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# Ecophysiological responses of water hyacinth exposed to $Cr^{3+}$ and $Cr^{6+}$

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ABSTRACT

Due to its wide industrial use, chromium (Cr) is considered a serious environmental pollutant of aquatic bodies. In order to investigate the ecophysiological responses of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] to Cr treatment, plants were exposed to 1 and 10 mM $Cr_2O_3$ ($Cr^{3+}$) and $K_2Cr_2O_7$ ($Cr^{6+}$) concentrations for two or 4 days in a hydroponic system. Plants exposed to the higher concentration of $Cr^{6+}$ for 4 days did not survive, whereas a 2 days treatment with 1 mM $Cr^{3+}$ apparently stimulated growth. Analysis of Cr uptake indicated that most of the Cr accumulated in the roots, but some was also translocated and accumulated in the leaves. However, in plants exposed to $Cr^{6+}$ (1 mM), a higher translocation of Cr from roots to shoots was observed. It is possible that the conversion from $Cr^{6+}$ to $Cr^{3+}$, which immobilizes Cr in roots, was not total due to the presence of $Cr^{6+}$, causing deleterious effects on gas exchange, chlorophyll *a* fluorescence and photosynthetic pigment contents. Chlorophyll *a* was more sensitive to Cr than chlorophyll *b*. $Cr^{3+}$ was shown to be less toxic than $Cr^{6+}$ and, in some cases even increased photosynthesis and chlorophyll content. This result indicated that the $F_v/F_0$ ratio was more effective than the $F_v/F_m$ ratio in monitoring the development of stress by $Cr^{6+}$. There was a linear relationship between *q*P and $F_v/F_m$. No statistical differences were observed in NPQ and chlorophyll *a*/*b* ratio, but there was a tendency to decrease these values with Cr exposure. This suggests that there were alterations in thylakoid stacking, which might explain the data obtained for gas exchanges and other chlorophyll *a* fluorescence parameters.



## 1. Introduction

In most natural environments, the heavy metal content of the soil and water is low and does not cause significant phytotoxicity. However, the increasing contamination and subsequent accumulation of heavy metals in the environmental, due to human activities such as mining, extensive use of fertilizers and sewage waste production may have serious consequences for normal plant growth (Vernay et al., 2007).

Heavy metal phytotoxicity is controlled by a number of factors, including the element's uptake site, bioavalability, competition for binding sites and ionic speciation (Ralph and Burchett, 1998; Panda and Choudhury, 2005). This phytotoxicity can lead to the production of reactive oxygen species (ROS), which can be dismutated by antioxidant enzymes (Vitória et al., 2001; Gratão et al., 2005; Tamás et al., 2008). Krupa and Basynski (1995) discussed some hypotheses concerning the possible mechanism of heavy metal toxicity on photosynthesis and presented a list of key enzymes of photosynthetic carbon reduction, which were inhibited in heavy metal-treated plants.

Anthropogenic activities have led to Cr contamination in aquatic and terrestrial ecosystems. Cr is the seventh most abundant metal in the earth's crust (Panda and Choudhury, 2005). Cr occurs in several oxidation states ranging from $Cr^{2+}$ to $Cr^{6+}$, with the trivalent ($Cr^{3+}$) and hexavalent ($Cr^{6+}$) states being the most stable and common. $Cr^{6+}$ usually occurs associated with oxygen as chromate ($CrO^{2-}_4$) or dichromate ($Cr_2O^{2-}_7$) and is considered to be more mobile and toxic than $Cr^{3+}$, which on the other hand, is less soluble in water and is required in trace amounts as an inorganic nutrient for animals (Lien et al., 2001). $Cr^{6+}$ and $Cr^{3+}$ are taken up by plants and many organisms (anaerobic bacteria and plants). Plants are capable of reducing $Cr^{6+}$ to $Cr^{3+}$ and there is also evidence that no conversion occurs for Cr species in the nutrient solution before uptake by plant roots (Shanker et al., 2005). Although $Cr^{3+}$ is less toxic than $Cr^{6+}$, it too induces oxidative stress (Panda and Choudhury, 2005).

Cr phytotoxicity can result in inhibition of nutrient balance, changes in antioxidant enzymes activities, degradation of pigment,

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alteration of chloroplast and membrane ultrastructure, decrease in $CO_2$ assimilation and modification of chlorophyll *a* fluorescence parameters (Vajpayee et al., 2000; Panda and Choudhury, 2005; Arduini et al., 2006; Vernay et al., 2007). Cr stress can also affect photosynthesis in terms of $CO_2$ fixation, electron transport, photophosphorylation and enzyme activities (Shanker et al., 2005). Maintaining heavy metals (e.g. Cr) in the root system appears to maintain the level of biomass production and reduce harmful symptoms in photosynthesis (Soltan and Rashed, 2003). Decreases in total chlorophyll, chlorophyll *a* and *b*, and carotenoids have been well documented under Cr stress in plants (Panda and Khan, 2003; Vernay et al., 2007). Carotenoids and chlorophyll absorb radiant energy and part of this is emitted as chlorophyll fluorescence. The proportion of radiant energy emitted in the form of fluorescence is low under plant optimum conditions. However, in many situations, fluorescence increases under stress conditions, and there are also changes in the characteristics related to fluorescence.

In terrestrial plants, the negative action of Cr on photosynthesis is well documented (Shanker et al., 2005; Vernay et al., 2007), whilst for aquatic plants their potential in removing metals ions from aquatic environments has received more attention (Lu et al., 2004; Mangabeira et al., 2004). A group of plant species (termed hyperaccumulators) have the ability to accumulate non-essential metals, such as Cr, and apparently do not show damage. Water hyacinth [*Eichhornia crassipes* (Mart.) Solms] is a floating macrophyte hyperaccumulator species native of South America that, due to its fast growth and large biomass production, is particularly useful in the phytoremediation process and monitoring of heavy metals in aquatic environments (Lu et al., 2004).

Disorganization of the chloroplast ultrastructure and inhibition of electron transport processes due to Cr and a diversion of electrons from the electron-donating side of PSI to $Cr^{6+}$ is a possible explanation for the Cr-induced decrease in photosynthetic rate (Shanker et al., 2005). There is little reported evidence of a correlation between PSII activity, $CO_2$ assimilation and heavy metal accumulation under conditions of excess Cr in aquatic plants. Moreover, most reports on Cr in plants have concentrated on its effects on growth, uptake, toxicology and translocation. We investigated the effect of added $Cr^{6+}$ and $Cr^{3+}$ on the responses of the photosynthetic apparatus in water hyacinth, both in $C0_2$ assimilation, as measured by leaf as exchanges, and for the function of photosynthetic apparatus, as assessed by chlorophyll *a* fluorescence. Additionally, photosynthetic pigment contents and Cr translocation from roots to shoots were simultaneously analyzed.

## 2. Material and methods

### 2.1. Plant material and chromium treatments

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms] samples were collected from the Imbé River located at 21°01′ 08″ S, 74°19′52″W in the southeast region of Brazil, in May, June and July 2007 and transferred to 10 L capacity polyethylene pots containing 7 L of nutrient solutions (Hoagland and Arnon, 1950) in a glasshouse. The pots were placed in a randomized position and two different forms of Cr were used in the experiments: $Cr_2O_3$ ($Cr^{3+}$) and $K_2Cr_2O_7$ ($Cr^{6+}$) at 1 and 10 mM concentrations for 0, 2 and 4 days. The plants were grown under greenhouse conditions with a photoperiod of 11 h (light period) and 13 h of darkness, mean temperatures of 25 °C during the day (light) time and 18 °C during the night (dark) time. The relative humidity was kept at 70 ± 5% and a photosynthetic photon flux density (PPFD) of 1500 μmol $m^{-2}$ $s^{-1}$ was used.

### 2.2. Chromium analysis

After 4 days of Cr exposure, plants samples were washed gently and exhaustively with distilled–deionized water to remove adsorbed culture medium. Plants were divided into roots and leaves, frozen in liquid nitrogen and freeze-dried for 48 h (LABCONCO 260337 Freeze Dry System). All plant parts were digested as described by Klumpp et al. (2002) and resuspended in 0.5 M $HNO_3$. An atomic absorption spectrometer (AA-120 Varian Techtron) was used to determine the Cr content. The values were expressed in μg $g^{-1}$ dry matter.

### 2.3. Chlorophyll a fluorescence measurement

The measurements of the chlorophyll *a* fluorescence were carried out between 9:30 a.m. and 11:00 a.m. using a pulse amplitude modulation fluorimeter (FMS2, Hansatech Instruments Ltd., Norfolk, UK). Ten intact and healthy leaves from each treatment were kept in the dark for 30 min and then exposed to the weak, modulated beam light (approximately 6 μmol $m^{-2}$ $s^{-1}$ at 660 nm), followed by exposure for 0.8 s of high intensity (10000 μmol $m^{-2}$ $s^{-1}$) actinic white light, as adapted by Genty et al. (1989). The minimal fluorescence ($F_0$), the maximum fluorescence ($F_m$) and extinction coefficients: *q*P (photochemical quenching) and NPQ (non-photochemical quenching) were measured. The variable fluorescence ($F_v = F_m - F_0$), maximum quantum yield of PSII ($F_v/F_m$) and variable chlorophyll fluorescence ratio ($F_v/F_0$) were calculated according to Van Kooten and Snel (1990). The values are presented as the mean of ten measurements, representing ten replicates.

### 2.4. Gas exchange measurements

Net photosynthetic rate (*P*n), stomatal conductance ($g_s$) and substomatal $CO_2$ concentration (*C*i) were determined after 0, 2 and 4 days after Cr exposure. The treatments were measured with a wearable infrared gas analyzer (Ciras 2, PP-System, UK) with clamp-on leaf cuvette that exposed 2.4 $cm^2$ of leaf area. Light (PPFD), temperature and humidity were 1800 μmol $m^{-2}$ $s^{-1}$, 25 ± 2°C and 75%, respectively. $CO_2$ was maintained at a constant level of 380 μmol $mol^{-1}$. Light was imposed using the Ciras 2 LED light source (PP-System). Each measurement was carried out on five newly maturated leaves per plant and was repeated on five plants for each treatment ($n = 5$).

### 2.5. Photosynthetic pigments

Three discs were taken from each treatment and used to quantify the photosynthetic pigments. The three discs were sliced and placed in plastic tubes in the dark with a lid containing 5 ml dimethylsulfoxide reagent (DMSO) as organic solvent. After 5 days, the extract was analyzed in a spectrophotometer at wavelengths of 480 nm, 649 nm and 665 nm. The photosynthetic pigments were quantified for the samples from 4 days using the equations by Wellburn (1994) for carotenoids, chlorophyll *a* and chlorophyll *b*. The total chlorophyll, chlorophyll *a*/*b* and total chlorophyll/carotenoid ratios were calculated. All the laboratory procedures were carried out in a low light environment. The values were expressed in μmol $cm^{-2}$.

### 2.6. Statistical analysis

The results of the Cr accumulation, chlorophyll *a* fluorescence variables, gas exchange and photosynthetic pigments were ana-