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RESEARCH ARTICLE

Photosynthetic performance of *Salvinia natans* exposed to chromium and zinc rich wastewater

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Investigations were carried out to evaluate alterations in photosynthetic performance of *Salvinia natans* (L.) exposed to chromium (Cr) and zinc (Zn) rich wastewater. Accumulation of high levels of Cr and Zn in plants affected photosynthetic electron transport. Photosystem- (PS) II-mediated electron transport was enhanced in plants exposed to Cr rich wastewater while a decline was observed in Zn-exposed plants. Photosystem-I-mediated electron transport increased in plants exposed to Cr and Zn rich wastewater. Efficiency of photosystem II (F_v/F_m) measured by fluorescence did not show any significant change in Cr-exposed plants but a decrease was observed in Zn-exposed plants as compared to the control. The enhancement in PS I-induced cyclic electron transport in Cr and Zn exposed plants led to a build up of the transthylakoidal proton gradient (ΔpH) which subsequently helped in maintaining the photophosphorylation potential to meet the additional requirement of ATP under stress. The carbon assimilation potential was adversely affected as evident from the decrease in Rubisco (EC 4.1.1.39) activity. The alterations in photosynthetic electron transport affected stromal redox status and induced variations in the level of stromal components such as pyridine nucleotides in plants exposed to Cr and Zn rich wastewater. The present investigations revealed that alteration in the photosynthetic efficiency of *Salvinia* exposed to Cr could primarily be the result of a decline in carbon assimilation efficiency relative to light-mediated photosynthetic electron transport, though in the case of Zn-exposed plants both these factors were affected equally.
Key words: carbon assimilation, electron transport, heavy metals, photophosphorylation, redox status

Desempenho fotossintético de plantas de *Salvinia natans* expostas a água de esgoto rica em cromo e zinco: Avaliou-se o desempenho fotossintético de plantas de *Salvinia natans* L. expostas a água de esgoto rica em cromo (Cr) e zinco (Zn). O acúmulo de Cr e Zn em altos níveis afetou o transporte fotossintético de elétrons. O transporte de elétrons mediado pelo fotossistema (FS) II aumentou nas plantas expostas ao esgoto rico em Cr, observando-se o oposto nas plantas expostas ao esgoto rico em Zn. O transporte de elétrons mediado pelo FS I aumentou nas plantas expostas ao esgoto rico tanto em Cr como em Zn. A eficiência do FS II (F_v/F_m) avaliada por fluorescência não variou significativamente em resposta ao Cr, porém reduziu-se nas plantas expostas ao esgoto rico em Zn, em comparação com as plantas-controle. O aumento do transporte cíclico de elétrons associado ao FS I acarretou em aumento do gradiente transtilacoidal de prótons (ΔpH) que, por seu turno, esteve associado com o potencial de fotofosforilação para suportar as necessidades adicionais de ATP sob condições de estresse. O potencial de assimilação do carbono foi decrescido, conforme se deduz da menor atividade da Rubisco (EC 4.1.1.39). As alterações no transporte fotossintético de elétrons afetaram o status redox do estroma e acarretou variações nos níveis de componentes estromais, como nucleotídeos de piridina, nas plantas expostas ao esgoto rico em Cr e Zn. Nas plantas de *Salvinia* expostas ao Cr, as alterações no desempenho fotossintético foram primariamente resultantes de um declínio da eficiência de assimilação do carbono, enquanto nas plantas expostas ao esgoto rico em Zn tanto a eficiência de assimilação do carbono como o transporte de elétrons comprometeram igualmente o desempenho fotossintético.
Palavras-chave: assimilação do carbono, fotofosforilação, metais pesados, status redox, transporte de elétrons

INTRODUCTION

Heavy metals released during industrial activity form a major portion of the contaminants that accumulate in water bodies. Chromium in particular, being highly soluble and bioavailable, exerts toxic effects on biological systems arising from the possibility of free-diffusion across the cell membrane and its strong oxidative potential (Shanker et al., 2005) whereas Zn-induced damage to living systems mainly includes impairment in functioning of several essential enzymes involved in metabolism (Rout and Das, 2003).

Heavy metal exposure induces alterations in the basic metabolic events in plants. Photosynthesis is one of the major physiological processes known to be affected severely by heavy metal stress. The heavy metal-induced damage to the photosynthetic apparatus includes changes in photosynthetic electron transport and carbon fixation capacity (Clijsters and Van Assche, 1985; Krupa and Baszynski, 1995; Siedlecka et al., 1997; Sersen et al., 1998; Shanker et al., 2005). The changes in photosynthetic potential are regulated by enzymes of the ferredoxin-thioredoxin system which account for the stromal redox status of the plant (Scheibe et al., 2005; Schrader et al., 2007). Photophosphorylation which is energetically coupled to the flow of protons through ATPase and driven by the transthylakoidal proton gradient also seems to be affected by heavy metal exposure (Panda and Choudhury, 2005; Sharma and Dubey, 2005).

Aquatic plants possess an immense potential to remove heavy metals from wastewater (Dushenkov et al., 1995; Kara, 2005). Though extensive literature is available highlighting the potential of several *Salvinia* species for accumulation/removal of various heavy metals including Ni, Cu, As, Pb, Cr, Zn from wastewaters (Sen and Mondal, 1990**;** Sen and Bhattacharya, 1994**;** Nigam et al., 1998**;** Nichols et al., 2000**;** Hoffman et al., 2004; Hadad et al., 2007), the physiological factors and regulatory mechanisms related to the greater potential of plants for tolerating high levels of heavy metals are not known. Moreover, the exact mechanism of damage to the photosynthetic apparatus whether electron transport or carbon assimilation when *Salvinia natans* is exposed to heavy metals has not been studied. The present studies were carried out to assess the potential of *S. natans* for heavy metal uptake and evaluate the alteration in photosynthetic performance of plants on exposure to Cr and Zn rich wastewaters. Detailed investigations were carried out with the aim of assessing the impact of Cr and Zn rich wastewaters on (i) photosynthetic electron transport in *S. natans* using polarographic and fluorescence techniques; (ii) carbon assimilation potential through changes in ribulose-1,5-carboxylase/oxygenase (Rubisco) activity; (iii) photophosphorylation potential; and (iv) stromal redox status.

MATERIAL AND METHODS

Plant material and growth conditions: Salvinia natans L. (Salviniaceae), a free-floating aquatic fern was chosen as the study material. Plants collected from unpolluted water bodies were maintained in cemented pots (~1 m diameter) under natural light conditions. The temperature ranged from 30 to 32ºC. The wastewater samples collected from a Cr and Zn plating (electroplating) unit were used as the experimental solution. The wastewater samples possessed Cr (total) and Zn at a concentration of 50 mg L^{-1} and 35 mg L^{-1}, respectively. Tap water was used as control. The wastewater samples were also checked for the presence of other heavy metals. The physico-chemical characterization of wastewater was carried out following the standard protocols given in APHA (1998). The parameters such as chloride, total hardness, ammonium nitrogen, sulphate, and phosphate were measured following protocols as detailed in APHA (1998). Electrical conductivity (EC) and total dissolved solutes (TDS) were measured using a conductivity meter (Model 1601 E, Electronic, India). Ten plants of *Salvinia* were floated in tub containing 1 L of Zn rich wastewater. A similar set up was used for the control (tap water) and Cr rich wastewater. The plants were exposed to metal for 48 h and subsequently harvested for metal analysis. The physiological and biochemical studies were also carried out after 48 h of metal exposure.

*Heavy metal analysis***:** After harvest, the plant samples were digested using a mixture of nitric and perchloric acid (3:1) (Rai et al., 2003) and analyzed for heavy metal content using an atomic absorption spectrophotometer (AA-6300, Shimadzu Corporation, Kyoto, Japan). Water samples collected at different time intervals were also analyzed for heavy metal content.

Photosynthetic pigments: The photosynthetic pigments, namely chlorophyll a, chlorophyll b and total chlorophyll, were estimated according to Arnon (1949). The leaves were homogenized in 80% acetone and the homogenate centrifuged at 10,000 g_n for 10 min. The optical density of the supernatant was measured spectrophotometrically at 645, 663, 750 nm.

Photochemical activities: PSI- and PSII-mediated electron transport was measured in isolated thylakoids using an oxygen electrode (Oxygen Electrode, Hansatech Instruments, Norfolk, England). The thylakoids were isolated from leaves as per modified protocol of Mishra and Sabat (1995). The leaves were crushed in extraction buffer containing 300 mM sucrose, 10 mM NaCl, 10 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM ascorbate and 20 mM Tricine-NaOH (pH 7.5). The homogenate was filtered through four layers of muslin cloth and the filtrate centrifuged at 6000 g_n for 5 min. The pellet was suspended in 1 mL of suspension buffer containing 100 mM sucrose, 10 mM NaCl, 10 mM $CaCl_2$, 5 mM $MgCl_2$ and 20 mM Tricine-NaOH (pH 7.5). The reaction mixture for assaying PSII activity consisted of 50 mM HEPES buffer (pH 7.5) containing 400 mM sucrose, 5 mM $MgCl_2$, and 10 mM NaCl in a final volume of 1 mL. The activity was measured as oxygen evolution with water as electron donor and 500 µM phenyl *p*-benzoquinone (pBQ) as the electron acceptor (Atal et al., 1991). For measurement of PSI-mediated electron transport, the reaction mixture consisted of assay buffer along with 5 µM DCMU, 100 µM dichlorophenol indophenol (DCPIP), 1 mM ascorbate, 500 µM methyl viologen and 1 mM sodium azide (Atal et al., 1991). The chlorophyll content in thylakoids was estimated according to Arnon (1949). The thylakoids equivalent to 10 µg chlorophyll were used.

Fluorescence analysis: PSII efficiency was measured at room temperature using the Plant Efficiency Analyzer equipment (Handy PEA, Hansatech Instruments Ltd., Norfolk, England) with an excitation light intensity of 3000 µmol m^{-2} s^{-1} as per Appenroth et al. (2001). A single pulse of actinic light with a duration of 1 s was applied. All measurements were taken using 30 min dark-adapted intact leaves. The relative fluorescence values of initial (F_0), maximal (F_m) and variable (F_v) fluorescence were determined. The PSII efficiency was expressed as the F_v/F_m ratio. The specific energy fluxes such as absorption (ABS), electron transport (ET) and dissipation (DI) per reaction center (RC) were also measured (Aragão et al., 2005).

Rubisco activity: The activity (Rubisco) (EC 4.1.1.39) was measured in a crude leaf extract following Sato et al. (1980). The assay reaction mixture consisted of 50 mM Tris-HCl buffer (pH 8.0), 10 mM $NaHCO_3$, 5 mM $MgCl_2$, 2 mM NADH, 1 mM ATP, 1 mM DTT, 0.5 mM RuBP, 5 U glyceraldehyde-3-phosphodehydrogenase, and 5 U phosphoglycerate kinase in a final volume of 3 mL. The change in absorbance was measured at 340 nm.

Measurement of ΔpH: The formation of the transthylakoidal pH gradient (ΔpH) was measured in terms of quenching of the fluorescent dye 9-aminoacridine. The reaction mixture consisted of 10 mM Tricine-NaOH buffer (pH 7.8), 5 mM NaCl, 5 mM $MgCl_2$, 5 µM dye and thylakoids equivalent to 10 µg chlorophyll (Evron and McCarty, 2000). The fluorescence was measured using a microplate fluorescence reader (Flx-800, Biotek, Vermont, USA) at excitation and emission wavelengths of 360 and 430 nm, respectively. Values of ΔpH were calculated following Schludiner et al. (1972).

Photophosphorylation potential: The photophosphorylation reaction was measured in isolated thylakoids as per modified protocol of Lindon et al. (1993). The assay reaction mixture consisted of 25 mM Tris buffer (pH 7.8), 20 mM ADP, 2 mM KH_2PO_4, 1 mM $K_3Fe(CN)_6$ and 5 mM phenazine methosulfate (PMS). Thylakoids equivalent to 10 µg chlorophyll were added. The reaction mixture was illuminated for 3 min using saturating white light at an intensity of 1000 µmol m^{-2} s^{-1}. The concentration of ATP was determined enzymatically by reduction of NADP in presence of glucose, hexokinase and glucose-6-phosphate dehydrogenase (Lamprecht and Trautschold, 1976).

Measurement of pyridine nucleotides: $NADP^+$ and NADPH levels in isolated chloroplasts were measured according to enzyme cycling assay of Takahama et al. (1981). The reaction mixture consisted of 33 mM Tris buffer (pH 7.6), 2 mM EDTA, 0.1 mM DCPIP, 60 µM PMS, 3.3 mM glucose 6-phosphate and 10 µg enzyme glucose-

6-phosphate dehydrogenase. The reaction mixture was incubated for 5 min. The reaction was started by adding enzyme and the change in the absorbance was followed at 600 nm. The concentration of the pyridine nucleotides was determined according to standard curves prepared using known amounts of pyridine dinucleotides (Takahama et al., 1981).

From the measured concentrations of NADPH and NADP, the anabolic reduction charge (ARC) was calculated according to Backhausen et al. (2000):

ARC = [NADPH] / [NADP$^+$ + NADPH]

Measurement of NADP-malate dehydrogenase (NADP-MDH; EC 1.1.1.82) activity: The activity of enzyme was measured in isolated chloroplasts. The reaction mixture consisted of 100 mM Tris-HCl (pH 8.0), 1 mM EDTA, 14 mM β-mercaptoethanol, 2 mM oxaloacetate and 0.2 mM NADPH. The change in absorbance was measured at 340 nm. The activity of enzyme measured immediately accounted for initial (non-activated) activity, while total activity was measured after complete activation of enzyme by incubating chloroplast suspension with 20 mM dithiothreitol at pH 8.0 at room temperature. The final activity was expressed as the ratio of initial to total activity (activated) (Scheibe et al., 1986).

Data analysis: Analysis of variance (ANOVA) for all measured variables was performed using the software new MSTAT-C (version 2.1). The level of significance was measured using Duncan's multiple range test taking $P \leq 0.05$ as significant.

RESULTS

Heavy metal analysis: Various physico-chemical characteristics of wastewater samples were determined and their values are summarized in Table 1. Wastewater samples collected from an electroplating unit at different time intervals possessed Cr and Zn at the concentrations of 50 and 35 mg L^{-1}, respectively. Chromium and Zn rich wastewater samples did not show the presence of heavy metals such as Ni, Pb, As, Co but contained trace quantities of Cu, Cd, Mn, Zn (Table 2). *Salvinia* accumulated Cr and Zn to levels as high as 10.6 and 4.8 mg g^{-1} DW on exposure to wastewaters having 50 mg L^{-1} Cr and 35 mg L^{-1} of Zn, respectively (Figure 1).

Table 1. Physico-chemical characteristics of water, Cr and Zn wastewater samples measured at the time of experimental set up. EC = electrical conductivity; TDS = total dissolved solutes.

Parameters	Water	Cr	Zn
pH	6.5	4.5	5.6
EC (mS)	0.51	0.82	0.93
TDS (ppt)	0.53	0.52	0.83
Chloride (mg L^{-1})	91	87	107
Sulphate (mg L^{-1})	18	24	32
Phosphate (mg L^{-1})	9.8	8.5	11
Nitrogen (mg L^{-1}) (Ammonical)	2.8	4.3	4.8
Hardness (mg L^{-1})	200	230	223

Table 2. Heavy metal concentration of water, Cr and Zn wastewater samples measured at the time of experimental set up.

Metals	Concentration (mg L^{-1})		
	Water	Cr	Zn
Ni	0	0	0
Pb	0	0	0
Co	0	0	0
As	0	0	0
Cd	0.09	0.12	0.12
Cu	0	0.9	0.95
Mn	0.2	0.8	1.8
Cr	0.5	50	0.3
Zn	1.5	1.8	35

Photosynthetic pigments: Accumulation of Cr and Zn at high levels induced decreases in concentrations of chlorophyll a, chlorophyll b and total chlorophylls, though the decline was more significant in Zn-exposed plants (Table 3).

Photochemical activities: Primary photochemical activities measured polarographically using an oxygen electrode showed that PS-II-mediated photosynthetic electron transport was higher than the control in Cr-exposed *Salvinia* but lower than the control in plants exposed to Zn. The PS II activity of Cr-exposed plants showed a ~1.6 fold increase in comparison to the control. In contrast, a ~25% reduction was noted in Zn-exposed plants. The PS-I-mediated photochemical activity revealed a ~1.7 and 2.6 fold increase in plants exposed to Cr and Zn rich wastewater, respectively (Figure 2).

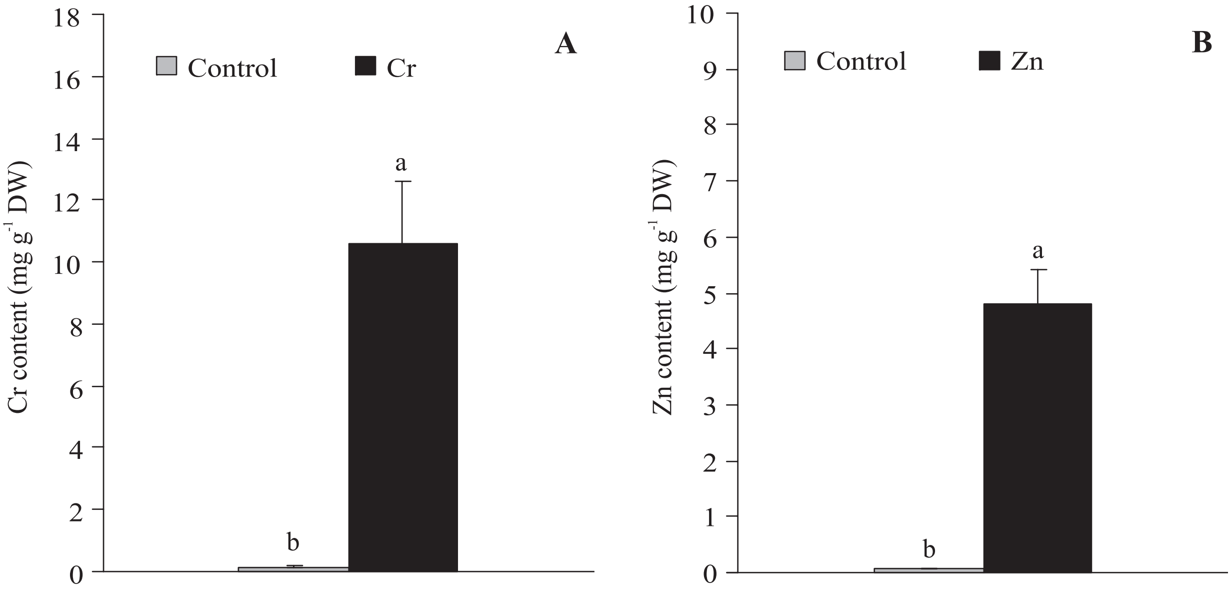

Figure 1. Chromium (**A**) and zinc (**B**) accumulation (mg g^{-1} DW) in *Salvinia* measured after 48 h of exposure to Cr and Zn rich wastewater. Each value represents the mean of five replicates ± SE. The different letters over bars indicate significant differences at $P \leq 0.05$.

Table 3. Photosynthetic pigments (mg kg^{-1} FW) of *Salvinia* measured after 48 h of metal exposure. Each value represents the mean of five replicates ± SE. Data followed by different letters in a column are significantly different at $P \leq 0.05$.

Treatments	Parameters		
	Chlorophyll a	Chlorophyll b	Total chlorophyll
Control	458 ± 47^a	149 ± 16^a	616 ± 64^a
Cr	427 ± 44^a	141 ± 16^a	574 ± 55^b
Zn	409 ± 43^b	129 ± 14^b	550 ± 56^b

Fluorescence analysis: The fluorescence technique is used to assess photosynthetic performance of plants non-invasively based on its relationship with the quantum efficiency of electron transport through PS II in leaves Both the F_0 and F_m values showed a significant decline in both Cr- and Zn-exposed plants. The F_v/F_m ratio declined in Zn-exposed plants in contrast to Cr-exposed ones where the values remained more or less equal to the control (Table 4). Plants exposed to Cr presented a slight increase in absorption per reaction center without any significant alteration in electron transport. The reaction center molecules of Cr-exposed plants utilized the absorbed light energy more efficiently, hence showing no significant alteration in electron transport. Accordingly, a low rate of energy dissipation was recorded. In contrast, damage to reaction center molecules in Zn-exposed plants adversely affected light absorption and hence utilization capacity, resulting in excessive energy dissipation (Table 4).

Rubisco activity: Activity of Rubisco, one of the major enzymes of the carbon fixation cycle, showed a significant decline of about 25% in Cr- and Zn-exposed plants (Fig. 3).

Transthylakoidal pH gradient (ΔpH) and photophosphorylation potential: The increase in PS-I-mediated electron flow observed in Cr- and Zn-exposed plants contributed to a build up of ΔpH in both Cr- and Zn-treated plants (Fig. 4A). On the other hand, the photophosphorylation potential did not show any significant change in plants exposed to Cr and Zn rich wastewater (Fig. 4B).

Pyridine nucleotides and NADP-malate dehydrogenase activity: Among the pyridine nucleotides, the levels of NADPH significantly increased in plants exposed to Cr. In contrast, a significant decline was observed in Zn-exposed plants (Table 5). Correspondingly, a considerable decline in $NADP^+$ levels was noted in plants

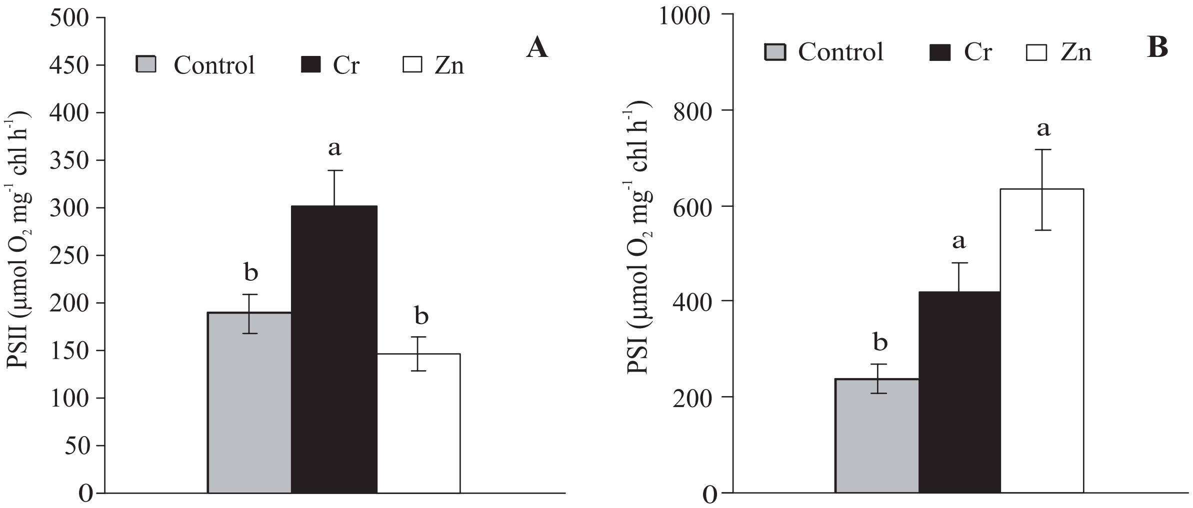

Figure 2. Alterations in PS II (**A**) and PS I (**B**) activity measured after 48 h of exposure to Cr and Zn rich wastewater. Statistics as in Figure 1.

Table 4. Changes in photosynthetic efficiency of plants measured through fluorescence after 48 h of metal exposure. The parameters measured include F_0 (initial fluorescence), F_m (maximum fluorescence), variable-to-maximum fluorescence ratio (F_v/F_m), absorption per reaction center (ABS/RC), electron transport per reaction center (ET/RC), and dissipation per reaction center (DI/RC). All the values are expressed in relative units. Statistics as in Table 3.

Treatments	Parameters					
	F_0	F_m	F_v/F_m	ABS/RC	ET/RC	DI/RC
Control	350 ± 34^{a}	1650 ± 178^{a}	0.78 ± 0.09^{a}	4.66 ± 0.56^{a}	1.76 ± 0.21^{a}	1.60 ± 0.20^{a}
Cr	303 ± 32^{b}	1215 ± 119^{b}	0.75 ± 0.08^{a}	5.11 ± 0.72^{b}	1.48 ± 0.50^{a}	1.38 ± 0.24^{a}
Zn	311 ± 32^{b}	1009 ± 120^{b}	0.69 ± 0.07^{b}	2.57 ± 0.3^{b}	1.53 ± 0.17^{a}	2.49 ± 0.34^{b}

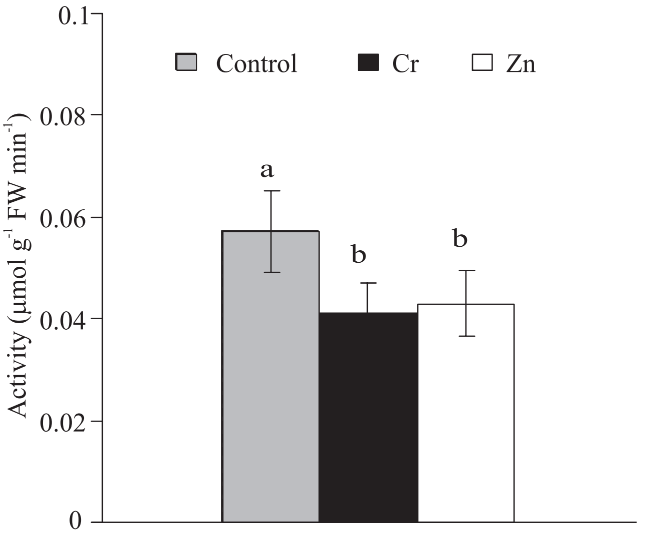

Figure 3. Rubisco activity of *Salvinia* measured after 48 h of exposure to Cr and Zn rich wastewater. Statistics as in Figure 1.

exposed to Cr and Zn. The anabolic reduction charge (ARC) ratio increased in plants exposed to Cr and Zn rich wastewater.

The NADP-MDH activity was enhanced in plants exposed to Cr and Zn rich wastewater (Table 5).

DISCUSSION

Salvinia exposed to Cr and Zn exhibited changes in photosynthetic potential. Alteration in levels of photosynthetic pigments affected the photosynthetic performance of *Salvinia*. The reduction in pigment levels in plants exposed to Cr and Zn rich wastewater could be the due to (i) reduced efficiency of enzymes involved in chlorophyll biosynthesis; (ii) decrease in availability of iron; and (iii) formation of metal substituted chlorophylls (Clijsters and Van Assche, 1985; Van Assche and Clijsters, 1990; Kupper et al., 1998). Similar responses of

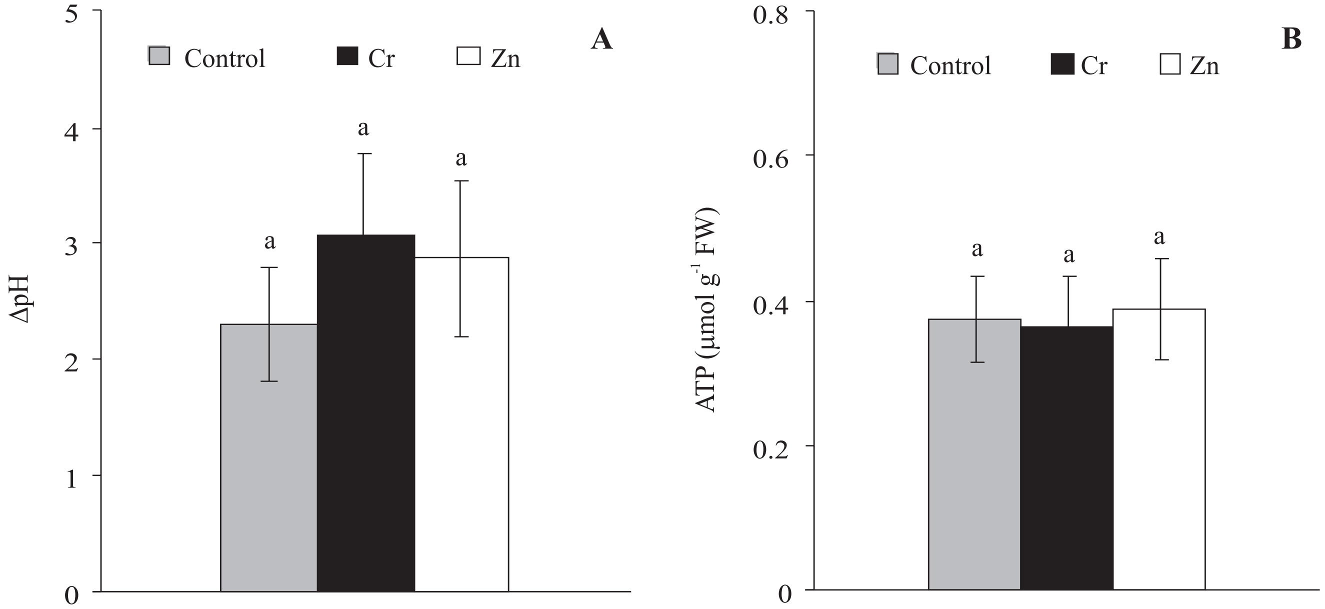

Figure 4. Changes in ΔpH (**A**) and photophosphorylation potential (**B**) of *Salvinia* measured after 48 h exposure to Cr and Zn rich wastewater. Statistics as in Figure 1.

Table 5. Alterations in pyridine nucleotide levels ($NADP^+$, NADPH), anabolic reduction charge (ARC), and NADP-malate dehydrogenase (NADP-MDH) activity measured after 48 h of metal exposure. Concentrations of $NADP^+$ and NADPH levels expressed as nmol g^{-1} FW, and NADP-MDH activity is expressed as the ratio of activated and non-activated enzyme activity. Statistics as in Table 3.

Treatments	Parameters				
	$NADP^+$	NADPH	NADPH/ $NADP^+$	ARC	NADP-MDH activity
Control	9.8 ± 1.2^a	5.17 ± 0.61^a	0.53 ± 0.07^a	0.34 ± 0.04^a	1.48 ± 0.17^a
Cr	7.14 ± 0.81^b	7.77 ± 0.91^b	1.08 ± 0.2^b	0.52 ± 0.07^b	1.79 ± 0.2^a
Zn	5.9 ± 0.7^b	4 ± 0.7^b	0.68 ± 0.08^a	0.40 ± 0.05^a	1.87 ± 0.2^a

reduction in photosynthetic pigments on exposure to heavy metals have been reported earlier in *Salvinia* species (Nichols et al., 2000; Al-Hamdani and Blair, 2004; Hadad et al., 2007). Among primary photochemical activities, PS-II-mediated electron transport remained more or less unaffected in plants exposed to Cr in contrast to that observed in Zn-treated plants. The PS II photochemical efficiency, measured as F_v/F_m, showed no significant change in Cr-exposed plants. Accordingly, the absorption of light energy and electron transport per reaction center was equal to that of the control in Cr-exposed plants. In contrast, Zn-treated plants showed a decline in F_v/F_m in comparison to their respective controls. A Zn-induced decrease in light quenching capacity might have resulted from damage to reaction center molecules and/or loss in chlorophyll content (Chen et al., 2008). Moreover, reduced efficiency for utilization of light energy adversely affected electron transport, hence increasing energy dissipation.

In contrast, PS-I-mediated photoreactions measured polarographically exhibited an increase in plants exposed to Cr and Zn rich wastewaters. This could well be an acclimation response to cope with the additional flow of electrons during stress (Makino et al., 2002). In general, plants exposed to abiotic stresses reveal down-regulation of linear electron flow and activation of cyclic electron transport, when linear electron flow becomes saturated (Fork and Herbert, 1993; Golding et al., 2004;

Scheibe et al., 2005). The activation of PS I can also be linked to the decreased stromal $NADP^+$/NADPH ratio (Rajagopal et al., 2003).

Salvinia exposed to Cr or Zn revealed a rise in ΔpH, which probably supported ATP synthesis under stress. It has been reported that enhancement in PS-I-mediated electron flow facilitates build up of the transthylakoidal proton gradient which acts as a driving force for ATP synthesis and helps in maintenance of photophosphorylation potential under stress (Backhausen et al., 2000; Makino et al., 2002). Moreover, the heavy metal-induced decrease in functioning of Calvin cycle enzymes also results in accumulation of ATP and NADPH leading to feedback inhibition of electron transport and increase in the transthylakoidal proton gradient (Siedlecka et al., 1997; Romanwoska et al., 2002; Siedlecka and Krupa, 2004). Adequate amounts of reducing equivalents such as NADPH and NADH are required for maintenance of stromal redox status (Backhausen et al., 2000). In this context, the increase in NADPH/$NADP^+$ ratio of Cr-exposed plants could be the consequence of either reduced utilization of NADPH resulting from a decline in Rubisco activity (thus preventing oxidation of stroma) or enhanced synthesis resulting from cyclic electron flow around PS I to meet the additional requirements of reducing equivalents during CO_2 fixation (Backhausen et al., 2000). Heavy metal-induced increases in NADPH levels have also been reported in aquatic plant species such as *Phragmites australis* (Pietrini et al., 2003). Alterations in NADPH levels have been reported in plants exposed to heat, chilling and other abiotic stresses (Schrader et al., 2004, 2007). In any case, since the ARC values were high in plants exposed to Cr and Zn rich wastewater, a low demand for NADPH in the stroma is to be expected. Similar responses of increase in redox status (NADPH/$NADP^+$) and phosphorylation potential of Scots pine seedlings exposed to high concentrations of metals such as Al (4 mM) have also been reported (Plucinska and Karolewski, 1994). The carbon fixation/assimilation potential was severely affected in plants exposed to Cr or Zn as evident from the decline in Rubisco activity. The deactivation of the enzyme after substitution of Mg^{2+} in the ternary Rubisco complex by metal cations can result in loss of carboxylation capacity (Van Assche and Clijsters, 1990). The decrease in CO_2 assimilation capacity of *Salvinia* has also been correlated with a decrease in photosynthetic pigments (Nichols et al., 2000; Al-Hamdani and Blair, 2004). Similar responses of decline in Rubisco activity have also been reported previously for a few aquatic species (Pietrini et al., 2003).

During photosynthesis, plants develop certain strategies to maintain homeostasis under stress. These mainly include activation of some thiol regulated chloroplast enzymes including NADP-MDH, the activation of which has been used as an indicator of redox state of stroma (Scheibe and Stitt, 1988). The enzyme catalyzes the formation of malate from oxaloacetate using NADPH to generate an electron acceptor, i.e. $NADP^+$, and its activity is switched off when NADPH is consumed by assimilatory processes in the chloroplast so that no reducing equivalents are exported (Scheibe et al., 2005). It is supposed that activation of the enzyme provides a way for reducing equivalents to allow some electron transport and proton pumping. *Salvinia* exposed to Cr and Zn did not exhibit a significant increase in NADP-MDH activity indicating that metal stress does not affect stromal status as adequate levels of NADPH and other reducing equivalents are maintained for CO_2 assimilation. It is supposed that closure of the malate valve under stress also prevents drainage of excess reducing equivalents from the chloroplast, hence preventing the oxidation of stroma (Scheibe and Stitt, 1988; Schrader et al., 2004). The effect of ARC and redox state on the enzyme activation has also been reported (Backhausen et al., 2000).

Conclusively, the present investigations revealed that changes in the photosynthetic potential of *S. natans,* irrespective of exposure to heavy metals Cr or Zn, could be primarily the result of alterations in photosynthetic electron transport or carbon assimilation without significant effects on photophosphorylation potential. The studies also suggest that CO_2 assimilation reactions are more sensitive to Cr exposure than photosynthetic electron transport and ATP-producing reactions, while in the case of exposure to Zn both CO_2 assimilation potential and photosynthetic electron transport are equally sensitive.

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