Short-term chromium(VI) stress induces different photosynthetic responses in two duckweed species, *Lemna gibba* L. and *Lemna minor* L.

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Abstract

Physiological responses of two duckweed species, *Lemna gibba* and *Lemna minor,* to hexavalent chromium [Cr(VI)] were studied in axenic cultures using short-term (48 h) treatments by $K_2Cr_2O_7$ (0–200 μM). Chlorophyll (Chl) fluorescence parameters and photosynthetic pigment composition of plants were screened to determine the effects of Cr(VI) exposures. The two duckweed species exhibited different sensitivity in the applied Cr(VI) concentration range. Chl fluorescence parameters of dark-adapted and light-adapted plants and electron transport inducibility were more sensitive to Cr(VI) in *L. minor* than in *L. gibba*. We also found fundamental differences in quantum yield of regulated, Y(NPQ), and nonregulated, Y(NO), non-photochemical quenching between the two species. As Cr(VI) concentration increased in the growth medium, *L. minor* responded with considerable increase of Y(NPQ) with a parallel significant increase of Y(NO). By contrast, in *L. gibba* only 200 μM Cr(VI) in the growth medium resulted in elevation of Y(NPQ) while Y(NO) remained more or less constant within the regarding Cr(VI) concentration range during 48 h. Photosynthetic pigment content did not change considerably during the short-term Cr(VI) treatment but decrease of Chl *a*/*b* and increase of Car/Chl ratios were observed in good accordance with the changes in Chl fluorescence parameters. The data suggest that various duckweed species respond with different sensitivity to the same ambient concentrations of Cr(VI) in the growth medium, and presumably to other environmental stresses too, which may have an influence on their competitive relations when heavy metal pollution occurs in aquatic ecosystem.

Additional keywords: chlorophyll fluorescence; Cr(VI); duckweed; electron transport rate; *Lemna gibba*; *Lemna minor*; non-photochemical quenching; rapid light curves.

Introduction

Despite of growing regulatory efforts toxic chemicals are released in ever-increasing amounts into natural bio-geochemical cycles. A considerable proportion of them eventually enter surface waters strongly affecting their biota. Unpredictable industrial accidents could result in high loads of toxic chemicals to the environment within short time intervals as happened to River Tisza in Hungary in 2000 when heavy-metal and cyanide contamination entered the river and caused ecological catastrophe (Lakatos *et al*. 2003). For such considerations it is essential to predict the possible effects of toxic substances on vital processes and species composition of

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Abbreviations: AL – actinic light; Car – carotenoids; Chl – chlorophyll; Cr(VI) – hexavalent chromium; E_k – light intensity at the onset of light saturation; F_o – minimal fluorescence of dark-adapted sample; F_o' – minimal fluorescence of light-adapted sample, measured after far-red pulse; F_m – maximal fluorescence of dark-adapted sample; FM – fresh mass; FR – far-red pulse; F_v – variable fluorescence of dark-adapted sample; F_v/F_m – variable to maximal fluorescence ratio of dark-adapted sample; F_v/F_o – variable to minimal fluorescence ratio of dark-adapted sample; ETR – electron transport rate; ML – measuring light; q_P – photochemical quenching; PFD – photon flux density; PSII – photosystem II; $rETR_{max}$ – relative maximum of the electron transport rate; RFD – relative fluorescence decrease; RLC – rapid light curve (without steady-state photosynthesis); SP – saturating light pulse; Y(II) and $\Delta F/F_m$' – photochemically converted proportion of the energy absorbed by PSII, *i.e.* actual photochemical efficiency; Y(NO) – yield of nonregulated non-photochemical loss of energy absorbed by PSII; Y(NPQ) – proportion of regulated non-photochemical loss of energy absorbed by PSII; α – initial slope of rapid light response curve, *i.e.* light-limited efficiency of ETR.

aquatic biota. Duckweed species are extensively used test organisms for assessment of potential impact of environmental chemicals in ecotoxicology and plant physiology (Environment Canada 1999). Duckweeds are free-floating plants showing wide distribution in different types of aquatic ecosystems. In spite of their small size, they exhibit large potential for vegetative reproduction and thereby rapid biomass growth. Being important elements in primary production and food chain, sensitivity of such aquatic macrophytes to various toxic chemicals may impact the functioning of the whole aquatic ecosystem.

Amongst heavy metals, the effects of chromium (Cr) on living organisms has received highlighted attention due to its strong toxicity and relatively less known mode of action. In aquatic ecosystems affected by anthropogenic activities the concentration of chromium could even reach mmol per liter order of magnitude (Perreault *et al*. 2009). Its oxidation form varies between 0 and +6 readily changing depending on redox circumstances of the environment. Under normal conditions only trivalent Cr(III) and hexavalent Cr(VI) forms are stable. Hexavalent chromium - the most toxic Cr form - usually exists as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ion (Hörcsik *et al*. 2007).

Plants are able to uptake Cr(VI) in higher concentration than Cr(III) with consequently greater translocation to shoots (Paiva *et al*. 2009). Following the uptake of chromate ion, which is similar to that of other chemically homologous anions, the destroying effects in cells take place as triggering oxidative burst. Due to potential destructive capability of heavy metals on biological membranes, photosynthetic pigments and proteins at cellular level (Cervantes *et al*. 2001, Panda and Choudhury 2005, Kučera *et al*. 2008), effects of Cr(VI) could be assessed by means of Chl fluorescence indirectly characterizing alterations in functioning of PSII (Schreiber *et al*. 1994, Ali *et al*. 2006, Hörcsik *et al*. 2007, Perreault *et al*. 2009).

In this study we investigated the effects of short,term Cr(VI) treatments on photosynthesis of two worldwide spread and frequently co-existing duckweed species, *L. gibba* L. and *L. minor* L.

Materials and methods

Axenic stock cultures of *L. gibba* L. and *L. minor* L. were maintained in 0.5 strength Hutner's medium (pH 6.3) (Lakatos *et al*. 1993) under controlled conditions (PFD = 50 ± 5 μmol m^{-2} s^{-1} continuous white illumination, 22 ± 2°C).

Short-term chromate exposures were performed for 48 h. 3–5 healthy colonies of stock cultures consisting of three or four fronds (as a total of 10–15 fronds, approx. 2 mg fresh mass of *L. minor,* 5 mg fresh mass of *L. gibba*) were transferred into 100 cm^3 Erlenmeyer flasks containing 50 cm^3 of growth medium. Cr(VI) was applied at the start of 48 h tests in final concentrations of 0, 50, 100, and 200 μM using $K_2Cr_2O_7$ stock solution. Treatments with every Cr(VI) concentration were performed in four replications under static conditions. Other culturing conditions corresponded to the maintenance of stock cultures. The experiments were repeated three times and the results of one representative experiment are shown in this paper.

Chl fluorescence parameters were measured with PAM 2000 Chl fluorometer (*WALZ GmbH*, Effeltrich, Germany) in intact plants placed on surface of 1.65 cm^3 pure 0.5 strength Hutner's medium in 2 cm^3 Eppendorf tubes. The surface of growth medium was covered by plants as properly as it was possible (number of fronds was 7–10 and 5–7 for *L. minor* and *L. gibba*, respectively. Plants were kept in these measuring tubes during dark adaptation and continuous light-acclimation periods. The *2010-F* fiberoptics of *PAM 2000* fluorometer was positioned in right angle above the surface of plant samples at a distance resulting in 0.2–0.4 mV F_o signal of dark-adapted control plants.

The measurement routine of Chl fluorescence parameters are presented in Fig.1. Minimal fluorescence (F_o) was measured after 20 min of dark adaptation using red modulated measuring light (ML). Then a saturating light pulse (SP: PFD = 6,000 μmol m^{-2} s^{-1}, white, 0.8 s) was applied to measure the maximal fluorescence (F_m) of plant samples. The difference between F_m and F_o was used for calculation of variable fluorescence (F_v).

After quenching of maximal Chl fluorescence in dark, plants were illuminated by continuous red actinic light (AL: PFD = 200 μmol m^{-2} s^{-1}) for 5 min. Fluorescence quenching during illumination was analysed by saturation pulse method. Saturating pulses (PFD = 6,000 μmol m^{-2} s^{-1}, white light, 0.8 s) were applied with 20-s intervals to determine the changes in maximal fluorescence (F_m') which were followed by far-red (FR) pulses to determine the values of F_o'. 5-min irradiation by actinic light proved to be long enough for both control and heavy-metal-treated plants of the species to reach nearly steady-state value of fluorescence (F_s'). Less than 5% variation was observed in values of Chl fluorescence parameters measured at the 4th and 5th min of continuous actinic illumination. Chl fluorescence parameters obtained after the last saturating pulse at the 5th min of illumination (Fig.1 *A*) were used for evaluation of responses of species to chromate treatments.

Use of this measurement routine with the *PAM 2000* fluorometer (Fig.1 *A*) allowed to estimate different Chl fluorescence parameters:

potential photochemical efficiency: F_v/F_m (Rosenqvist and van Kooten 2003),

estimation of the PSII photosynthetic capacity: F_v/F_o (Lichtenthaler *et al*. 2005),

photochemical quenching of variable fluorescence as

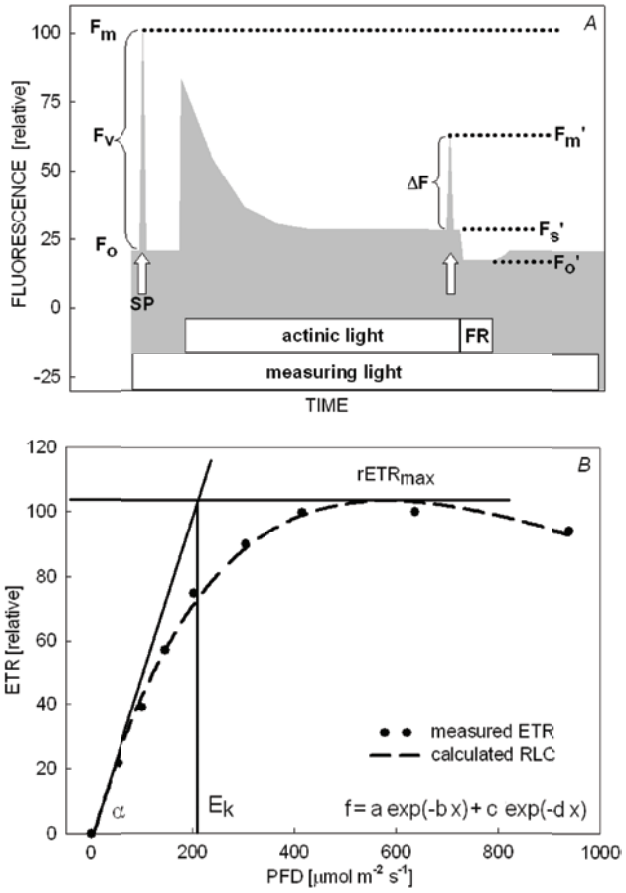

Fig. 1. *A*: Measurement routine of chlorophyll (Chl) fluorescence parameters of dark-adapted and light-adapted duckweed plants. Saturation-pulse method was used for analysis of parameters of illuminated samples. Duration of illumination by red actinic light (200 μmol m^{-2} s^{-1}) was 5 min. After continuous illumination a saturating pulse (6,000 μmol m^{-2} s^{-1}) was applied for estimation of Chl fluorescence quenching parameters. Nomenclature and calculation of Chl fluorescence parameters were used after Rosenqvist and van Kooten (2003). *B*: A rapid light curve (RLC) representing *L. minor* control plant with characteristic parameters of the curve. The curve was plotted on the measured ETR *vs*. PFD data points using *SigmaPlot*'s double exponential decay function.

an estimated proportion of open PSII reaction centres: $q_P = (F_m' - F_s')/(F_m' - F_o')$ (Rosenqvist and van Kooten 2003),

relative fluorescence decrease: $RFD = (F_m - F_s')/F_s'$ (Lichtenthaler *et al*. 2005),

yield of photochemical energy conversion (actual photochemical efficiency): $Y(II) = \Delta F/F_m' = (F_m' - F_s')/F_m'$ (Klughamer and Schreiber 2008),

yield of regulated non-photochemical energy dissipation: $Y(NPQ) = 1-\Delta F/F_m' - F_s'/F_m = (F_s'/F_m') - (F_s'/F_m)$ (Klughamer and Schreiber 2008),

yield of nonregulated non-photochemical energy dissipation: $Y(NO) = F_s'/F_m$ (Klughamer and Schreiber 2008).

Performance of photosynthetic electron transport was investigated by means of rapid light response curves (RLC) with 1-min-long light steps from 0 to 1,000 μmol(photon) m^{-2} s^{-1} after 20-min dark adaption, using *Mini-PAM* Chl fluorometer (*WALZ GmbH*, Effeltrich, Germany) and *2030-B* leaf-clip holder with right-angled fiberoptics. During these measurements plants were kept on moist filter paper in the leaf clip to avoid drying of fronds. Electron transport rate (ETR) was calculated using the equation: ETR [μmol(e^-) m^{-2} s^{-1}] = $\Delta F/F_m' \times$ PFD × 0.5 × 0.84 (Rosenqvist and van Kooten 2003) where 0.5 reflects the fraction of whole absorbed light utilized in PSII and 0.84 is the empiric proportion of absorbed incident light in a fully green leaf. The value of the latter one is presumably influenced by changes in Chl content under chromate stress. However, the estimation of relationship between absorption coefficient and Chl content was not in the focus of this work therefore we used the value of 0.84 for both control and treated plants.

Calculated ETRs were plotted *vs*. corresponding measured PFD values. Characteristic parameters of the empirical RLCs were mathematically calculated by "regression wizard" of *SigmaPlot v10.0* (*Systat Software, Inc*., San Jose, USA) with double exponential decay function (Fig. 1*B*). Initial slope of ETR response curve [α = μmol(electron) μmol(photon)$^{-1}$], calculated maximal ETR value [$rETR_{max}$ = μmol(e^-) m^{-2} s^{-1}] and light intensity at the onset of light saturation [E_k = μmol(photon) m^{-2} s^{-1}] of RLCs were calculated (Fig. 1*B*) (Saroussi and Beer 2007).

After Chl fluorescence measurements, the plant material was centrifuged by 3,000 rpm (850 × *g*) for 5 min to remove excess water. Fresh mass was measured with 0.0001 g accuracy and samples were subsequently stored at –80°C for further analyses.

Photosynthetic pigment content of plants was measured from 80% acetonic extracts by spectrophotometric method (*UV/VIS 1601*, *Shimadzu*, Japan) with 0.1 nm slit-width. Absorbances of extracts were measured at wavelengths 470, 663.2, and 646.8 nm and after correction with absorbance at 750 nm were used for calculation of concentrations of Chl *a*, Chl *b* and carotenoids by means of equations suggested by Wellburn (1994).

Effects of Cr(VI) treatments on every measured variable were characterized in four replicates (± SD) per concentration. Significance in differences among applied Cr(VI) concentrations were analyzed by *R 2.10.1* software (*R Foundation for Statistical Computing*, Austria) using *Welch*'s *t*-test. Differences are indicated as nonsignificant (NS), ^{a} $p<0.05$, ^{b} $p<0.01$, and ^{c} $p<0.001$ significance levels. Differences in responses of measured variables between *L. gibba* and *L. minor* were characterized by percentage changes of variables as compared to mean values of control plants.

Results

Short-term Cr(VI) treatment induced alteration of Chl fluorescence parameters of both duckweed species but significant interspecific differences were observed in the applied Cr(VI) concentration range.

Changes in potential (maximal) quantum yield of PSII (F_v/F_m) reflected much stronger chromate-induced inhibition of *L. minor* than that of *L. gibba* (Table 1). In *L. gibba* the decrease of F_v/F_m was only 1% and 2% at 50 and 100 μM Cr(VI), respectively, and amounted to its strongest level (17%) at 200 μM Cr(VI). In contrast, *L. minor* suffered 10% drop of F_v/F_m already at 50 μM Cr(VI) and exhibited further 27 and 37% decrease at 100 and 200 μM Cr(VI) in growth medium, respectively (Table 1). Variable to minimal fluorescence ratio (F_v/F_o) showed very similar pattern of inhibition as F_v/F_m in the applied Cr(VI) concentration range due to their analogous calculation (Table 1). However, F_v/F_o was more sensitive Chl fluorescence parameter of both species to Cr(VI) treatments than F_v/F_m. Cr(VI) induced two or three times higher inhibition of F_v/F_o than that of F_v/F_m. 200 μM Cr(VI) resulted in 47% decrease of F_v/F_o in *L. gibba* and 75% decrease of F_v/F_o in *L. minor* as compared to control plants (Table 1).

Differences in responses of F_v/F_m and F_v/F_o are due to different changes in F_m and F_o during Cr(VI) exposures. In both duckweed species reduction of F_m was found to be the main factor in decreasing variable fluorescence (Fig. 2). F_m values showed strong concentration-dependent decrease due to Cr(VI) treatments, reaching nearly 60% of control values at 200 μM Cr(VI) in both species. On the other hand, F_o responded to Cr(VI) diversely in the two species. In *L. gibba* even 200 μM Cr(VI) had no significant effect on F_o values. In *L. minor*, however, F_o showed significant increase with the increase of chromate concentration reaching 150% of control values at 200 μM Cr(VI) (Fig. 2).

Table 1. Potential photochemical efficiency (F_v/F_m and F_v/F_o) of dark-adapted plants (20 min) of *L. gibba* and *L. minor* measured after 48-h Cr(VI) treatments (0–200 μM). Values are means of 4 replicates, standard deviation (SD) are in parenthesis. *Letters* denote significant differences compared to control at levels: [a] $p<0.05$, [b] $p<0.01$, and [c] $p<0.001$.

μM Cr(VI)	*L. gibba*		*L. minor*	
	F_v/F_m	F_v/F_o	F_v/F_m	F_v/F_o
0	0.783 (0.002)	3.606 (0.039)	0.779 (0.005)	3.524 (0.103)
50	0.777 (0.003)[a]	3.472 (0.068)[a]	0.703 (0.011)[c]	2.367 (0.123)[c]
100	0.768 (0.004)[b]	3.311 (0.066)[c]	0.571 (0.038)[b]	1.342 (0.217)[c]
200	0.652 (0.039)[b]	1.904 (0.325)[b]	0.487 (0.045)[b]	0.869 (0.227)[c]

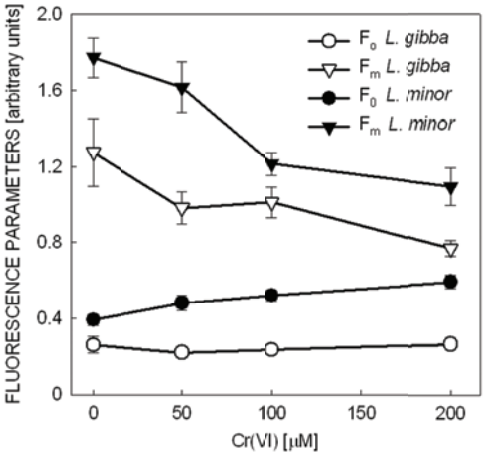

Fig. 2. Changes in F_o (*circles*) and F_m (*triangles*) of dark-adapted plants (20 min) of *L. gibba* (*open symbols*) and *L. minor* (*filled symbols*) plants measured after 48-h Cr(VI) treatments (0–200 μM). Symbols are means ± SD of 4 replicates.

Similarly to the so-called dark-adapted Chl fluorescence parameters, light-adapted Chl fluorescence parameters also indicated strong inhibition in functioning of PSII and reflected different sensitivities of the two duckweed species to chromate stress (Table 2).

Increasing Cr(VI) concentration in the growth medium induced a continuous reduction of relative fluorescence decrease (RFD) of both species (Table 2). After 48-h exposure, RFD decreased less significantly at every Cr(VI) concentration in *L. gibba* than in *L. minor*. 50 μM Cr(VI) resulted in only 4% reduction of RFD in *L. gibba*. Exposure of *L. gibba* plants to 100 and 200 μM Cr(VI) induced 12% and 15% decrease of RFD, respectively (Table 2). In *L. minor* a significant decrease (22%) of RFD was observed after 48-h exposure to 50 μM Cr(VI), and increase of Cr(VI) concentration to 100 and 200 μM accelerated the reduction of RFD (by 39% and 55% as compared to control, respectively).

In *L. minor* even 50 μM Cr(VI) induced significant decrease (15%) in q_P and exposure to 100 μM Cr(VI) doubled the decrease (31%). Further increase of Cr(VI) concentration to 200 μM resulted only in slight additional change (41% decrease in q_P compared to control, Table 2). In contrast, in *L. gibba* Cr(VI) induced significant decrease of q_P only at 100 and 200 μM concentrations within 48-h treatment (5% and 15% decrease, respectively, Table 2).

Table 2. Effect of 48-h Cr(VI) treatments (0–200 μM) on relative fluorescence decrease (RFD) and photochemical quenching (q_P) of *L. gibba* and *L. minor* plants illuminated by actinic light (200 μmol m^{-2} s^{-1}) for 5 min. Values are means of 4 replicates, standard deviations (SD) are in parenthesis. *Letters* denote significant differences compared to control at levels: [a] $p<0.05$, [b] $p<0.01$, and [c] $p<0.001$.

μM Cr(VI)	*L. gibba* RFD	q_P	*L. minor* RFD	q_P
0	2.24 (0.08)	0.789 (0.023)	2.22 (0.09)	0.746 (0.015)
50	2.15 (0.06)	0.775 (0.022)	1.75 (0.06)[c]	0.633 (0.017)[c]
100	1.98 (0.10)[b]	0.741 (0.019)[a]	1.35 (0.09)[c]	0.510 (0.018)[c]
200	1.91 (0.06)[b]	0.667 (0.008)[c]	0.99 (0.05)[c]	0.451 (0.029)[c]

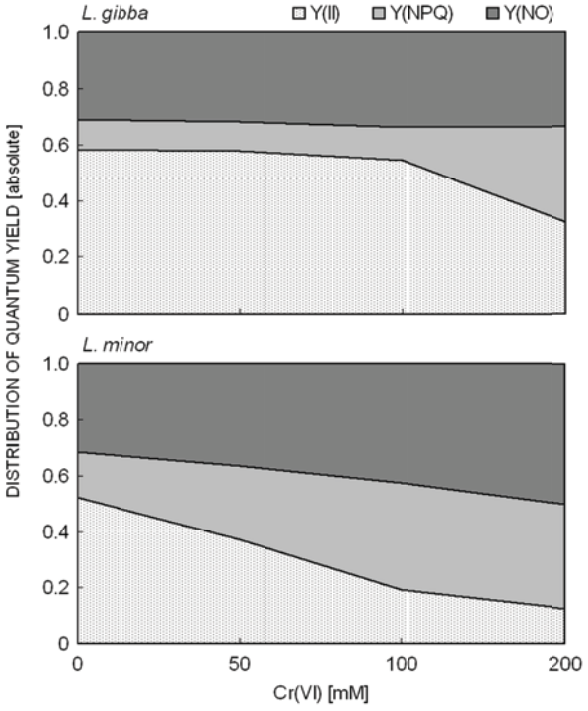

Fig. 3. Effect of 48-h Cr(VI) treatments (0–200 μM) on distribution of quantum yield among photochemical energy conversion, Y(II), regulated non-photochemical quenching, Y(NPQ), and nonregulated non-photochemical quenching, Y(NO), of *L. gibba* and *L. minor* test plants after illumination by actinic light (200 μmol m^{-2} s^{-1}) for 5 min ($n = 4$).

Effective quantum yield of PSII, Y(II), of light-acclimated plants measured at quasi steady-state also decreased in line with increasing concentrations of chromate in the growth medium (Fig. 3). Y(II) of *L. minor* reflected stronger chromate-induced damages to PSII (28, 67, and 76% inhibition at 50, 100, and 200 μM chromate, respectively) as compared to *L. gibba* (1, 7, and 41% at 50, 100, and 200 μM chromate, respectively).

Quantum yields of regulated, Y(NPQ), and non-regulated, Y(NO), nonphotochemical energy losses also showed different responses of the two duckweed species to Cr(VI) (Fig. 3). In *L. gibba* Y(NO) remained more or less stable (~10% NS increase at 200 μM) irrespectively of the applied chromate concentration. Y(NPQ) of *L. gibba* was also insensitive to Cr(VI) at 50 and 100 μM Cr(VI). However, 200 μM Cr(VI) increased Y(NPQ) of *L. gibba* three times higher (317%) than the control value (Fig. 3). Regarding these parameters, *L. minor* responded differently and exhibited significant increases in both Y(NPQ) and Y(NO). After 48-h treatment, Cr(VI) induced increases of Y(NO) of *L. minor* to 117, 137, and 162% of control values at 50, 100, and 200 μM, respectively. Y(NPQ) of *L. minor* also showed significant increases at 50 and 100 μM Cr(VI) exposures (134 and 167%, respectively). However, the exposure of *L. minor* plants to 200 μM chromate did not induce further increase of Y(NPQ) in this species, the value of this parameter was similar as in 100 μM Cr(VI) treatment (Fig. 3).

Table 3. Effects of 48-h Cr(VI) treatments (0–200 μM) on maximal photon-use efficiency (α), relative maximum of electron transport rate ($rETR_{max}$) and light-saturation coefficient (E_k) of rapid light curves (RLCs) in *L. gibba* and *L. minor* test cultures. Values are means of 4 replicates, standard deviations (SD) are in parenthesis. *Letters* denote significant differences compared to control at levels: [a] $p<0.05$, [b] $p<0.01$, and [c] $p<0.001$.

μM Cr(VI)	*L. gibba* α[μmol(e^-) μmol(photon)$^{-1}$]	$rETR_{max}$ [μmol(e^-) m^{-2} s^{-1}]	E_k [μmol(photon) $m^{-2}s^{-1}$]	*L. minor* α[μmol(e^-) μmol(photon)$^{-1}$]	$rETR_{max.}$ [μmol(e^-) m^{-2} s^{-1}]	E_k [μmol(photon) m^{-2} s^{-1}]
0	0.338 (0.026)	83.8 (4.0)	249 (8)	0.289 (0.015)	52.9 (2.7)	184 (18)
50	0.347 (0.007)	66.4 (1.9)[b]	191 (3)[b]	0.234 (0.035)	25.4 (3.0)[c]	109 (11)[b]
100	0.340 (0.002)	64.4 (2.8)[b]	189 (9)[b]	0.157 (0.036)[a]	13.7 (2.5)[c]	89 (14)[b]
200	0.246 (0.039)[a]	31.6 (6.0)[c]	129 (7)[c]	0.075 (0.017)[c]	5.4 (0.5)[c]	74 (10)[b]

Table 4. Concentration of chlorophylls (Chl) and carotenoids (Car), Chl *a*/Chl *b* and Car/Chl ratios in *L. gibba* and *L. minor* after 48-h exposure to Cr(VI) (0–200 μM). Values are means of 4 replicates, standard deviations (SD) are in parenthesis. *Letters* denote significant differences compared to control at levels: [a] $p<0,05$ [b], $p<0,01$, and [c] $p<0,001$.

μM Cr(VI)	Chl *a*+*b* [mg g^{-1}(FM)]	Chl *a*/*b* [g g^{-1}]	Car [mg g^{-1}(FM)]	Car/Chl [g g^{-1}]
L. gibba				
0	1.31 (0.04)	3.18 (0.04)	0.291 (0.012)	0.223 (0.006)
50	1.19 (0.03)[b]	3.08 (0.03)[a]	0.270 (0.006)[a]	0.226 (0.002)
100	1.08 (0.11)	3.02 (0.06)[a]	0.254 (0.028)	0.234 (0.003)[a]
200	1.05 (0.07)[b]	2.88 (0.02)[c]	0.243 (0.013)[b]	0.232 (0.003)[a]
L. minor				
0	1.124 (0.075)	3.44 (0.03)	0.255 (0.016)	0.227 (0.001)
50	0.950 (0.052)[a]	3.24 (0.07)[a]	0.228 (0.015)	0.240 (0.005)[a]
100	0.929 (0.036)[b]	3.16 (0.07)[b]	0.226 (0.006)[a]	0.243 (0.005)[b]
200	0.894 (0.050)[b]	3.21 (0.05)[b]	0.215 (0.002)[a]	0.241 (0.012)

Analyses of rapid light responses indicated chromate-induced alteration at electron transport level. Significant changes of α value were observed at 100 and 200 μM Cr(VI) in *L. minor* (46 and 74% decreases, respectively) and at 200 μM Cr(VI) in *L. gibba* (28%) as compared to control plants (Table 3). $rETR_{max}$ responded more sensitively in both species after 48 h exposure to 200 μM Cr(VI), showing 62 and 90% decrease in *L. gibba* and *L. minor*, respectively. Lower concentrations of Cr(VI) also induced significant decreases in $rETR_{max}$, 50 and 100 μM Cr(VI) resulted in much smaller decreases of $rETR_{max}$ value (21 and 23%, respectively) in *L. gibba* than in *L. minor* (52% at 50 μM and 74% at 100 μM). Cr(VI) treatments also declined the onset of light saturation (E_k) strongly (Table 3). At 200 μM Cr(VI) E_k decreased by 48% in *L. gibba* and by 60% in *L. minor* compared to control plants. At lower chromate concentrations less drastic inhibition of E_k was observed (in *L. gibba* 23% at 50 μM and 24% at 100 μM Cr(VI), in *L. minor* 41% at 50 μM and 52% at 100 μM Cr(VI).

48-h exposures to Cr(VI) did not affect photosynthetic pigments as strongly as Chl fluorescence parameters (Table 4). Concentrations of Chls and carotenoids decreased in the presence of Cr(VI) but differences in effects of various Cr(VI) concentrations could not be confirmed statistically or showed weak significance in both species (Table 4). In general, among chlorophylls Chl *a* was the more sensitive compound to Cr(VI) treatments in both species. It was reflected in decreasing Chl *a*/*b* ratios (max. 10%) as compared to control plants but no statistical differences were observed among the Cr(VI) concentrations. Compared to chlorophylls, carotenoids exhibited higher stability to Cr(VI) treatments which reflected a growing need for defence processes under stress. Cr(VI) resulted in slightly elevated (1–5%) Car/Chl ratios without statistical significance or with a weak significance in both species (Table 4). Regarding photosynthetic pigments, the two duckweed species responded very similarly to Cr(VI) treatments.

Discussion

Being highly toxic oxidizing agent, Cr(VI) has potential impact on photosynthetic processes of plants which may appear within shorter time interval than the effect on biomass growth of plants. In this study, by using Chl fluorescence parameters and rapid light curves, we found that Cr(VI) altered significantly photosynthesis of two duckweed species within 48 h.

Comparing inhibitions of F_v/F_m and F_v/F_o by Cr(VI), the latter fluorescence parameter proved to be more sensitive to Cr(VI) stress suggesting that F_m and F_o have not the same sensitivity to Cr(VI) (Vernay *et al*. 2007, Paiva *et al*. 2009). Separate analyses of changes in F_m and F_o showed that decrease in variable fluorescence under Cr(VI) treatment mainly resulted from the reduced maximal fluorescence in both species. However, in *L. minor* increase of F_o was also significant contributor to the decrease of F_v. This indicated damages of PSII reaction centres (Ali *et al*. 2006) and ultrastructure of thylakoid membrane affecting electron transport, too (Paiva *et al*. 2009). These results are in good accordance with the reduction in total Chl content and Chl *a*/*b* ratios observed in our study. Decrease of photosynthetic pigments due to chromate stress may appear as the consequence of both direct oxidative destruction of photosynthetic pigments and inhibition of their *de novo* synthesis (Panda and Choudhury 2005, Shanker *et al.* 2005). Our results suggest that in short term (1–2 days) the effects of Cr(VI) on the functioning of photosynthetic apparatus involve rather the oxidative damages to photosynthetic pigments than the inhibition of synthesis, although these changes are small and do not show statistically confirmed differences among Cr(VI)

concentrations. Chromate-induced change in Chl *a*/*b* ratio was also described as a result of damages in peripheral antennae complexes of photosystem (Panda and Choudhury 2005, Shanker *et al.* 2005, Vernay *et al*. 2007, Paiva *et al*. 2009). In the presence of Cr(VI) *L. minor* showed increases of F_o in concentration-dependent manner which reflects higher sensitivity of this species to Cr(VI). As the applied treatment period was short, Cr(VI) presumably could not fundamentally interfere with the Chl synthesis so the measured rise in F_o values might evolve from inhibited energy transfer from antenna to reaction centre. On the other hand, due to the Cr(VI)-induced damages of reaction centres the average antenna size per functioning reaction centre, however, increase overloading the electron transport capacity in chloroplasts (Perreault *et al*. 2009). Such an overcharge could result in growing demand for alternative energy conversion (Ali *et al*. 2006) explaining the measured strong elevation in regulated non-photochemical quenching in both species which took place with a simultaneous increase of nonregulated non-photochemical quenching in *L. minor*. Slightly elevated Car/Chl ratios in both duckweed species indicated higher stability of carotenoids under short-term Cr(VI)-stress. Enhanced production of carotenoids in plants exposed to chromate treatments has also been reported (Panda and Choudhury 2005, Perreault *et al*. 2009) which may contribute to the effective defence against over-excitation and oxidative degradation in PSII when Cr(VI) is present in the growth medium in longer term.

In both species, Y(II) in light-acclimated plants responded even more sensitively to Cr(VI) than F_v/F_m (Subrahmanyam 2008) indicating that beside pigment-protein complexes other components of PSII, such as oxygen evolving complex and D1 protein of reaction centres, might also be the targets of chromate-induced oxidative burst (Ali *et al*. 2006, Hörcsik *et al*. 2007). Cr(VI) induced larger inhibition of Y(II) at corresponding concentrations than that of RFD parameter (reflecting efficiency of the overall photosynthesis, Lichtenthaler *et al.* 2005) which suggested that inhibition might occur in the electron transport chain (Subrahmanyam 2008, Paiva *et al*. 2009).

Vulnerability of electron transport to Cr(VI) was confirmed by RLC-measurements. Even the lowest Cr(VI) concentration resulted in stronger inhibition of ETR (21% in *L. gibba* and 52% in *L. minor*) compared to F_v/F_m, F_v/F_o, and Y(II). Such disorders narrow the capacity of electron transport chain resulting in depressed $rETR_{max}$ values (Ali *et al*. 2008, Perreault *et al*. 2009) with increasing degrees if Cr(VI) concentration is elevated in the growth medium. However, at lower illumination levels this capacity seems to be sufficient to utilize absorbed energy resulting in relatively less altered α values of both duckweed species if Cr(VI) concentration is also low.

As a general tendency of changes observed in both species, chromate treatments lowered the relative number of open PSII reaction centres under actinic light, *i.e*. the photochemical quenching (q_P) of Chl fluorescence.

The most evident difference in response of the two duckweed species to Cr(VI) was found in the pattern of quantum yield of photochemical, regulated [Y(NPQ)] and nonregulated non-photochemical [Y(NO)] energy dissipation within the applied (0–200 μM) Cr(VI) range. Parallel with the decrease in efficiency of energy transfer from antennae to PSII reaction centres and electron transport chain a shift occurred from this pathway to the non-photochemical energy quenching mechanisms. It has been reported earlier that hexavalent chromium alters the distribution of excitation energy *via* regulated and nonregulated non-photochemical dissipation (Ali *et al*. 2008, Perreault *et al*. 2009). Proportions of both Y(NPQ) and Y(NO) responded differently to Cr(VI) in *L. gibba* and *L. minor*. Although in both species Y(NPQ) increased with the applied Cr(VI) concentrations but species differed in the concentration of Cr(VI) where this parameter reached its maximum, that was 100 and 200 μM in the case of *L. minor* and *L. gibba,* respectively. Total Car content and Car/Chl ratio did not show clear Cr(VI) dose-dependent changes in neither species which suggests that other alternative defence mechanism than heat dissipation is involved in the short-term responses to chromate. On the other hand, nonregulated energy dissipation increased only in *L. minor* within 48-h chromate treatments indicating more vulnerable photosynthesis and/or higher Cr(VI) uptake rate of this species. It is likely that chromate might provoke similar changes in the pathways of non-photochemical quenching of *L. gibba* also during longer treatment periods since RLC-measurements indicated the same decreasing trend of ETRs in both species.

In conclusion, both duckweed species responded sensitively to Cr(VI) under tested culture conditions but most of their measured physiological parameters exhibited different dose-dependent responses to Cr(VI). Maintenance of physiological stability under Cr(VI) treatments was more effective in *L. gibba* than in *L. minor* reflecting higher Cr(VI) tolerance of the former species in short term. This arises two important considerations: (*1*) firstly, use of the two species in ecotoxicological testing of environmental chemicals may result in different outcomes concerning their toxicity, (*2*) secondly, advantages from larger tolerance of *L. gibba* to Cr(VI), and presumably to other stresses too, versus *L. minor* may manifest themselves in competition between these coexisting species in their natural habitats when environmental constrains occur.

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