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# Hexavalent Chromium Induced Inhibition of Photosynthetic Electron Transport in Isolated Spinach Chloroplasts

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Additional information is available at the end of the chapter

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## 1. Introduction

Plants, being sessile, are often exposed to various kinds of harsh environmental conditions which adversely affect growth, metabolism and yield. Among various abiotic stresses, drought, salinity, gaseous pollutants and heavy metals are important environmental stressors which severely affect plant growth. Photosynthesis is essentially the only mechanism of energy input into living world. The decline in productivity in many plant species subjected to harsh environmental conditions is often associated with a reduction in photosynthetic capacity. Heavy metals have been increasing in the environment (air, water and soil) as a result of rapid industrialization, urbanization and agricultural runoff. Many of these metals have adverse effects on growth and metabolic processes in plants, including reduction in chlorophyll content, chloroplast degeneration, reduced photosynthesis and inhibition of enzyme activities.

Heavy metals influence photosynthesis by affecting pigments, electron transport activities and Calvin cycle enzymes. Of the various metals, Pb, Cu, Cd, Ni, Hg and Zn have been extensively studied in relation to their effects on plant photosynthesis [1-2]. Long term exposure of whole wheat plants to cadmium (Cd) affected chlorophyll and chloroplast development in young leaves [3]. However Pandey et al [4] reported significant increase in chlorophyll content in 20  $\mu\text{M}$  Cr (VI) treated Indian mustard seedlings. Chloroplast membranes, particularly thylakoids, have been investigated as the sites of action of heavy metals. Photosynthetic electron transport within thylakoid membranes has been the primary target site of heavy metal action [5].

Light is shown to play a role in the binding of heavy metals to the chloroplast membranes and to inhibit photosynthetic electron transport. The accessibility of Cu (II) to site of inhibition in chloroplast membranes was much more rapid and to a greater extent in the light than in the

dark [6]. A reduction in the inhibitory effects of Cu (II) upon leaving the membranes for short periods of time in the dark led them to presume that in the dark Cu (II) was being irreversibly bound to non-inhibitory site in the membranes, which prevented it from binding with inhibitory sites in the light [6]. The effect of Zn (II) on inhibition of oxygen evolution was somewhat identical in both light and dark [7]. However, Baker et al. [8] found a greater and tighter binding of zinc in the pea thylakoid membranes incubated in dark or exposed to low light as compared to those exposed to saturating light. Cu (II) inhibition increased in spinach chloroplasts with the time of light exposure [9]. Essentiality of light for the damage due to Cu (II) was also demonstrated in spinach PSII particles [10].

Chromium (Cr) is a toxic element that occurs in highly variable oxidation states. Chromium is found in all phases of the environment, including air, water and soil. Chromium content in naturally occurring soil ranges from 10 to 50 mg. kg<sup>-1</sup> depending on the parental material. In ultramafic soils (serpentine), it can reach up to 125 g. kg<sup>-1</sup> [11]. Cr and its compounds have multifarious industrial uses. They are extensively employed in leather processing and finishing [12], in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture and 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. These anthropogenic activities have led to the widespread contamination of Cr in the environment. Very few studies have reported ameliorative measures for Cr toxicity in crop plants. *Pluchea indica* showed a good potential of phytoremediation, as it presented high levels of Cr accumulation and translocation to the leaves [13]. Mellem et al [14] found that *Amaranthus dubius* tolerate high Cr(VI) concentrations showing good potential for phytoremediation. Furthermore, Gardea-Torresdey et al [15] found that *Convolvulus arvensis* L. had capability to accumulate more than 3800 mg of Cr kg<sup>-1</sup> dw tissue, showing that this specie can be used in phytoremediation of Cr(VI) contaminated soils. Khan (16) reported the potential of mycorrhizae in protecting tree species *Populus euroamericana*, *Acacia arabica* and *Dalbergia sisso* against the harmful effects of heavy metal and phytoremediation of Cr contamination in tannery effluent-polluted soils. The poor translocation of Cr from roots to shoots is a major hurdle in using plants and trees for phytoremediation.

Despite toxicity of chromium (Cr) to human and animals due to its instant exposure or via its incorporation in food chain, the mechanism of action of this element in the photosynthetic electron transport activity is not critically studied. Of the various species of Cr, trivalent Cr (III) and hexavalent Cr (VI) forms are of biological relevance due to their wide occurrence in the environment. While Cr (VI) is most biologically toxic oxidation state of chromium [17], Cr (III) is less toxic and is also an essential trace element in human nutrition [18]. The chemistry of Cr in respect to plant accumulation from the environment revolves around the reduction of Cr (VI) to Cr (III), the oxidation of Cr (III) to Cr (VI) and the relative stability of Cr (III) compound once formed [19].

Growth inhibition in Cr treated *Lemna gibba* was shown to be associated with an alteration of the PS II electron transport [20]. The net photosynthesis of primary and secondary leaves of *Phaseolus vulgaris* was decreased by Cr (VI) [21]. Decline in net CO<sub>2</sub> assimilation of leaves in Cr (VI) treated pea plants was found associated with depressed activities of both PS I and PS II. On the other hand, study on isolated pea chloroplasts showed that exogenously added Cr (VI) had markedly inhibited PS I activity while PS II activity was marginally affected [22]. This

result was in contrast to the effects of most of the heavy metals (*Pb*, *Cu*, *Cd*, *Ni*, *Hg* and *Zn*) on photosynthetic electron transport where photosystem II was preferentially inhibited [23-25]. *Cd* treated pea and broad bean plants showed modified PS II activity due to damaged oxygen-evolving complex and caused disassembly of PS II itself [26]. Binding studies with radiolabeled herbicide revealed that the  $Q_B$  pocket activity was also altered. Päsikkä et al [27] while studying effect of *Cu* on photoinhibition of PS II in vitro in bean and pumpkin thylakoids concluded that the primary effect of excess *Cu* on the photoinhibition of PS II is caused by inhibition of electron donation to  $P680^+$ , which leads to donor-side photoinhibition. The *in vitro* study of Desmet et al [28] indicated reduction of Cr (VI) to a lower oxidation state of Cr by spinach chloroplasts in dark. The authors also showed that  $CrO_4^{2-}$  behave as an electron acceptor of photosynthesis in light.

The present study was undertaken to assess the impact of hexavalent Cr on isolated spinach chloroplasts, role of light in Cr binding and to resolve the site(s) of inhibition of Cr (VI) in photosynthetic electron transport.

## 2. Materials and methods

Chloroplasts were isolated from field grown spinach (*Beta vulgaris* L.) following the method of Navari-Izzo et al [29] with some modifications. Leaves were homogenized in a medium (1:3, w/v) containing 330 mM sucrose, 50 mM HEPES-KOH (pH 7.5), 5 mM  $MgCl_2$ , 10 mM NaCl. The homogenate was filtered through 4 layers of muslin cloth and centrifuged at 1100x g for 3 min. The pelleted chloroplasts were purified by layering on the top of a Percoll gradient (10 ml each of 40 and 80% Percoll prepared with isolation medium) and centrifuged for 30 min at 4000x g. After centrifugation the lower band containing intact chloroplasts was separated, washed gently and finally suspended in the isolation medium. The intactness of the chloroplasts was estimated in the range of 90 to 95% by ferricyanide-dependent  $O_2$  evolution [30]. Chloroplast fragments were prepared by giving osmotic shock to intact chloroplasts followed by their re-suspension in hypotonic medium [31].

PSII particles with high rate of oxygen evolution were prepared by the method of Berthold et al [32]. Chlorophyll was determined by method of Porra et al [33].

For inhibition study, thylakoid membranes or PSII particles ( $30 \mu g \text{ ml}^{-1}$ ) were incubated at 20° C with different concentration of  $K_2Cr_2O_7$  in a reaction vessel for 5 min in the dark or in the presence of  $10 \mu \text{mol m}^{-2} \text{ s}^{-1}$  red light provided by LED, (Quantum Devices, Inc., USA) under continuous stirring. After treatment, the aliquots were centrifuged (at 5000x g for thylakoid and at 40000 g for PSII particles) and pellets after washing twice with assay buffer finally suspended in 1 ml assay medium to monitor electron transport activity. The conditions of assay for electron transport reactions using artificial electron donors and acceptors have been given under separate heads below. The oxygen evolution and consumption during different reactions were monitored polarographically in a total volume of 1 ml using a Clark type electrode (Hansatech, UK). A projector lamp (Kindermann-Germany) provided saturated light intensity under the condition of different electron transport assay.

### 2.1. Whole chain electron transport rate

The electron transport through the whole chain of photosynthesis, i.e. from  $\text{H}_2\text{O}$  to MV was measured as  $\text{O}_2$  uptake. Assay medium, 1 ml, contained 30 mM HEPES, 10 mM NaCl, 5 mM  $\text{NH}_4\text{Cl}$ , 1 mM  $\text{MgCl}_2$ , 0.1 mM sodium azide, and 0.1 mM MV adjusted at pH 7.5. Thylakoid were added to the above reaction mixture to a final concentration of 30  $\mu\text{g}$ .

### 2.2. PS I rate

PS I rate in lysed chloroplasts was monitored in a 1 ml reaction mix comprising of 50 mM HEPES (pH 7.5), 0.2 mM DCPIP, 2.0 mM ascorbate, 5 mM  $\text{NH}_4\text{Cl}$ , 2  $\mu\text{M}$  DCMU, 0.1 mM MV, 0.1 mM Na azide and 30  $\mu\text{g}$  thylakoid/ml. DCPIP reduction was measured spectrophotometrically at 600 nm in Shimadzu UV-VIS 1601 spectrophotometer.

### 2.3. PS II rate

PS II rate in lysed chloroplasts was monitored in 1 ml reaction mix consisting of 30 mM HEPES (pH 7.0), 10 mM NaCl, 1 mM  $\text{MgCl}_2$ , 0.5 mM DMBQ and 30  $\mu\text{g}$  thylakoid/ml.

### 2.4. $\text{DQH}_2$ to MV and TMPD bypass

The photo reduction of MV with duroquinol as the donor was seen in reaction mix containing 30 mM HEPES (pH 7.5), 10 mM NaCl, 5 mM  $\text{NH}_4\text{Cl}$ , 1 mM  $\text{MgCl}_2$ , 0.1 mM sodium azide, 0.1 mM MV, 4  $\mu\text{M}$  DCMU and 0.5 mM duroquinol (freshly prepared in ethanol) and 30  $\mu\text{g}$ /ml thylakoids. For TMPD bypass, 30  $\mu\text{M}$  TMPD and 1  $\mu\text{M}$  DBMIB were added.

### 2.5. Reactions with PS II particles

The standard assay medium for PS II membranes consisted of 350 mM sucrose, 50 mM MES (pH 6.0), 2 mM  $\text{MgCl}_2$ , 15 mM NaCl, and 0.5 mM DMBQ or 0.2 mM  $\text{SiMO}_4$  as electron acceptors. Ferricyanide was not used as it interfered with Cr. DMBQ was dissolved in ethanol and  $\text{SiMO}_4$  in 50% aqueous dimethyl sulfoxide.

### 2.6. Spectrophotometric measurements of PS II electron transfer activity in the presence and in the absence of Diphenylcarbazide (DPC)

In some experiments effect of Cr and light was seen on isolated spinach PS II particles by measuring the rate by which PS II particles reduce dichlorophenol-indophenol (DCPIP) in the presence and in the absence of the artificial electron donor diphenyl carbazide (DPC). DCPIP reduction was measured in the reaction water to DCPIP and DPC to DCPIP as a decrease in absorbance at 600 nm in a 2 ml reaction mixture containing 200 mM sucrose, 30 mM MES (pH 6.5), 1 mM  $\text{MgCl}_2$ , 10 mM NaCl, 60  $\mu\text{M}$  DCPIP in the presence and absence of 0.5 mM DPC. Because Cr would react with DPC, PS II particles were washed twice with PS II buffer and pelleted. The light saturated rate of DCPIP reduction was measured by illuminating the PS II particles with the PPFD of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of red light in a spectrophotometer cuvette. The



absorbance reading was taken at 600 nm in Shimadzu UV-VIS 1601 spectrophotometer at 30-s intervals during the 2-min assays.

## 2.7. Cr estimation in thylakoids

Chromium was determined in isolated chloroplasts. The chloroplast pellets were dried at 48 °C for 48 h. Determination of Cr was made by atomic absorption spectrometry (AAnalyst 300, Perkin Elmer, USA) on nitric acid: perchloric acid (3:1, v/v) digests of pelleted chloroplasts using background correction. Stock chromium certified solutions (Sigma) and blanks were run in parallel to validate the quality of metal analyses.

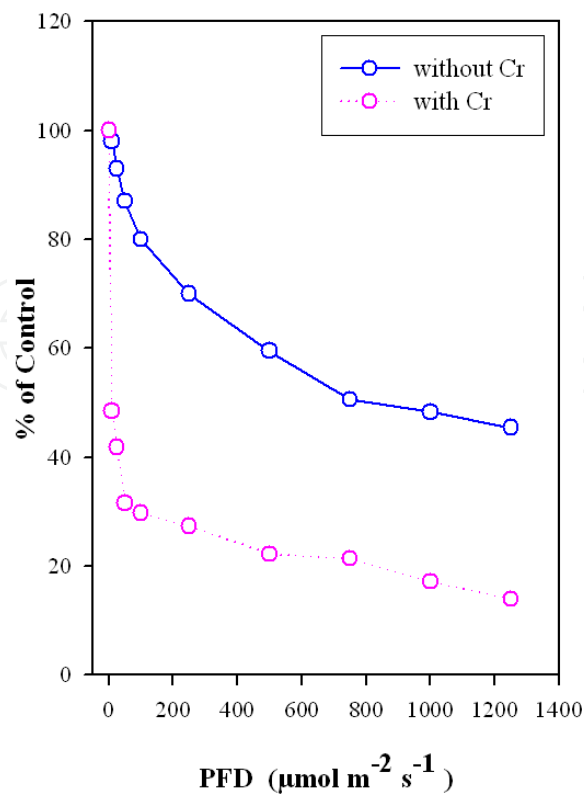
## 2.8. Hydroxyl radical estimation

Formation of hydroxyl radicals was measured under illumination in a stirred reaction vessel with 30 µg chl ml<sup>-1</sup> at 25 °C in the presence of 0.7 M DMSO according to Babbs et al [34]. The medium for illumination was 50 mM MES (pH 6.0), 2 mM MgCl<sub>2</sub> and 10 mM NaCl. After centrifugation (3 min at 10,000 g), the pH of the supernatant was adjusted to 2.5 by adding HCl. The colour reaction was started by adding 0.14 mM fast blue BB salt (prepared fresh) to the chloroplast supernatant. The methane sulphinic acid (MSA) content was then calculated from the absorbance at  $\lambda_{\text{max}}$  425 nm using the extinction coefficient of 14.5 mM<sup>-1</sup> cm<sup>-1</sup>.

## 3. Results & discussion

Presence of chromium ions in the assay medium did not allow instant and accurate monitoring of electron transport activities in the isolated spinach chloroplasts due to their reactivity with the reagents (DMBQ, DCPIP, DPC and ascorbate) used in analyzing different electron transport reactions (data not shown). The other uncertainty in monitoring of the electron transport rates in the presence of chromium was that the chromium inhibition of electron transport activity was more effective at acidic pH while optimal electron transport rates were recorded at neutral to slightly basic pH of the assay medium. It was found therefore critical to eliminate chromium from the assay medium. With a view to avoid interference of free Cr ions during assay, we incubated spinach chloroplasts for different duration with varying concentrations of Cr and prior to monitoring electron transport activity, the treated thylakoids were washed with the assay buffer to remove unbound Cr. The resulting Cr induced inhibition in the electron transport activity was irreversible even after treatment with 5 mM EDTA (data not shown).

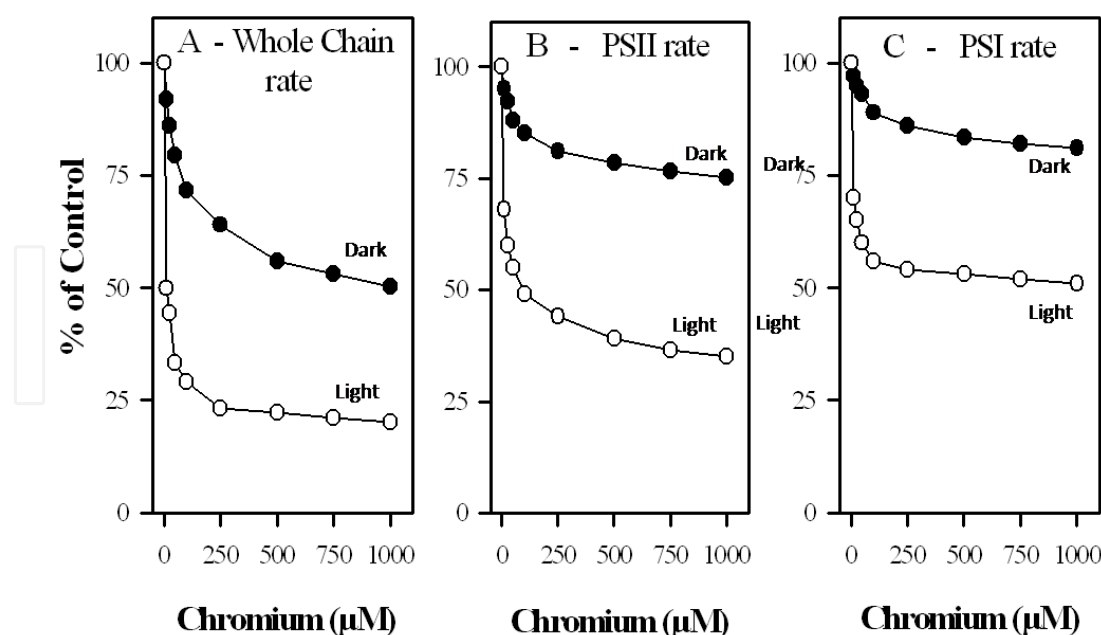
Light is known to play a major role in heavy metal induced toxicity. Jagerschold et al [35] found that strong illumination inhibited PS II electron transport and degraded D1 protein in chlorine depleted medium. With a view to see the effects of light, we incubated spinach chloroplasts under different per mutation combination of PAR and chromium concentrations (preliminary data not shown). The light dependence of chromium toxicity in the chloroplasts incubated for 5 min with 10 µM Cr (VI) on whole chain electron transport activity is shown in Figure 1.



**Figure 1.** Whole chain electron transport rate (Water to MV) of spinach chloroplasts showing light dependence curve in absence and presence of Cr (10  $\mu\text{M}$ )

Cr (VI) treatment of the chloroplasts in the light had a remarkable impact on photoreduction of methyl viologen. The inhibition of 8% whole chain electron transport activity observed in the chloroplasts incubated for 5 min with 10  $\mu\text{M}$  Cr (VI) in dark, increased to ca 68% when chloroplasts were incubated for 5 min with 10  $\mu\text{M}$  Cr (VI) at 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (Figure 2 A). Exposure of chloroplasts to and above 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the presence of 10  $\mu\text{M}$  Cr (VI) caused a gradual inhibition leading to 86% loss in whole chain electron transport activity at 1250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 2 A). These results indicated that even low PPFD during incubation of chloroplasts with chromium was substantially effective in inactivating electron transport reactions. In order to validate our finding we monitored rate of electron flow from water to MV, water to DMBQ (PSII) and ascorbate + DCPIP to MV (PSI) subsequent to incubation of chloroplasts at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 5 min with different concentrations of Cr (VI) (Figure 2 A-C).

The pretreatment of the chloroplasts with Cr (VI) at the PPFD as low as 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  caused an abundant increase in the inhibition of whole chain as well as partial electron transport reactions. While the lowest Cr (VI) concentration (10  $\mu\text{M}$ ) in dark incubated chloroplasts decreased the whole chain, PSII and PSI electron transport activities by 8, 5 and 3%, the corresponding inhibition in the chloroplasts incubated at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light amounted to 50, 32 and 30%, respectively (Figure 2 A-C). While the  $I_{50}$  value for inhibition in dark incubated chloroplasts was 1000  $\mu\text{M}$  Cr (VI) for water to MV, it was only 10  $\mu\text{M}$  Cr (VI) for corresponding reaction in the chloroplasts incubated at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The  $I_{50}$  Cr (VI) concentrations for PSII and PSI inhibition in the chloroplast incubated at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was 100 and 1000  $\mu\text{M}$  Cr (VI)



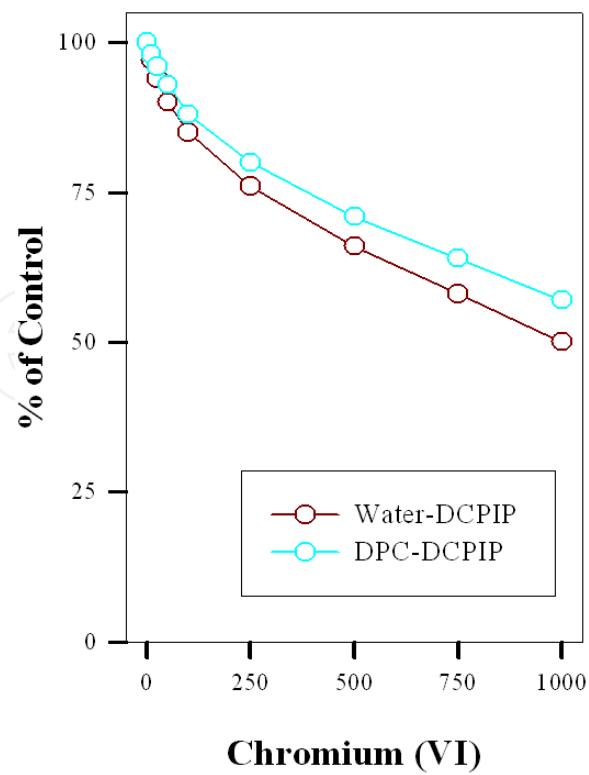
**Figure 2.** Effect of different concentrations of Cr on isolated spinach chloroplasts incubated either in dark or 10 µmol light. A: whole chain rate (Water to MV), B: PS II rate (Water to DMBQ) and C: PS I rate (ASC/DCPIP to MV). Control rate of whole chain reaction: 200 µmol mg chl<sup>-1</sup> h<sup>-1</sup>; control rate of PS II reaction: 90 µmol mg chl<sup>-1</sup> h<sup>-1</sup>; control rate of PS I: 350 µmol mg chl<sup>-1</sup> h<sup>-1</sup>.

respectively which otherwise could not be achieved in chloroplasts incubated in dark even with 2000 µM Cr (VI).

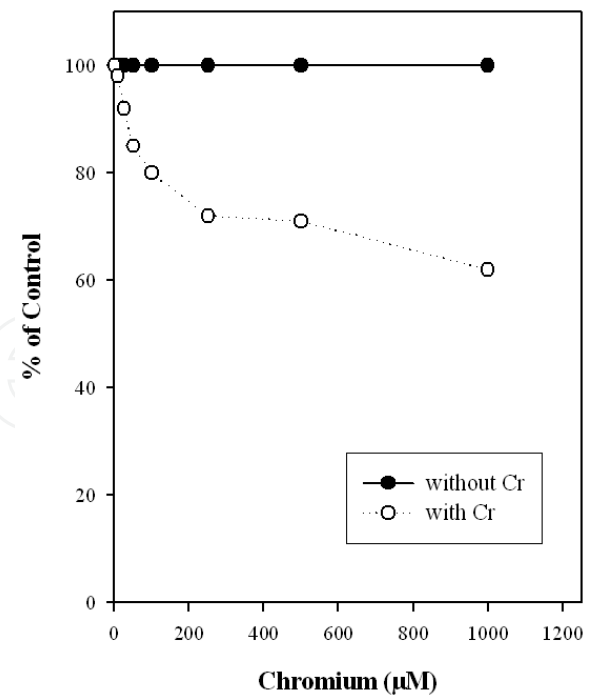
In the past, most workers located the target of heavy metal inhibition of PSII to its oxidizing side [5]. In the present study, the assay of partial electron transport reaction was carried out with DCPIP as an electron acceptor. DCPIP accepts electrons from the plastoquinone pool of the electron transport chain and its photo-reduction is considered primarily a PS II reaction [7]. Cr (VI) treated PSII particles showed a gradual reduction in water to DCPIP rate. And DPC to DCPIP reaction of PS II did not show any significant recovery in rates from H<sub>2</sub>O to DCPIP in the isolated PSII particles (Figure 3). These reactions ruled out the possibility of Cr effects on water oxidation complex.

As the DPC as electron donor could not reverse the PSII inhibition by Cr (VI), we may assume that the Cr (VI) if inactivate PSII donor side, that must be after DPC electron donating site i.e. the P680 and/or the acceptor side. It is possible that Cr impaired the PSII photochemistry by an interaction at or beyond the PSII primary electron carrier donor, TYR, (redox active Tyr of the D1 protein). It was, however, worth noting that in the present study Cr (VI) inhibited the DCMU-insensitive H<sub>2</sub>O-SiMO<sub>4</sub> (silicomolybdate) activity. Although, silicomolybdate is an artificial electron acceptor with a very controversial binding site in PSII, this acceptor has been long considered the only specific one for testing the PSII donor side in a Hill reaction [36-38]. We found gradual reduction (5 to 32%) in water to silicomolybdate rate as the Cr concentration increased (Figure 4). Our results on above reaction carried out with Tris-washed PSII particles clearly excluded the Q<sub>B</sub> site and the water splitting system as the main Cr-inhibitory targets in PSII.



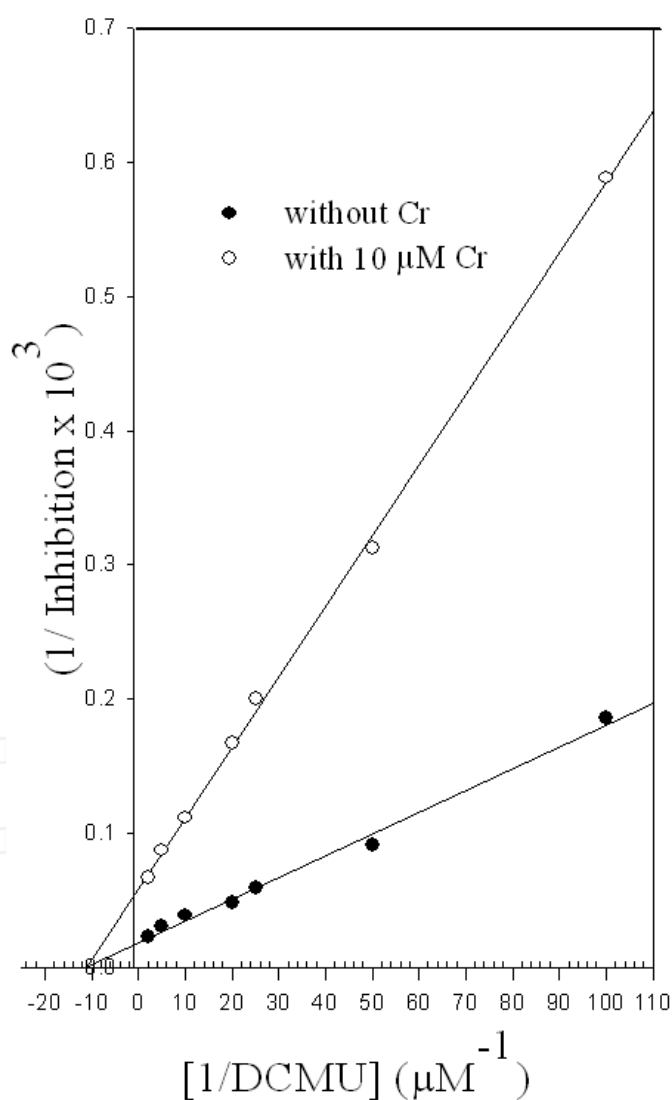


**Figure 3.** Effect of DPC on DCPIP reduction in Cr treated spinach PS II particles incubated in  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  light for 5 min.



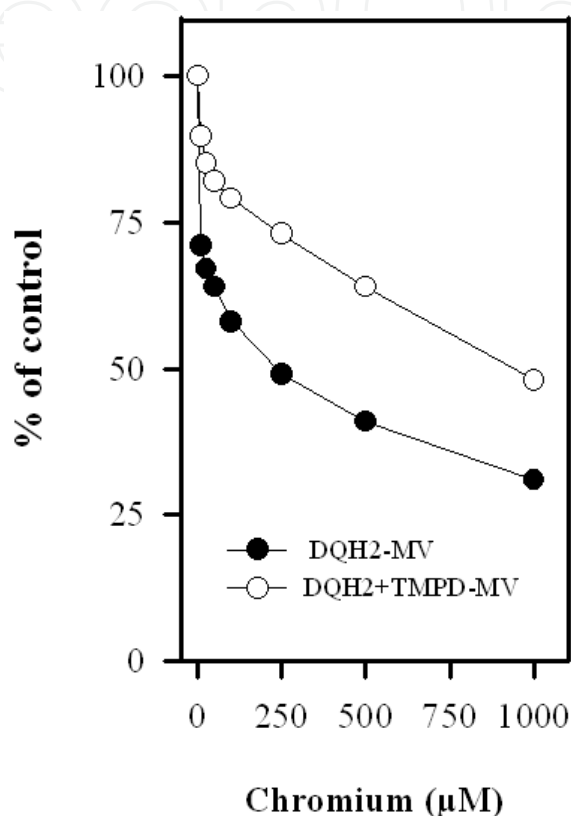
**Figure 4.** Effect of different concentrations of Cr on water to silicomolybdate rate of PS II particles isolated from spinach chloroplasts. 100% control activity was about  $150 \mu\text{mol O}_2 \text{ evolved mg chl}^{-1} \text{h}^{-1}$ .

That the Cr (VI) inhibitory site is located before DCMU inhibitory site we studied the DCMU-inhibition pattern in the presence of different concentration of Cr by using Lineweaver-Burk plot. The data obtained were plotted  $1/\text{inhibition}$  vs.  $1/[\text{DCMU}]$  in the presence of different concentration of Cr (VI) and saturating amount of DMBQ. We obtained linear regression curves with the same intersect on the X axis, that implies that Cr (VI) is a non-competitive inhibitor with respect to DCMU (Figure 5). These results indicated that Cr (VI) binding site did not overlap with that of the DMBQ electron acceptor site and that of the DCMU binding site. These findings further suggested that the Cr (VI) binding site might be at the level of the Pheo- $Q_A$ -Fe domain, separated from the  $Q_B$  niche (the DMBQ electron acceptor site). DCMU was shown to be competitive inhibitor with respect to  $Q_B$  is in agreement with the location of this herbicide-binding site at the level of  $Q_B$  niche.



**Figure 5.** Reciprocal plot of the inhibitory effect of DCMU on the inhibition of oxygen evolution activity by Cr (VI). The DCMU-inhibition was measured in the absence of Cr (VI) and in the presence of 10  $\mu\text{M}$  Cr (VI) and is given as  $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$ . DMBQ was used as electron acceptor at a concentration of 0.5 mM.

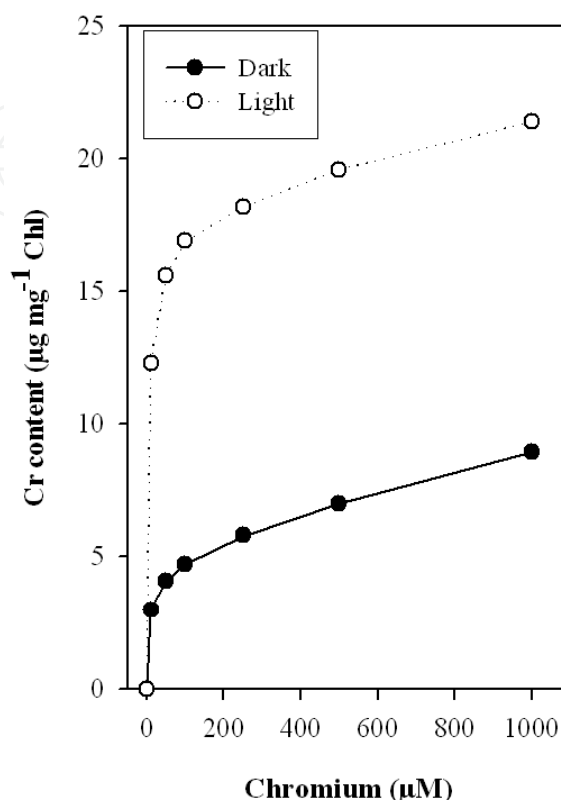
In order to see if any component between the two photosystems was affected by Cr (VI), the electron transport from reduced plastoquinone to methyl viologen was monitored by using duroquinol as the donor for MV reduction. At 10 – 1000  $\mu\text{M}$  Cr (VI) a 30-70% inhibition was found (Figure 6). There was restoration of 17-24% of electron flow by addition of TMPD which has been shown to bypass the native plastoquinone site. Thus the results indicated that the components between the two photosystems were partly affected by Cr (VI).



**Figure 6.** Effect of different concentrations of Cr on electron transport rate between the two photosystems in spinach chloroplasts

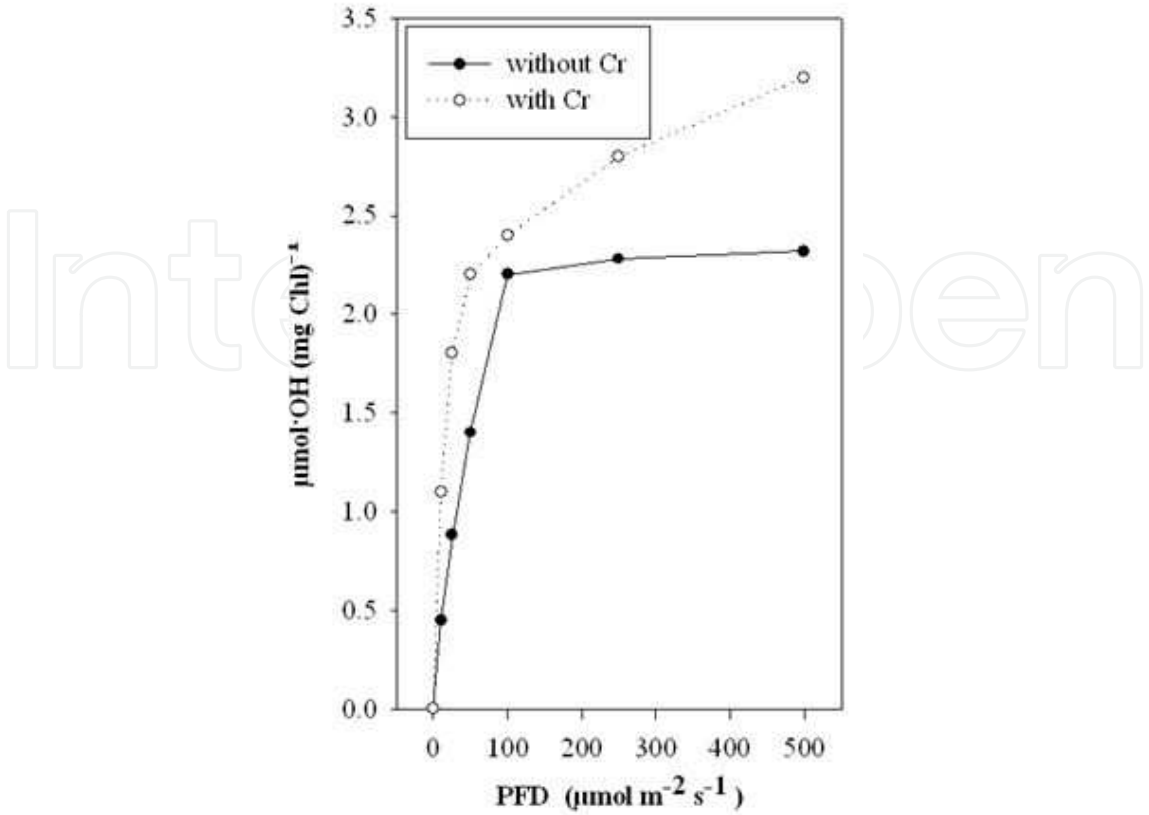
The possibility of Cr (VI) binding to the thylakoids during dark and light incubation might be a cause of different degree of inhibition, was examined by determining the amount of bound chromium. The membranes were washed thrice with incubation buffer containing  $\text{MgCl}_2$ , NaCl and MES at pH 6.5 to remove weakly associated Cr and then analyzed their Cr content. The amounts of Cr tightly bound to the thylakoid membranes treated with different concentrations of Cr (VI) in the dark and at low PPFD of  $10 \mu\text{mol m}^{-2}\text{s}^{-1}$  are presented in Figure 7. The results showed that thylakoid membranes treated in light had more bound Cr than those treated in dark. The differences in the binding of Cr to the thylakoid membranes were significantly higher in light than in the dark at all the Cr (VI) concentrations (Figure 7). Moreover, the binding of Cr to the membranes as a function of Cr (VI) concentrations both in dark and light showed a gradual increase from 3 and  $12.3 \mu\text{g Cr mg}^{-1} \text{Chl}$  at  $10 \mu\text{M}$  Cr (VI) to 9 and  $21.4 \mu\text{g Cr mg}^{-1} \text{Chl}$  at  $1000 \mu\text{M}$  Cr (VI) concentrations, respectively. It is possible that

low PAR during Cr exposure to thylakoids may induce conformational and dynamic movements of the complete thylakoid membrane system that may facilitate transport of Cr into the granal region to bind with functional components of electron transport chain.



**Figure 7.** Binding of Cr to thylakoid membranes in light and dark

Chromium enhanced formation of lethal hydroxyl radical in light treated thylakoids (Figure 8). In order to investigate the mechanism of Cr (VI) mediated reactive oxygen species formation, we studied the effect of several oxygen radical scavengers on the oxygen evolution activity of Cr and light treated thylakoid membranes. With respect to treated thylakoids (10 µM Cr (VI) and 10 µmol m<sup>-2</sup> s<sup>-1</sup> light), where the activity was 53 % after 5 min of illumination, the activity was restored by ca. 10% by SOD and CAT (Table 1). We also used dimethyl sulfoxide (DMSO), a molecular probe for OH radical. It provided strong protection and activity was restored to 74% (Table 1). When DMSO, SOD and CAT were used together, they provided the best protection and activity was restored to 81% (Table 1). Ali et al [20] reported effect of Cr (VI) on *Lemna gibba* PS II photochemistry. The authors showed that Cr inhibitory site was located at oxygen evolving complex and Q<sub>A</sub> reduction. The inhibition of PS II electron transport and formation of ROS by Cr were highly correlated with the decrease in the D1 protein and OEC proteins. There is also evidence that ROS may induce direct degradation of D1 peptide bonds [37]. Thus our results implicate superoxide in the inhibition mechanism and give evidence that hydroxyl radicals are formed via metal-catalyzed Haber-Weiss reaction and contribute to the inhibitory mechanism.



**Figure 8.** Effect of PFD on Cr (10 μM) induced generation of hydroxyl radical in isolated spinach thylakoids.

Protective system	Activity (% of control)
Without additions	58.81±1.8
Catalase (2000 U ml <sup>-1</sup> )	64.26±1.6
SOD (300 U ml <sup>-1</sup> )	62.84±1.1
7% DMSO	74.40±1.6
7% DMSO + Catalase (2000 U ml <sup>-1</sup> ) and SOD (300 U ml <sup>-1</sup> )	81.52±2.4

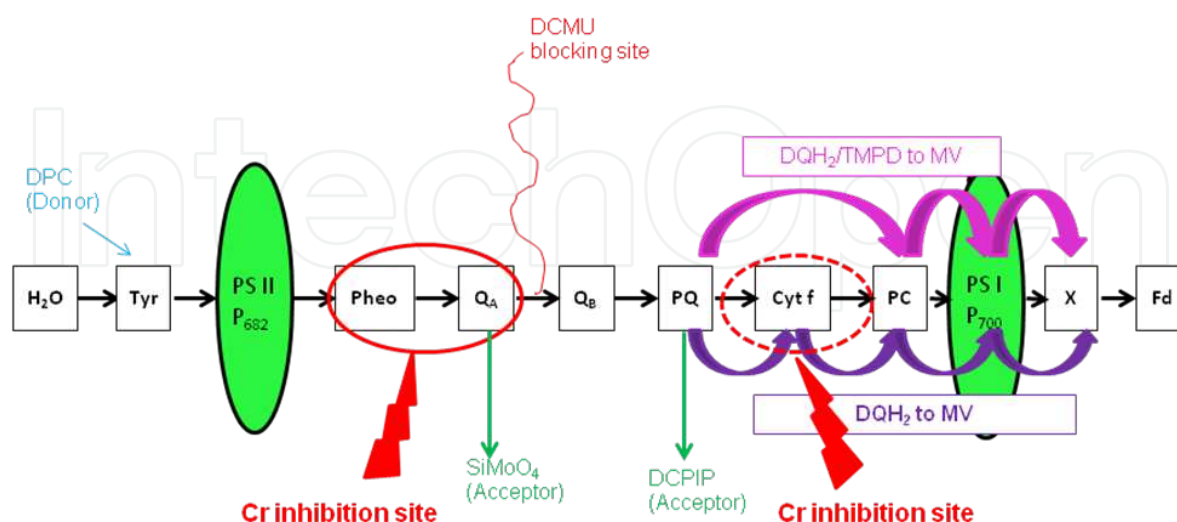
**Table 1.** Effects of combined systems for protection against free radicals on the inhibition of electron transport activity (H<sub>2</sub>O-MV) following Cr (10μM) +10μmol m<sup>-2</sup> s<sup>-1</sup> treatment of thylakoid membranes.

4. Conclusion

The present study showed that Cr (VI) inhibits both PS II and PS I electron transport of isolated spinach chloroplasts (Figure 9). Within the PS II, Pheo-Q<sub>A</sub> region was more affected. Water oxidation complex was not affected by Cr (VI). Light as low as 10 μmol m<sup>-2</sup> s<sup>-1</sup> enhanced Cr



(VI) induced inhibition of electron transport. Our results also indicated that hydroxyl radical may be a major contributing factor towards decreased electron transport rate.



**Figure 9.** Schematic diagram of sites of Cr inhibition of photosynthetic electron transport in isolated spinach chloroplasts. The major sites of inhibition are at Pheo-Q<sub>A</sub> domain and between two photosystems. Diagram also shows DQH<sub>2</sub> to MV and TMPD bypass rate. (for explanation please see the text). Electron transport chain components: Tyr tyrosine, P<sub>682</sub> reaction centre chlorophyll for PS II, Pheo pheophytin, Q<sub>A</sub> a plastoquinone tightly bound to PS II, Q<sub>B</sub> another plastoquinone loosely bound to PS II, PQ plastoquinone, Cyt f cytochrome f, PC plastocyanin, P<sub>700</sub> reaction centre chlorophyll for PS I, X primary electron acceptor of PS I, Fd ferredoxin

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