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Ecophysiological responses of *Eichhornia crassipes* (Mart.) Solms to As^{5+} under different stress conditions

A.C. MENEGUELLI-SOUZA+, A.P. VITÓRIA, T.O. VIEIRA, M.S.O. DEGLI-ESPOSTI, and C.M.M. SOUZA

Laboratório de Ciências Ambientais, Centro de Biociência e Biotecnologia, Universidade Estadual do Norte Fluminense, 28013 – 602, Campos dos Goytacazes, RJ, Brazil

Abstract

Arsenic is a critical contaminant that is released into the environment through geochemical processes and anthropic actions. Two independent hydroponic experiments were performed to evaluate the ecophysiological responses of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] to As under various stress conditions. In experiment 1, water hyacinth was exposed to As^{5+} at concentrations of 0, 0.2, 2.0, and 20 mg L^{-1} for 0, 2, and 4 d; in experiment 2, water hyacinth was exposed at concentrations of 0, 0.025, 0.05, and 0.1 mg L^{-1} for 0, 10, and 20 d. In both experiments, As accumulation in plant tissue was proportional to its increase in the nutrient solution; As concentrations were higher in roots than in shoots. Detrimental effects of As on gas exchange were observed and were more pronounced in experiment 1. In experiment 1, at the beginning on the second day of exposure, significant decreases of maximum photochemical efficiency of PSII (F_v/F_m), variable chlorophyll fluorescence (F_v/F_0), and photosynthetic pigment contents were observed in plants exposed to 2.0 and 20 mg(As^{5+}) L^{-1}. It indicated that damage to the photosynthetic apparatus had occurred. No changes in F_v/F_m, F_v/F_0, and contents of photosynthetic pigments were observed in the plants grown in the presence of 0.2 mg(As^{5+}) L^{-1} (in experiment 1) or after any of the treatments in experiment 2, indicating plant tolerance. Elevated nonphotochemical quenching was observed in experiment 2 after 20 d of exposure to As; it was as a part of protection mechanisms of the photosynthetic apparatus in these plants. The results obtained here indicate that the use of water hyacinth for As^{5+} removal from highly impacted environments is limited but that it is effective in remediating sites with a low contamination.

Additional key words: carotenoid; chlorosis; photosynthesis; remediation; senescence; trace element.

Introduction

Arsenic is widespread in environment and is one of the most toxic contaminants to human health (Jain and Ali 2000). This metalloid has several oxidation states, but in natural waters, it is mostly present in inorganic form as As^{3+} and As^{5+}. Environmental problems related to As result from its mobilization under natural conditions (Schmöger *et al.* 2000) and from several anthropic activities, such as insecticide application and mining (Azcue and Nriagu 1995). When As comes from anthropic activities, its presence in the medium increases (Smedley and Kinniburgh 2001), generally resulting in toxic As concentrations in aquatic and terrestrial ecosystems.

Some plant species are able to adapt to sites that are enriched in trace elements. Tolerance to trace elements can be constitutive or induced by exposure (Verkleij and Schat 1992). In general, plants are tolerant to As concentrations in the medium up to 50 mg L^{-1} (Jiang and Sing 1994). However, plant species differ in their sensitivity to As. Arsenic phytotoxicity depends on several factors, such as its concentration in the medium, chemical species, absorption pathway, and bioavailability (Martínez-Sánchez *et al.* 2011). As can exhibit toxic effects on

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+Corresponding author; e-mail: annalizameneguelli@hotmail.com
Abbreviations: Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DMSO – dimethylsulfoxide; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_0 – variable chlorophyll fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_P – photochemical quenching.
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photosynthesis at the level of electron transport, CO_2 fixation, enzyme activities, photosynthetic pigments, and PSII functional activity (Päivöke and Simola 2001, Singh *et al*. 2009, Garg and Singla 2011). However, at low concentrations, tolerance mechanisms may be triggered, such as an increase in antioxidant enzyme activity, thermal dissipation (carotenoids and NPQ) and photochemical (q_P) of excess energy, thereby avoiding the formation of singlet oxygen in the PSII reaction center (Burns *et al*. 2003, Vitória *et al*. 2010, 2011; Pereira *et al.* 2011).

However, some plant species, called hyperaccumulators, tolerate high concentrations of trace elements, such as As, in their tissues (Meharg and Hartley-Whitaker 2002). Ladder brake (*Pteris vittata)* was the first plant species reported to be the As hyperaccumulator, accumulating between 3,280 and 4,980 mg kg^{-1} in its biomass (Ma *et al.* 2001). Water hyacinth (*Eichhornia crassipes*) is a free-floating macrophyte and is useful for the phytoremediation of contaminated aquatic environments due to its high biomass production and rapid propagation (Giri and Patel 2012).

As contents in the medium vary with the source of contamination and the environmental matrix to which the element is associated; it is higher in sediments, where it can reach between 161 and 4,709 mg(As) kg^{-1} (Borba *et al.* 2003, Rezende *et al.* 2011). Lower As concentrations between 2×10^{-3} and 3 mg L^{-1} are found in the water column (Borba *et al.* 2004, Gonçalves *et al.* 2007). Permissible values in water, according to its use, were established to be between 1.4×10^{-4} and 0.5 mg(As) L^{-1} by the National Environmental Council (CONAMA 2005).

The goal of the present study was to determine and compare photosynthetic adaptation strategies of water hyacinth to As under a variety of contamination conditions and to evaluate the energy cost of tolerance mechanisms to the plant. A wide range of stress conditions was tested. With longer exposure times at concentrations close to the permissible levels established by the legislation, it is expected that the plant should be able to adapt its energy content to develop As tolerance mechanisms. In contrast, it is expected that short periods of exposure associated with high As concentrations do not allow the plants to maintain spending its resources on tolerance processes.

Materials and methods

Plant material and As treatment: Water hyacinths [*Eichhornia crassipes* (Mart.) Solms] of young and healthy appearance were collected at the Lagoa do Campelo, located in Campos dos Goytacazes, the state of Rio de Janeiro, Brazil (21°39'01"S, 41°11'W), during April 2011 (autumn) and January 2013 (summer). The plants were grown under greenhouse conditions in 10 L polypropylene pots containing 8 L of a nutrient solution (Hoagland and Arnon 1950, Smart and Barko 1985). The pots were distributed randomly. As was added in the form of Na_2HAsO_4 (As^{5+}). In experiment 1, the plants were subjected to 0, 0.20, 2.0, and 20 mg(As^{5+}) L^{-1} for 0, 2, and 4 d. Experiment 2 was designed according to results obtained in the experiment 1; the plants were subjected to 0, 0.025, 0.050, and 0.10 mg(As^{5+}) L^{-1} for 0, 10, and 20 d. All treatments were performed in triplicates (n = 3). Hydrogen potential, electrical conductivity, and dissolved O_2 were monitored throughout the experiments.

As determination: In order to determine As in plant tissues, the plants were harvested at the end of the experiments, separated into roots and shoots, and dried at 60°C for one week. When the plant material became dry, it was macerated in liquid nitrogen. Acid digestion was performed using hydrogen peroxide (H_2O_2) and a 1:1 mix of sulfuric and nitric acids (H_2SO_4:HNO_3). The digestion was performed under pressure in a *Mars Xpress microwave* (*CEM*, USA). As was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a *Liberty Series II* spectrometer (*Varian*, Ausralia). Values were expressed in mg(As) kg^{-1} (dry mass, DM).

Gas exchange: Net photosynthetic rate (P_N), stomatal conductance (g_s), and substomatal CO_2 concentration (C_i) were measured between 08:00 and 11:00 h using an infrared gas analyzer (*Ciras-2*, *PP Systems*, UK). Plants were measured under PPFD of 2,000 µmol m^{-2} s^{-1}, CO_2 concentration of 380 µmol mol^{-1}, 25 ± 3°C, and 80% humidity. Measurements were performed in two intact and healthy leaves from each plant for all treatments (n = 3).

Chlorophyll (Chl) *a* fluorescence measurements were performed between 12:00 and 13:00 h using a modulated light portable fluorometer (*FMS2*, *Hansatech*, UK). Six leaves from each treatment (n = 3) were kept in the dark for 30 min and then exposed to a modulated beam of light [approximately 6 µmol(photon) m^{-2} s^{-1} at 660 nm], followed by a saturating pulse of white actinic light [10,000 µmol(photon) m^{-2} s^{-1}], which was applied for 0.8 s (adapted from Genty *et al.* 1989). Minimal fluorescence (F_0), maximum fluorescence (F_m), photochemical quenching (q_P), and nonphotochemical quenching (NPQ) were measured. Variable fluorescence ($F_v = F_m - F_0$), F_v/F_m, and F_v/F_0 were calculated according to van Kooten and Snel (1990). The measurements were performed on the same leaves used for the gas-exchange measurements.

Photosynthetic pigments: Three leaf discs (n = 3) were collected from each treatment to quantify Chl *a* and *b* and carotenoids (Car). Discs were cut, placed in capped plastic tubes containing 5 mL of dimethyl sulfoxide (DMSO) as an organic solvent, and stored in the dark for five days. Samples were measured using a spectrophotometer (*Model*

TCC-240A, *Shimadzu*, Japan) at 665, 649, and 480 nm. Chl *a* and *b* and Car were calculated according to Wellburn (1994). Total Chl concentration and Chl *a*/*b* and total Chl/Car ratios were calculated. All laboratory work was performed under low light. All values are expressed in µmol cm^{-2}.

Statistical analysis: As quantification, gas exchange, Chl *a* fluorescence, and photosynthetic pigment data were subjected to a two-way analysis of variance (*ANOVA*), and the means were compared using *Tukey*'s test ($p<0.05$).

Results

Visual evaluation: Signs of chlorosis and necrosis were observed in plants 4 d following the addition of 2.0 and 20 mg(As^{5+}) L^{-1} (experiment 1) (Fig. 1*C*,*D*). In experiment 2, signs of chlorosis and necrosis were observed 20 d after addition of 0.025 mg(As^{5+}) L^{-1} (Fig. 1*F*).

As concentration: Higher concentrations were observed in roots compared to shoots in both experiments (Table 1). As root to shoot translocation was higher in the plants subjected to 2.0 mg(As^{5+}) L^{-1} in experiment 1 (day 4) and for plants exposed to 0.10 mg(As^{5+}) L^{-1} in experiment 2 (day 20).

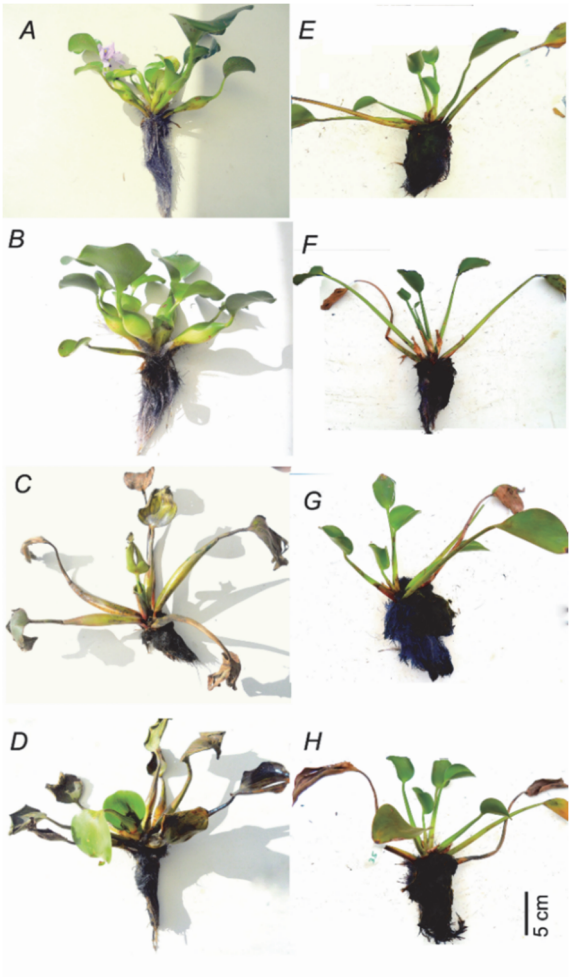

Fig. 1. Visual aspects of water hyacinth exposed to As^{5+}. *Left column* – experiment 1 (after 4 d of As exposure). *Right column* – experiment 2 (after 20 d of As exposure). *A* – control plant; *B*,*C*,*D* – plants exposed to 0.20, 2.0, and 20 mg(As^{5+}) L^{-1}, respectively. *E* – control plant; *F*,*G*,*H* – plants exposed to 0.025, 0.050, and 0.10 mg(As^{5+}) L^{-1}, respectively.

Table 1. Arsenic concentration [mg kg^{-1}(DM)] in water hyacinth exposed to 0, 0.20, 2.0, and 20 mg(As^{5+}) L^{-1} for 4 d (experiment 1) and 0, 0.025, 0.050, and 0.10 mg(As^{5+}) L^{-1} for 20 d (experiment 2). Each value represents the mean ± SD (n = 3). *Uppercase letters*: comparing the same treatment in different parts of the plant. *Lowercase letters*: comparison of different treatments in the same part of the plant. Values followed by *different letters* differ significantly ($p<0.05$).

Treatment [mg(As^{5+}) L^{-1}]	As [mg kg^{-1}(DM)] Shoots	Roots
Control	0 ± 0.00^{Ac}	2.66 ± 3.31^{Ac}
0.20	51.14 ± 29.62^{Bb}	524.83 ± 176.24^{Ab}
2.0	$1{,}626.80 \pm 540.00^{Aa}$	968.31 ± 178.09^{Ab}
20	$1{,}841.66 \pm 501.67^{Ba}$	$2{,}915.28 \pm 55.39^{Aa}$
Control	0 ± 0.00^{Ab}	1.39 ± 0.33^{Ac}
0.025	1.09 ± 0.36^{Bb}	28.34 ± 3.01^{Ab}
0.050	3.43 ± 1.08^{Bb}	19.92 ± 2.30^{Ab}
0.10	11.70 ± 0.66^{Ba}	34.55 ± 2.49^{Aa}

Gas-exchange parameters: In experiment 1, the highest P_N decrease was observed after 2 d of exposure to 2.0 and 20 mg(As^{5+}) L^{-1}. This decrease was still observed on day 4 in these treatments (Fig. 2*A*). In addition, increased C_i and decreased g_s were observed for these treatments (Fig. 2*B*,*C*). The lowest P_N for experiment 2 was observed after 20 d of plant exposure to 0.05 and 0.10 mg(As^{5+}) L^{-1} (Fig. 2*D*), followed by an increase in C_i (Fig. 2*E*). However, the decrease in P_N was less pronounced for experiment 2 than that for experiment 1. A significant decrease in g_s was observed in experiment 2 after 20 d of As exposure in all treatments (Fig. 2*F*).

Analysis of Chl *a* fluorescence parameters: In experiment 1, significant decreases in F_v/F_m and F_v/F_0 were observed in the presence of 2.0 and 20 mg(As^{5+}) L^{-1} starting from 2 d after exposure (Table 2). On day 4, F_v/F_m varied between 0.86 (control) and 0.34 (20 mg L^{-1}), and F_v/F_0 varied between 5.79 (control) and 0.51 (20 mg L^{-1}). Significant q_P decreases were observed in the presence of 2.0 mg L^{-1} (0.84) and 20 mg L^{-1} (0.86) starting after 2 d of exposure. In experiment 2, F_v/F_m, F_v/F_0, and q_P were stable

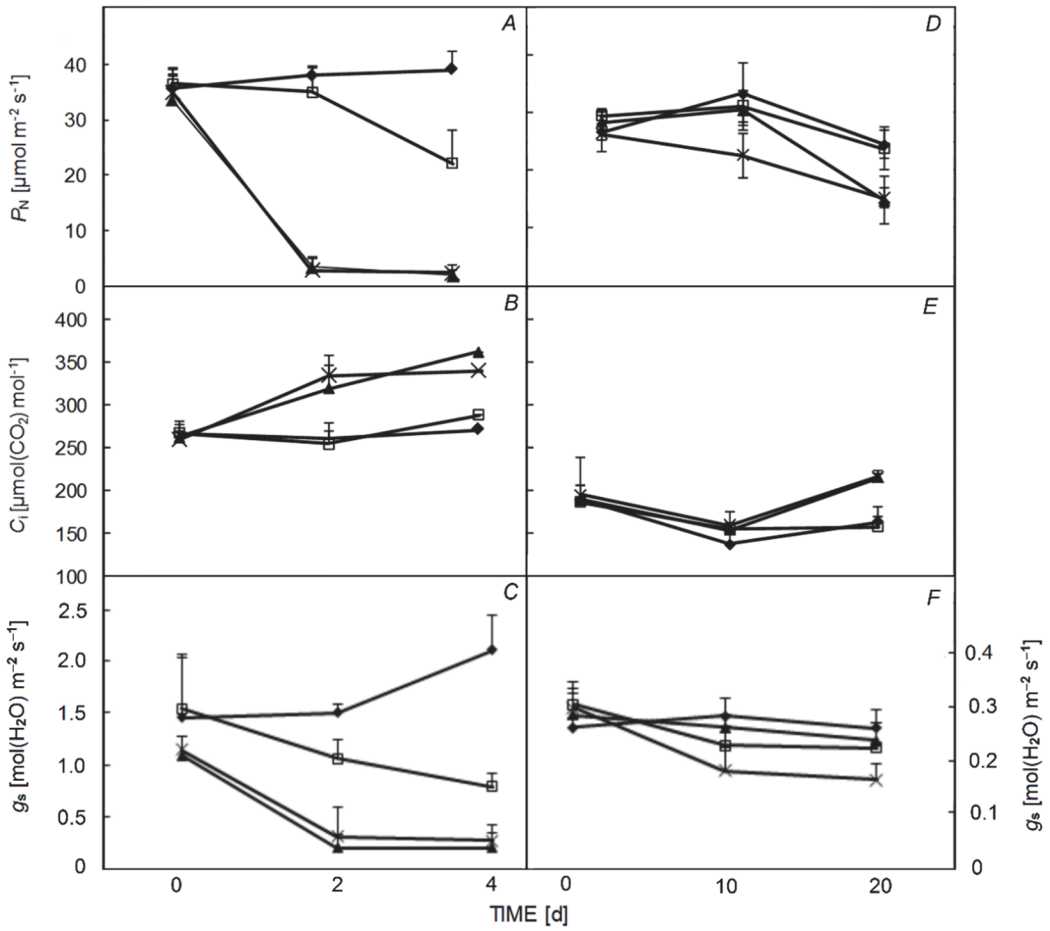

Fig. 2. Effects of As^{5+} exposure on net photosynthetic rate (*A*,*D*), substomatal CO_2 concentration (*B*,*E*), and stomatal conductance (*C*,*F*) in water hyacinth leaves. *Left column* – experiment 1. *Right column* – experiment 2. Plants were grown with 0 (♦), 0.20 (□), 2.0 (▲), and 20 mg(As^{5+}) L^{-1} (×) for 0, 2, and 4 d and with 0 (♦) 0.025 (▲), 0.050 (□), and 0.10 mg(As^{5+}) L^{-1} (×) for 0, 10, and 20 d. P_N – net photosynthetic rate, C_i – intercellular CO_2 concentration, g_s – stomatal conductance. Data points represent the averages ± SD (n = 3).

in all treatments and only varied significantly with time. In experiment 1, NPQ increased significantly in the presence of 2.0 and 20 mg(As^{5+}) L^{-1} on day 2 and decreased significantly on day 4. In experiment 2, NPQ increased in the presence of 0.050 and 0.10 mg(As^{5+}) L^{-1} after 20 d following the addition. For both experiments, F_v/F_0 was more sensitive than F_v/F_m in detecting variations due to the presence of As^{5+}.

Photosynthetic pigments: In experiment 1, Chl *a* and *b* concentrations significantly decreased in the presence of 2.0 and 20 mg(As^{5+}) L^{-1} (Table 3). The Chl *a*/*b* and total Chl/Car ratios decreased in the presence of 20 mg L^{-1}. No changes in pigment concentrations were observed during experiment 2.

Discussion

The uptake and accumulation of As by several plant species are well documented (Ma *et al*. 2001, Robinson *et al.* 2003, Xue and Yan 2011, Farnese *et al.* 2014). In the present study, the accumulation of As in plant tissue and its translocation into the shoots were mostly determined by the As concentration in the growth medium, not by the duration of exposure. The higher retention of As in the roots, which was observed in the present study, might be due to (*1*) the ability of As to be bond to root cell wall components (Salt *et al.* 1998), (*2*) a decrease in the conduction of As *via* water flow through the xylem (Stoeva *et al.* 2005), and (*3*) the complexation of As with thiol groups (As^{5+} is reduced to As^{3+} when it enters cells, Mathews *et al.* 2010). Arsenite has high affinity for thiol groups, which are responsible for its sequestration into vacuoles in roots (Zhao *et al.* 2009). The importance of roots as a preferential site of trace element accumulation has direct effects on photosynthesis, which is crucial for

Table 2. Chlorophyll *a* fluorescence parameters of the leaves in water hyacinth (dark-adapted) exposed to 0, 0.20, 2.0, and 20 mg(As^{5+}) L^{-1} for 0, 2, and 4 d and 0, 0.025, 0.050, and 0.10 mg(As^{5+}) L^{-1} for 0, 10, and 20 d. Each value represents the mean ± SD (n = 3). F_v/F_m – maximum quantum efficiency; F_v/F_0 – variable chlorophyll fluorescence; q_P – photochemical quenching; NPQ – nonphotochemical quenching. *Uppercase letters* indicate significant differences in the same treatment at different exposure times while *lowercase letters* differ treatments at the same time of exposure (p<0.05).

Time [d]	Treatment [mg(As^{5+}) L^{-1}]	F_v/F_m	F_v/F_0	q_P	NPQ
0	Control	0.84 ± 0.01^{Aa}	5.43 ± 0.48^{Aa}	0.98 ± 0.01^{Aa}	0.08 ± 0.00^{Aa}
	0.20	0.86 ± 0.01^{Aa}	5.96 ± 0.41^{Aa}	0.94 ± 0.03^{Aa}	0.09 ± 0.00^{Aa}
	2.0	0.86 ± 0.01^{Aa}	6.21 ± 0.41^{Aa}	0.95 ± 0.04^{Aa}	0.09 ± 0.00^{Ba}
	20	0.85 ± 0.01^{Aa}	5.79 ± 0.41^{Aa}	0.94 ± 0.05^{Aa}	0.11 ± 0.00^{Aa}
2	Control	0.87 ± 0.01^{Aa}	6.55 ± 0.38^{Aa}	0.97 ± 0.01^{Aa}	0.06 ± 0.00^{Aa}
	0.20	0.86 ± 0.01^{Aa}	6.23 ± 0.35^{Aa}	0.97 ± 0.01^{Aa}	0.07 ± 0.00^{Aa}
	2.0	0.72 ± 0.09^{Bb}	2.94 ± 1.59^{Bb}	0.84 ± 0.05^{Bb}	0.29 ± 0.00^{Ab}
	20	0.68 ± 0.13^{Bb}	2.56 ± 1.24^{Bb}	0.86 ± 0.01^{Bb}	0.36 ± 0.00^{Ab}
4	Control	0.85 ± 0.01^{Aa}	5.79 ± 0.22^{Aa}	0.98 ± 0.02^{Aa}	0.13 ± 0.01^{Aa}
	0.20	0.84 ± 0.01^{Aa}	5.12 ± 0.53^{Aa}	0.98 ± 0.01^{Aa}	0.06 ± 0.00^{Aa}
	2.0	0.50 ± 0.05^{Cb}	0.99 ± 0.18^{Bb}	0.76 ± 0.08^{Bb}	0.01 ± 0.00^{Ba}
	20	0.34 ± 0.01^{Cb}	0.51 ± 0.03^{Bb}	0.86 ± 0.01^{Bb}	0.01 ± 0.00^{Aa}
0	Control	0.89 ± 0.01^{Aa}	8.28 ± 0.69^{Aa}	0.97 ± 0.02^{Aa}	0.09 ± 0.00^{Aa}
	0.025	0.89 ± 0.01^{Aa}	7.89 ± 0.84^{Aa}	0.95 ± 0.03^{Aa}	0.08 ± 0.00^{Aa}
	0.050	0.89 ± 0.01^{Aa}	8.02 ± 1.11^{Aa}	0.95 ± 0.02^{Aa}	0.06 ± 0.00^{Ba}
	0.10	0.89 ± 0.01^{Aa}	8.24 ± 0.53^{Aa}	0.94 ± 0.01^{Aa}	0.07 ± 0.00^{Ba}
10	Control	0.84 ± 0.01^{Ba}	5.56 ± 0.05^{Ba}	0.92 ± 0.03^{Ba}	0.06 ± 0.00^{Aa}
	0.025	0.85 ± 0.01^{Ba}	5.68 ± 0.30^{Ba}	0.92 ± 0.03^{Aa}	0.10 ± 0.00^{Aa}
	0.050	0.86 ± 0.00^{Aa}	6.21 ± 0.23^{Ba}	0.90 ± 0.02^{Ba}	0.08 ± 0.00^{Aba}
	0.10	0.86 ± 0.01^{Aa}	6.06 ± 0.37^{Ba}	0.89 ± 0.03^{Ba}	0.08 ± 0.00^{Ba}
20	Control	0.81 ± 0.02^{Ba}	5.45 ± 0.31^{Ba}	0.92 ± 0.01^{Ba}	0.09 ± 0.00^{Aa}
	0.025	0.81 ± 0.03^{Ca}	5.14 ± 0.15^{Ba}	0.91 ± 0.02^{Aa}	0.16 ± 0.00^{Aa}
	0.050	0.82 ± 0.04^{Ba}	4.83 ± 0.70^{Ca}	0.92 ± 0.02^{ABa}	0.17 ± 0.00^{Aa}
	0.10	0.81 ± 0.02^{Ba}	4.25 ± 0.42^{Ca}	0.92 ± 0.01^{ABa}	0.19 ± 0.00^{Aa}

Table 3. Chlorophyll (Chl) *a*, *b*, and carotenoids (Car) content, Chl *a*/*b* and total Chl/Car ratios in water hyacinth exposed to 0, 0.20, 2.0, and 20 mg(As^{5+}) L^{-1} for 4 d and 0, 0.025, 0.050, and 0.10 mg(As^{5+}) L^{-1} for 20 d. Each value represents the mean ± SD (n = 3). Values followed by *different letters* differ significantly (p<0.05).

Treatment [mg(As^{5+}) L^{-1}]	Chl *a*	Chl *b*	Total Chl	Car	Chl *a*/*b*	Total Chl/Car
Control	43.46 ± 4.24^{A}	14.70 ± 1.56^{A}	58.16 ± 5.75^{A}	8.88 ± 0.90^{A}	2.96 ± 0.09^{A}	6.55 ± 0.09^{A}
0.2	43.67 ± 2.07^{A}	14.33 ± 0.88^{A}	58.00 ± 1.61^{A}	9.79 ± 0.89^{A}	3.05 ± 0.30^{A}	5.97 ± 0.30^{A}
2.0	36.62 ± 0.00^{B}	10.95 ± 0.00^{B}	47.67 ± 0.00^{B}	8.10 ± 0.00^{A}	3.35 ± 0.35^{A}	5.87 ± 0.00^{A}
20	21.22 ± 5.79^{B}	8.42 ± 1.30^{B}	29.64 ± 7.02^{C}	8.19 ± 0.31^{A}	2.50 ± 0.34^{B}	3.13 ± 0.19^{B}
Control	50.70 ± 0.01^{A}	16.30 ± 0.00^{A}	66.96 ± 0.01^{A}	11.17 ± 0.00^{A}	3.11 ± 0.00^{A}	6.04 ± 0.01^{A}
0.025	54.85 ± 0.00^{A}	17.20 ± 0.00^{A}	72.05 ± 0.01^{A}	11.61 ± 0.00^{A}	3.20 ± 0.00^{A}	6.23 ± 0.00^{A}
0.05	46.63 ± 0.00^{A}	15.10 ± 0.00^{A}	62.72 ± 0.00^{A}	10.77 ± 0.00^{A}	3.10 ± 0.00^{A}	5.74 ± 0.00^{A}
0.10	49.05 ± 0.01^{A}	16.30 ± 0.00^{A}	65.40 ± 0.01^{A}	11.65 ± 0.00^{A}	3.00 ± 0.00^{A}	5.62 ± 0.00^{A}

biomass gain (Soltan and Rashed 2003).Visual symptoms of trace element phytotoxicity, such as necrosis (Singh *et al.* 2006) and chlorosis (Soltan and Rashed 2003), are related to the presence of these elements in shoot tissues and to decreases in essential nutrient uptake (Singh *et al.* 2005, Souza *et al.* 2009).

Significant changes in the physiological responses of vascular plants are caused by trace element accumulation in their tissues (Jana 1988). The main mechanism responsible for the toxic effects of As is oxidative stress due to oxidation and reduction reactions that occur at the cellular level (Meharg and Hartley-Whitaker 2002). A marked drop in P_N of maize plants was observed in the presence of 5 mg(Na_3AsO_4) L^{-1} and was related to the decrease in g_s (Stoeva *et al.* 2003). This finding was also observed in both experiments reported here. The direct effect of As on

g_s might be due to the disruption of water uptake and transport that results from changes in roots during As uptake (Stoeva *et al.* 2003). Increased C_i and decreased g_s indicate that CO_2 fixation is compromised both at the biochemical and ecophysiological levels (Stoeva and Bineva 2003, Gusman *et al.* 2013).

The decreased P_N due to the presence of As^{5+} , which was observed in experiment 1, might be caused by changes in PSII function (as indicated by the values of F_v/F_m and F_v/F_0) and by the decrease in photosynthetic pigment concentrations. Values of F_v/F_m between 0.75 and 0.85 indicate that the photosynthetic apparatus is efficient (Bolhar-Nordenkampf *et al.* 1989), and the value of F_v/F_0 lies in the range between 4 and 6 in healthy plants (Roháček 2002). Lower values than those above were observed in experiment 1 and in oat plants (*Avena sativa*) treated with high As concentrations (Stoeva and Bineva 2003). Decreases in the above ratios indicate photoinhibitory damage, which is caused by excess incident photon fluxes that occur in plants subjected to a wide variety of environmental stresses (Björkman *et al.* 1987). F_v/F_0 has been suggested to be more sensitive in detecting small differences in PSII quantum yield compared to F_v/F_m (Oliveira *et al.* 2002, Paiva *et al.* 2009, Vitória *et al.* 2010), which was consistent with the results obtained in our experiment 1. Decreased values of F_v/F_m and F_v/F_0 under As^{5+} stress might be due to decreased Chl concentration, as observed for *Jussiaea repens* in the presence of Cd (Li *et al.* 2008). The reactive oxygen species increase in the presence of As; these species might interact with Chl and oxidize it (Schützendübel and Polle 2002). The decrease in the Chl *a*/*b* ratio observed in experiment 1 indicated that Chl *a* was more affected than Chl *b*. A similar finding was observed for *Elodea densa* in the presence of Cu (Maleva *et al.* 2012). Changes in this ratio indicate modifications in thylakoid membranes that are reflected in the photosynthetic capacity (Päivöke and Simola 2001).

The fact that Chl *a* fluorescence and the photosynthetic pigment concentrations did not change in experiment 2 and in plants exposed to 0.20 mg(As^{5+}) L^{-1} (experiment 1) might be due to the low As concentration reported in the leaves (Table 1). Similar results were found in *Isatis cappadocia* and *Lactuca sativa* exposed to low As concentrations (Gusman *et al* 2013, Karimi *et al.* 2013). Similarly, there was no significant change of the F_v/F_m ratio in the macrophytes, such as *Vallisneria gigantea*, *Azolla filiculoides*, and *Lemna minor*, which may be related to the low removal rate of As, ranging between 2 and 17% in this study (Iriel *et al.* 2015). The maintenance of the photosynthetic pigment concentrations explains the stability of F_v/F_m and F_v/F_0 because changes in these parameters indicate decreased Chl concentrations or photooxidation (He *et al*. 2001). The high Chl *a*/*b* ratio indicated that the thylakoid stacking level was maintained (Table 3) (Lichtenthaler *et al.* 2006). The fact that this ratio and the total Chl/Car ratio did not change indicated that the pigments were resistant to the toxic elements (Maleva *et al.* 2012).

The decrease of F_v/F_0 may be the result not only of changes in the Chl concentration, as discussed above, but also due to F_0 increase (not shown). F_0 is the fluorescence intensity at the stage when all PSII reaction centers are open. When the Chl concentration is stable or reduced, the F_0 increase may reflect the loss of efficiency of energy transfer from LHCs to PSII (Krause and Weis 1991).

To minimize the production of oxidant molecules in the photosynthetic apparatus, NPQ dissipates excess energy as heat, avoiding oxygen oxidation and protecting the plant against photooxidative damage (Li *et al.* 2000). In experiment 1, it was possible that the plants increased NPQ after 2 d of As exposure in an attempt to dissipate energy as heat and to protect the photosynthetic apparatus, as described largely under various types of stress (Lage-Pinto *et al.* 2008, Rabelo *et al*. 2013, Vieira *et al*. 2015). In some cases, the increase of heat dissipation (NPQ) can improve the plant performance and lead to a better adaptation to stress conditions. However, *E. crassipes* exposed for a short time (2 d) to Cr did not show this mechanism of energy dissipation (Paiva *et al.* 2009). Although *E. crassipes* increased NPQ when exposed to As for 2 d, this mechanism was unable to sustain the plant functionality for longer time (4 d, represented by the decrease of F_v/F_m, F_v/F_0, and all gas-exchange parameters, Table 2, Fig. 2) and the senescence process was observed (Fig. 1). According to Demmig-Adams and Adams (1996), decreases in F_v/F_m under stress conditions are related to increases in heat dissipation (NPQ). Similarly, in experiment 2, the NPQ increase, which was observed after 20 d, reflected some of the protection mechanisms employed by the photosynthetic apparatus of these plants.

A positive correlation between NPQ and Car concentrations has been reported for water hyacinth in the field (Lage-Pinto *et al*. 2008), indicating that energy dissipation by the plants is a probable mechanism for the energy decrease observed in the electron transport chain. However, Car concentrations did not change in experiment 1. This finding might be related to the experimental conditions because there was no time for the plants to protect their photosynthetic apparatus from stress. Vitória *et al.* (2010) suggested that energy dissipation mechanisms are a long-term adaptation in water hyacinth plants. In experiment 2, it is possible that the increase in NPQ has been sufficient to dissipate the excess excitation energy at the PSII reaction center, thus rendering energy expenditure by the plant to increase the Car content unnecessary. Other tolerance mechanisms to oxidative stress caused by As might be activated, *e.g*., an increase in the synthesis of thiols, such as phytochelatins, and increased antioxidant enzyme activity (Srivastava *et al*. 2007).

The results of hydroponic experiments are essential for understanding environmental contamination because these experiments allow the simulation of medium conditions in controlled assays. However, in hydroponics, the plant response varies with the conditions imposed, resulting in

punctual responses about particular stress. In the present study, experiments were performed in order to enable understanding of water hyacinth tolerance to various contamination conditions. Regarding the conditions tested in experiment 1, the exposure of water hyacinth to high As concentrations over short periods is more common when the source of contamination is anthropic, for example, due to toxic residues from metal industry. Regarding the conditions tested in experiment 2, water hyacinth exposure to low As concentrations over longer time periods are closer to As exposure originating from natural sources, such as the weathering of auriferous sulfide rocks. In conclusion, our results indicated that the use of water hyacinth to remediate highly impacted environments is limited because significant damage to the photosynthetic apparatus was observed in experiment 1. In this experiment, reduction of stomatal conductance, decline in PSII function, and the concentration of photosynthetic pigments determined the stress caused by As. However, the As tolerance observed in experiment 2 and in plants exposed to 0.20 mg(As^{5+}) L^{-1} in experiment 1 indicated that As can be removed efficiently by water hyacinth in environments experiencing low contamination.

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