same light source as the sample tubes. With the dark control tube as the blank, the absorbance at 560 nm was measured by a UV-vis spectrophotometer (Model UV-1206, Shimadzu, Japan). The increase in the absorbance of the light control (without the enzyme extract) was regarded as 100% inhibition and 1 unit of SOD activity was set at 50% inhibition. The enzyme

activity of the sample was calculated by determining the percentage of inhibition per minute. SOD activity in the leaf and root sample was then normalized to the total protein content of the respective tissue.

For the measurement of peroxidase (POD; donor H_2O_2 oxidoreductase, EC1.11.1.7) activity, 2.9 ml of 0.05 mM phosphate buffer + 1.0ml (0.05 mM) guaiacol, pH 7.8, was mixed with 0.1 ml of enzyme extract and allowed to stand at room temperature for three minutes. One microliter (1.0 ml) of 2% (v/v) hydrogen peroxide was added to activate the reaction. The absorbance at 470 nm was measured at every 30 second interval for five minutes, and an increase of 0.01 absorbance units per minute was equated to one unit of peroxidase activity. The activities of SOD and POD were expressed as units per mg of protein (Umg^{-1} protein).

Catalase (CAT, H_2O_2: H_2O_2 oxidoreductase, EC 1.11.1.6) activity was measured adapting the method of Beer and Sizer (1952) with minor modifications. The reaction mixture (1.5 ml) consisted of 100 mmol/l phosphate buffer (pH 7.0), 0.1 mmol/l EDTA, 20 mmol/l H_2O_2 and 50 μl enzyme extract. The reaction was started by the addition of the extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 mol/l cm), the results expressed as CAT units per minute and mg of protein. Protein concentration was determined according to Bradford (1976) using bovine serum albumin as a standard.

Lipid peroxidation measurement

Malondialdehyde is one of the final decomposition products of lipid peroxidation and as such has been used as an index for the status of lipid peroxidation. Thiobarbituric acid reactive substances representing the lipid peroxidation product were extracted through the homogenization of a 0.2 g leaf sample in 5 ml of solution containing 20% tri-chloroacetic acid and 0.5% 2-thiobarbituric acid. The mixture was heated at 95°C for 30 minutes and the reaction was arrested by putting on ice. The cooled mixture was centrifuged at 5000 × g for ten minutes at 25°C and the absorbance of the supernatant at 532 and 600 nm was recorded. After subtracting the nonspecific turbidity at 600 nm, the MDA concentration was determined by its molar extinction coefficient -- 155 mmol/l cm (Kosugi and Kikugawa 1985).

Statistical analysis

Data analysis was accomplished using two statistical programs, namely, Microsoft Excel 2007 package and SPSS 13.0, Chicago, IL, USA. Differences were analyzed using one-way ANOVA followed by Duncan's post-hoc test comparisons. The differences were considered statistically significant when P-value was less than 0.05.

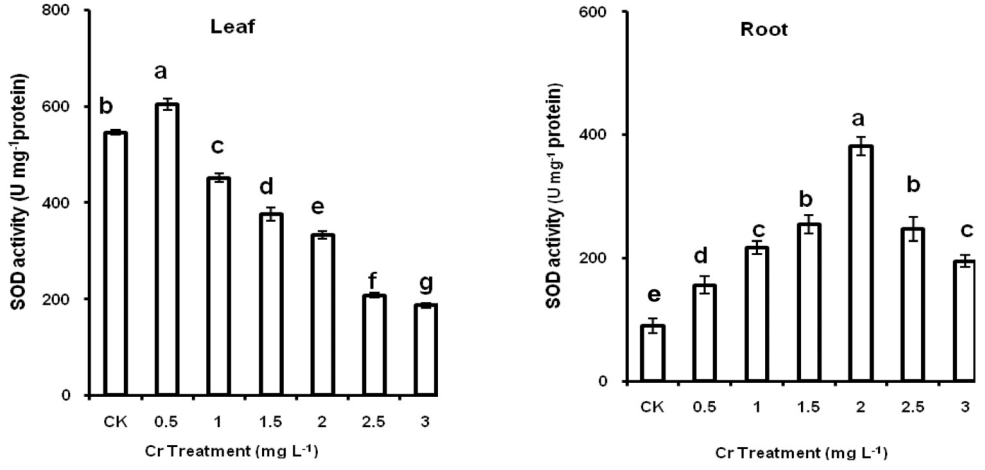
RESULTS AND DISCUSSION

The changes in the activities of antioxidant enzyme SOD in leaves and roots are shown in Fig. 1(a, b). SOD activity in leaves increased significantly under the treatment of 0.5 mg L^{-1} Cr concentration, but sharply decreased under the treatment of high (3 mg L^{-1}) Cr concentration. However, there was a significant difference in leaf SOD activity between the highest Cr concentration and the control samples. On the other hand, SOD activity in the roots of *K. candel* peaked at 2 mg L^{-1}; at the highest Cr concentration, roots showed slightly lower enzyme activity values. There was significant difference in root SOD activity between the highest Cr concentration and the control samples. Our observations are similar with early findings by Gwozdz et. al., 1997 and Dixit et. al., 2002. The decline in SOD activity from treatment 1 to 3 mg L^{-1} indicated that the oxygen scavenging function of SOD in leaf was damaged and Cr toxicity symptoms were appeared in leaf. The responses of POD activity to Cr stress varied with exposure to different Cr levels. POD activity in leaves increased significantly under the treatment of 0.5 mg L^{-1} Cr concentration in comparison with the control, but sharply decreased under the treatment of high (3 mg L^{-1}) Cr concentration (Fig. 2a). At 2 mg L^{-1} Cr, POD activity in roots of *K. candel* was much higher than that of the

control. At 2 mg L^{-1}, the POD activity in root of *K. candel* peaked at 193.04 U mg^{-1} protein and then decreased at 2.5 mg L^{-1} to 3 mg L^{-1} Cr concentration which was still significantly higher than that of the control (Fig. 2b).

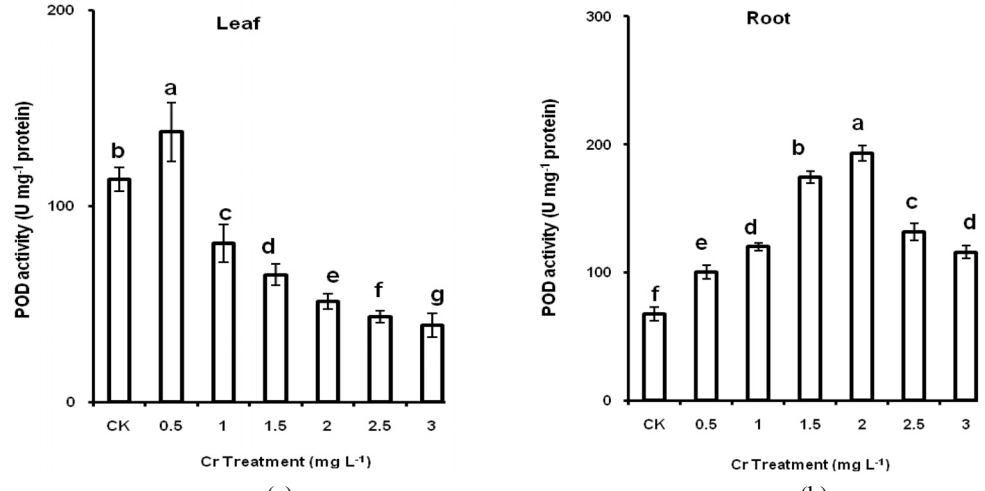
The CAT is an important heme-containing enzyme that catalyses the dismutation of H_2O_2 to H_2O and O_2 and is localized in the peroxisomes. CAT is an indispensable enzyme required for ROS detoxification in plants. In leaf, CAT activity increased at the Cr treatment of 0.5 mg L^{-1}, but for the treatment of 1 mg L^{-1} through to 3 mg L^{-1}, the CAT activity decreased rapidly (Fig. 3a). In roots, CAT activity of *K. candel* peaked at 2 mg L^{-1} and decreased to control level at 2.5 mg L^{-1} to 3 mg L^{-1}, which was still significantly higher than that of control (Fig. 3b). CAT is an iron-porphyry biomolecule, the decreased activity of CAT indicated that Cr is either interacting with iron in metabolic pool or affecting the availability of active form of iron (Sharma et. al., 2003).

Both in roots and leaves, an increase in MDA content was observed with increase in Cr concentration and exposure periods (Fig. 4a, b). The maximum MDA content both in root and leaf tissues was found in samples treated with 3



(a) (b)

Fig. 1. Effect of Cr on SOD activity in leaves (a) and roots (b) of *K. candel.* (Mean ± S.D.). Different superscripts on bars show significant (P <0.05) difference between the means according to Duncan's posthoc test).

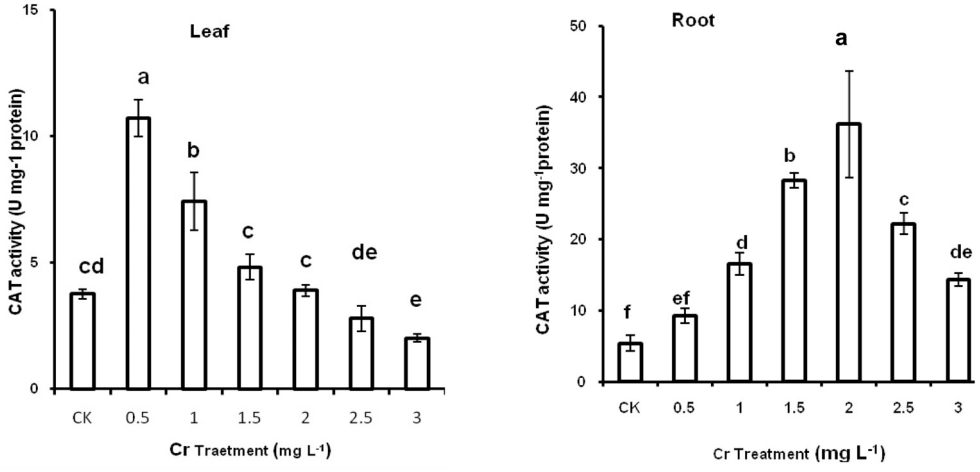


(a) (b)

Fig. 2. Effect of Cr on POD activity in leaves (a) and roots (b) of *K. candel.* (Mean ± S.D.). Different superscripts on bars show significant (P <0.05) difference between the means according to Duncan's test).

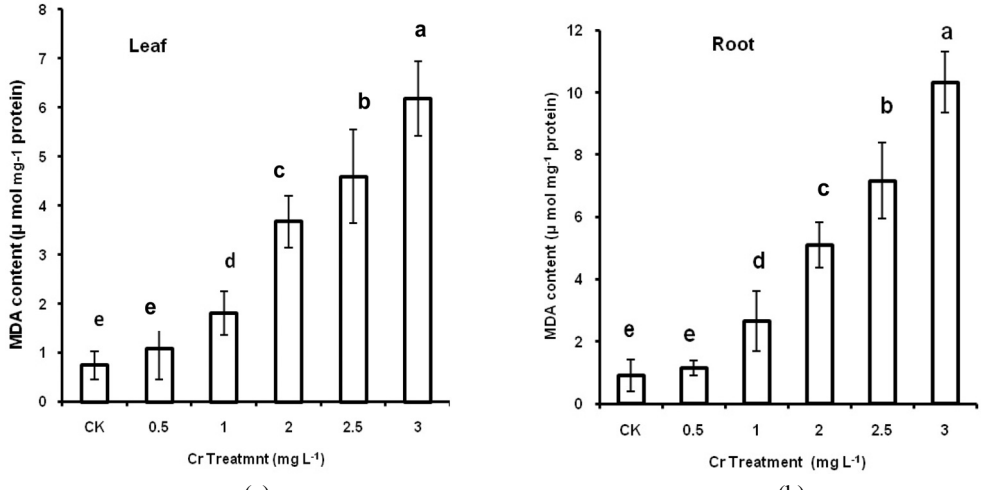
mg L^{-1} Cr. There was no significant difference observed between the MDA levels for samples treated with lowest Cr concentration (0.5 mg L^{-1}) and that of the control. Thus, it suggusts that Cr causes oxidative stress at higher levels of Cr concentrations in the treatment, which is evident from enhancement in lipid peroxidation.

After three months of cultivation under Cr stress the symptoms of Cr toxicity became visible in the seedling. The initial symptom of Cr toxicity appeared to cause chlorosis in *K. candel* seedlings. Chlorosis appeared in the upper leaves of seedlings, as an effect of Cr, probably due to the retardation of Fe and Zn translocation. The primary toxic effect seemed to be membrane damage due to the high oxidative potential of Cr. Severe necrosis was observed in the lower leaves of *K. candel* seedling with increased of Cr concentrations. The statistical analysis showed that the toxic effects on the seedling stem height, leaf number and biomass varied significantly ($P < 0.05$) with increasing Cr concentration (Table 2). Toxic effects of Cr on plant growth and development include alterations in the growth of roots, stems and leaves, which may affect total dry matter production and yield. Our findings



(a) (b)

Fig. 3. Effect of Cr on CAT activity in leaves (a) and roots (b) of *K. candel.* (Mean ± S.D.). Different superscripts on bars show significant ($P < 0.05$) difference between the means according to Duncan's test).



(a) (b)

Fig. 4. Effect of Cr on malondialdehyde activity in leaves (a) and roots (b) of *K. candel.* (Mean ± S.D.). Different superscripts on bars show significant ($P < 0.05$) difference between the means according to Duncan's test).

showed that the lower to higher concentration of Cr caused the reduction of the biomass of the seedlings. Davies et. al., (2002) also found that Cr is toxic to higher plants at lower concentration. Sokola et. al., (2010) found that the spread in chromium contents of plants is 0.006 to 18 mg L^{-1}. They also mentioned that the phytotoxicity occurs at 0.5 mg L^{-1} of Cr concentration.

Many studies (e.g., chromium, cadmium, copper, nickel, lead, mercury, and arsenic) have been performed on the enhancement of plant tolerance to oxidative stress by modifying the plant antioxidant defense system (Vitoria et. al., 2001; Sinha et. al., 2005; Rahman et. al., 2009). Our results showed that SOD activity in roots of *K. candel* peaked at 2 mg L^{-1}, while at the highest level of Cr concentration enzyme activity values decline. Increases in SOD activity were observed in the leaves at 0.5 mg L^{-1}, but at higher concentrations of Cr treatment (1 to 3 mg L^{-1}) SOD activity in leaves decreased rapidly. The decline in SOD activity at Cr concentration 1 to 3 mg L^{-1} in leaves suggested that the oxygen scavenging function of SOD was damaged. In contrast, SOD activity in roots peaked at 2 mg L^{-1} Cr concentration suggesting better protection of root tissues than that of leaves against oxidant damage. While SOD in root was active in scavenging the superoxide produced by Cr, a lack of SOD activity in the leaf may have contributed to the build-up of superoxide radicals in the leaf tissue. However, both leaves and root tissues were prone to oxidant damage at higher levels of Cr concentrations and SOD could not protect tissues against oxidative damage. Thus it can be concluded that the presence of excess Cr can cause oxidative stress in plants and subsequently increase the antioxidant responses as a result of increased production of highly toxic oxygen free radicals. Our findings conform with observation of Panda and Choudhury (2005) in that high amount of Cr in plants led to oxidative stress, inducing changes in the activity and content of some components of the antioxidative pathways.

POD activity in the leaves of *K. candel* peaked at 0.5 mg L^{-1}, while at the highest Cr concentration leaves showed lower enzyme activity values. On the other hand POD activity in the roots peaked at 2 mg L^{-1} and declined after that. Consequently it was observed that, *K. candel* eliminates H_2O_2 produced by peroxidation of membrane lipids more efficiently at Cr concentration of 0.5 mg L^{-1} in leaves and 2 mg L^{-1} in roots.

CAT is the most important oxidoreductase, which scavenges H_2O_2 to O_2 and H_2O. The major function of CAT is to metabolize the peroxide liberated in the peroxisome following the conversion of glycolate during photorespiration (Liu et. al., 2008). Our data revealed that, CAT participated in active H_2O_2 reduction at 2 mg L^{-1} concentration of Cr treatment in roots and 0.5 mg L^{-1} concentration of Cr treatment in leaves.

In the leaves, an increase in CAT, POD, and SOD activities was noticed especially after three months' exposure

Table 2. Visible symptoms on seedlings of three months old *K. candel* grown in green house with increasing Cr concentrations.

Cr in nutrient solution (mgL^{-1})	Visible symptoms	Stem growth Reduction (%)
CK	No symptoms	0
0.5	No obvious symptoms	5.59
1.0	Chlorosis	9.44
1.5	Chlorosis, reddish-brown discoloration of the leaf blades	11.88
2.0	Chlorosis (+), necrosis	22.36
2.5	Chlorosis (++), necrosis (+), root worsened with less root hair	32.51
3.0	Chlorosis (+++), necrosis (++), senescence, root becomes shorter and thicker, root hairs sparser and color is black and brown, and stem deep brown in color, Plant stared to die.	34.79

Note: Relative symptom intensity is given in brackets. Percent of growth reduction are given to the 0 mg L^{-1} Cr treatment.

at the lower Cr concentration levels. In contrast, these activities were more evident in roots at a range of Cr concentrations (0.5 to 2 mg L^{-1}). Also, roots showed much early rapid response to lower levels of Cr stimulation. This phenomenon may be explained by the fact that roots being the first plant organ that come in contact with Cr accumulate most part of the heavy metal. Thus, the difference in the enzyme activities between the roots and leaves might be due to the lower amount of available Cr in the aerial part than that of the hypogeal part. Roots contain more Cr than leaves. The reasons for greater accumulation of Cr in roots is due to accumulation of Cr in vacuoles of root cells, thus rendering it less toxic, which may be natural toxicity response of plant (Shankar et. al., 2005). POD activity increased much more in roots than in leaves can be explained by the probability that the glutathione/ascorbate cycle was operating at a high rate in order to detoxify the ROS formed in the roots. Huffman and Alloway (1973), also found that Cr absorbed by plants grown in culture solutions remained primarily in the roots and is poorly translocated to the leaves.

This study showed that Cr led to the change of SOD, POD, and CAT activities at different concentrations. The activity of antioxidant enzymes in leaves of *K. candel* seedlings indicates that enzymes engaged in antioxidant defense in certain level especially at low concentration of Cr treatments. Furthermore, it was also noticed that POD was activated in the root and decomposed H_2O_2. However, in leaf POD did not take a part actively to decompose the H_2O_2. Moreover, Cr inhibited the POD activity in leaves.

CAT activity decreased in response to high concentrations of Cr. In the present study, it was seen that CAT participated in active H_2O_2 reduction in low concentration of Cr treatment. The decrease in the activities of these enzymes cause weakening of leaf cell membrane trigerring a leaf-fall. Membrane lipids are especially prone to attack by free radicals. Protonation of superoxide radical can produce hydroperoxyl radical (•OH, H_2O_2), which can convert fatty acids to toxic lipid peroxides, destroying the biological membranes (Foyer et. al., 1994).

This study showed that MDA level rose significantly high when plants were treated with solution of higher levels of Cr concentrations. This suggests that high level of endogenous Cr induced production of superoxide radicals, leading to increased lipid peroxidation. Increased MDA levels in root of *K. candel* as compared to leaf indicated an increased lipid peroxidation of cell membrane. The increased lipid peroxidation causes toxicity in leaf and root tissues that affects metabolic processes of plant resulting in chlorosis, necrosis including loss of biomass. Other studies on impact of Cr on plants physiology also argued similar conclusion. For example, Panda and Choudhury (2005), asserted that Cr stress can induce metabolic modifications in plants, such as alterations in photosynthesis, degradation of photosynthetic pigments and induction of oxidative stress. Furthermore, Cr promotes reduction of leaf area and biochemical changes responsible for the inhibition of chlorophyll synthesis (Vajpayee et. al., 1999) and disorganization of the chloroplast ultrastructure (Panda and Choudhury, 2005) those were contributing to reduction of biomass and growth of *K. candel* and ultimately causing death.

CONCLUSION

The Cr adversely affected several antioxidative enzymes activities. Our results showed that Cr led to the change of SOD, POD, CAT and MDA activities at different concentrations. The activity of antioxidant enzymes in leaves of *K. candel* seedlings indicates that enzymes engaged in antioxidant defense at certain level especially, at low concentration of Cr treatments. However, at the highest amount of Cr concentration, the activities of SOD, POD and CAT were decreased both in leaf and root and an increase in MDA content was observed. The reduction in the activities of antioxidative enzymes in *K. candel* at the high concentration of Cr treatments indicates that Cr tolerance characteristics of this plant can not be attributed to antioxidative defense. The Cr stress can induce metabolic modifications in plants causing chlorosis, necrosis and biomass loss possibly due to alterations in photosynthesis, degradation of photosynthetic pigments and induction of

oxidative stress, and virtually all of these factors could contribute to seedling death.

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(Received November 13, 2010; Accepted December 28, 2010)