

# Influence of chromium in *Laguncularia racemosa* (L.) Gaertn f. physiology

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## ABSTRACT

(Influence of chromium in *Laguncularia racemosa* (L.) Gaertn f. physiology). Most studies of chromium have been realized in the sediments and in mangrove trees. However, little is known about its effect on growth and metabolism in this vegetation. *L. racemosa* plants were grown in solution with 20% of ionic strength of Hoagland and Arnon containing 100 mM NaCl and supplemented with 0.00, 0.05 and 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup>. The growth, the pigments and gas exchange measurements showed no changes in response to doses of chromium. After 30 days the initiation of treatments, plants of *L. racemosa* had average 0.559 g of total dry weight, 1.34 mg L<sup>-1</sup> of total chlorophyll and 7.9 μmol m<sup>-2</sup> s<sup>-1</sup> of A (CO<sub>2</sub> photosynthetic assimilation). The photochemical potential efficiency was affected by the intermediate dose of 0.05 mg L<sup>-1</sup> of Cr<sup>+3</sup> (*Fv/Fm*=0.73) and it was associated with improved translocation of chromium from root to leaves. *L. racemosa* accumulated Cr<sup>+3</sup> in the root, especially in the highest dose (560 mg g<sup>-1</sup> DW) representing twenty-eight times the value obtained in control. The concentration of chromium in stem and leaves was not influenced by the tested doses. There was a reduction of fructose and sucrose in the largest dose of chromium, suggesting the drain of these carbohydrates to meet the energy demand of root absorption. The results suggest that *L. racemosa* owns characteristics of a chromium-tolerant species.

**Keywords:** Carbohydrates, fluorescence, gas exchange, growth, heavy metal, mangrove.

## INTRODUCTION

Mangrove ecosystems are widely distributed in tropical, which is settled in marshy soils and influenced direct of tides (MacFarlane, Koller and Blomberg, 2007). It is developed for large areas hacking by several rivers, canals and lakes, in regions generally flat, coastal and tropical climate. They are highly productive and play a vital role as a major primary producer within estuarine systems. Mangrove systems serve as habitat and nursery area for many juvenile fish, crustaceans and mollusks of interest both social and economic (Ramos

and Geraldo, 2007). In addition to its ecological importance, the mangrove plays the role of filter in the purification of water, keeping in the plant roots the organic materials and metals transported by estuarine waters (Macfarlane et al., 1999). Thus, mangrove systems act as a reservoir of metals and values of metals in soil at concentrations considered harmful to living organisms (Schaeffer-Novelli, 1995; Vannucci, 2001).

The tolerance has been in part attributed to the ability of mangroves to exclude metals or regulate uptake of metals

in the root and limit translocation to the shoot (MacFarlane, Pulkownik and Burchett, 2003). A number of studies have found metals to be accumulated predominantly in root tissue, rather than in foliage (Peters et al., 1997; MacFarlane, Koller and Blomberg, 2007). The data seem to suggest that concentrations of translocated metals are low with bio-concentration factors (BCF; ratio of leaf metal to corresponding sediment metal concentration) ranging from <0.01 to 0.06 for mangrove trees (Lacerda, 1997). Other studies suggest that mangroves may accumulate and translocate some metals with leaf BCFs greater than one (Chen et al., 2003) thereby meeting established criteria for hyperaccumulating species (Baker and Whiting, 2002).

The high organic matter content, iron oxides, aluminum and manganese as well as the presence of humic acids associated with low cation exchange capacity and the low redox potential, also contribute to the complexation of heavy metals in sediments of mangroves (Ong Che, 1999; Preda and Cox, 2002; Ramos e Silva et al., 2006). This interaction allows the expression of multiple plasticity of vegetation, increasing the tolerance to high levels of metals (Cuzzuol and Campos, 2001).

It is recognized that the tolerance to metals is related to the partitioning of elements in different plant organs and cellular components (Chiu and Chou, 1991). Another form of tolerance is the excretion of metal by glands located on the leaves and/or regulation of the absorption and excretion by the root system (Bernini et al., 2006).

Most studies of chromium are limited to analyzing the sediment or the internal concentration in different plant organs. When the issue is the effect of this metal in plant growth and cell metabolism in coastal environments plants, little is known.

Chromium is not considered an essential trace element, without any participation in the plant metabolism (Dixit et al., 2002). However, at toxic levels, it can affect growth and cause permanent damage to the root system (Scoccianti et al., 2006). Inhibition of seed germination, degradation of photosynthetic pigments, changes in nutrient balance and induction of oxidative stress are other effects on plants under toxic effect of chromium (Panda, 2003).

A real understanding of the physiological mechanisms of plants under the influence of chromium is of great importance

to the environmental biomonitoring and the conservation of coastal resources, especially the mangrove trees of the region exposed to chromium at high levels, taking  $0.05 \text{ mg L}^{-1}$  as the limit base permitted by Brazilian legislation for estuarine and marine water (CONAMA, 2005). Considering that chromium is one of the most abundant heavy metals in the estuarine ecosystem of Vitória Island, Brazil (Jesus et al., 2004), there is a lack of information on the impact of this element in mangrove. Thus, this study aims to evaluate the effect of chromium on plant growth and carbon metabolism of *L. racemosa*.

## MATERIAL AND METHODS

**Plant material and experimental design:** Robust and homogenous plants of *Laguncularia racemosa* (L.) Gaertn. f., containing three pairs of leaves,  $10 \pm 0.5$  cm of length and  $0.426 \pm 0.085$  g of total dry weight, were collected in Dom Luiz Gonzaga Fernandes Natural Hall Park ( $20^{\circ}19'09''\text{S}$ ,  $40^{\circ}20'50''\text{W}$ ), located in Lameirão Ecological Station, Vitória city, Espírito Santo State, Brazil.

One hundred and fifty plants were carefully removed from the sediment and immediately immersed in distilled water, rinsed in running water and transferred to plastic pots (1L) containing Hoagland and Arnon (1938) solution 20% of ionic strength in hydroponic system with constant aeration, under environmental temperature and photoperiod in greenhouse with 50% of shade. The nutrient solution was changed weekly, and after thirty days the dead plants were discarded. From the initial batch, seventy-nine plants with good morphological appearance were cultivated during three weeks in 20% of Hoagland solution containing 100 mM of NaCl and 5.5 pH as preliminary results and compatible to the estuarine environments where the values vary from 7 and 514 mM of NaCl (Sobrado, 2004).

After this period of adjustment, the treatments with  $\text{Cr}^{+3}$  at concentrations of 0.00, 0.05 and  $0.50 \text{ mg L}^{-1}$  were performed using as source the basic chromium sulphate ( $\text{Cr}_2(\text{OH})_2(\text{SO}_4)_2$ ), normally used in tanning. The experimental design was built up with randomized blocks and each plot was formed by two black plastic pots (1 L) covered with a perforated styrofoam support with four plants per pot totaling 24 plants per treatment. Weekly, the solutions were exchanged to prevent the salinization, the depreciation of the chemical elements and the chromium concentration increase.

**Analysis of growth:** After 30 days, three plants per plot were randomly selected for growth analysis, measuring the length of stem, number of leaves, number of internodes, total leaf area, and fresh and dry weight of plant organs. The biological material was packaged in oven at 60°C up to obtaining the constant mass. The leaf area was measured using the LI-COR 3100C area meter. With these data, it was determined the reason root:shoot, leaf area ratio ( $LAR = A/M$ ) with the values of leaf area and total dry weight of the plant and also the specific leaf weight ( $SLW = M/A$ ) measured by the ratio of leaf dry weight and total leaf area (Hunt, 1978).

**Pigments, gas exchange and potential photochemical efficiency:** The levels of total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid pigments were determined by the method of Arnon (1949). Samples of 1 mg of the first fully expanded leaf apical were homogenized with pistil in 10 ml of acetone 80% (v/v) for extraction of pigments. The operation was performed under low light and temperature to prevent the chlorophyll degradation by light or by enzymatic action. The reading was held in spectrophotometer to estimate the levels of chlorophyll *a*, chlorophyll *b* and carotenoids. The calculations were made based on equations derived by Hendry and Grime (1993) and the concentration of pigments was expressed in mg.g FW<sup>-1</sup>.

$$\text{Chlorophyll } a \text{ (mg.g}^{-1}\text{)} = [(12.7 \times A_{663} - 2.69 \times A_{645})/1000FW] \times V$$

$$\text{Chlorophyll } b \text{ (mg.g}^{-1}\text{)} = [(22.9 \times A_{645} - 4.68 \times A_{663})/1000FW] \times V$$

$$\text{Total chlorophyll (mg.g}^{-1}\text{)} = [(8.02 \times A_{663} + 20.2 \times A_{645})/1000FW] \times V$$

The carotenoid concentrations were determined by the equation of Price and Hendry (1995) for quantification of carotenoids ( $\mu\text{mol.gFW}^{-1}$ ) =  $[(A_{480} + 0.114 \times A_{663}) - (0.638 \times A_{645}) \times V/112.5 \times \text{FW of the leaf (g)}]$ , where *V* = volume of the sample (ml), *A* = absorbance and *FW* = fresh weight.

Using the SPAD chlorophyll meter of Minolta (Soil Plant Analysis Development), four readings in four different points of the leaves were made, two readings near the apex and two near the base on both sides of the leaf. The measure system used was the average among the four leaf values, totalizing 36 readings, four for each treatment in the third leaf node completely expanded.

Measurements of gas exchange were performed on the same leaves where the analysis of pigments and other assessments were made. The CO<sub>2</sub> photosynthetic assimilation

(*A*), stomatal conductance (*g<sub>s</sub>*), transpiration (*E*) and leaf to air vapor pressure deficit ( $DPV_{\text{leaf-air}}$ ) were determined using a portable system of gas exchange measurements, model LI-6200 (LI-COR, Lincoln, NE, USA), on leaves of the third pair completely expanded, counted from the apex. The determinations were made at 8:00 h in the morning by applying photosynthetic active radiation of 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and concentration of environmental CO<sub>2</sub>.

The potential photochemical efficiency measurements were performed on the same leaves and at the same time using the non-modulated Fluorimeter, model SAP (Hanstech, King's Lynn, Norkfolk, UK) that determines the characteristics of rapid fluorescence - initial fluorescence (*F<sub>o</sub>*), maximum fluorescence (*F<sub>m</sub>*), variable fluorescence (*F<sub>v</sub>*) and photochemical efficiency of photosystem II (*F<sub>v</sub>/F<sub>m</sub>*). After 30 minutes of adaptation to darkness, each leaf disc was exposed to a pulse of saturation of luminous intensity equal to 1680  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during five seconds.

**Soluble non-structural carbohydrates:** Leaf segments were homogenized in nitrogen liquid and the extraction of non-structural carbohydrates was made in 80% ethanol to 70°C. The determination of glucose, fructose and sucrose followed the enzymatic method, reducing NAD<sup>+</sup> estimated at 340 nm by Elisa reader (Stitt et al., 1989). Aliquots of the samples were homogenized with buffer (100 mM imidazole pH 7.4, 5 mM MgCl<sub>2</sub>, 2 mM NAD<sup>+</sup>, 1 mM ATP and 2U of G<sub>6</sub>PDH), adding 1.5 U of hexokinase for the estimation of glucose. 3 U of phosphoglucose isomerase has been added for determination of fructose while sucrose was determined by appending 5 U of β-fructosidase. In the end of this reaction, 3 U of phosphoglucose isomerase was added to estimate the sucrose through the activity of β-glucofructose.

**Internal concentration of chromium:** Two plants per plot, totalizing 18 samples per treatment, were dried in paper filter for 24 hours. The roots, the stems and leaves were separated, washed and dried in oven (60°C), then ground, sieved in a 1.0 mm mesh and stored in glass flasks at ambient temperature. The extraction of chromium was performed by acid digestion in nitric acid and perchloric acid in a cold digester tube for three to four hours. After this period, the material (1 g DW) was transferred to the digester block and the temperature was increased gradually to 160°C for 40 minutes and then 210°C for 20 minutes, as the method described by Silva (1999). The concentration of chromium was determined by atomic

absorption spectrophotometer (AAS) using a hollow cathode lamp of chromium as an energy source. A calibração do equipamento, em cada troca de lâmpada, foi realizada utilizando padrões a partir de soluções estoques. The calibration of the equipment in exchange for each lamp was made using patterns from stock solutions ( $50 \text{ ppm} \pm 0.5\%$ ).

**Statistical analysis:** Data were submitted to analysis of variance (ANOVA) using the program ASSISTAT version 7.4 beta (Silva and Azevedo, 2007), and the Tukey test at 5% of probability was applied when necessary. For the analysis of the  $\text{Cr}^{+3}$  effect in gas exchange, the photochemical efficiency, the concentration of pigments and carbohydrates and analysis of plant growth was applied randomized blocks, and the double factorial scheme was applied for internal analysis of  $\text{Cr}^{+3}$ .

## RESULTS AND DISCUSSION

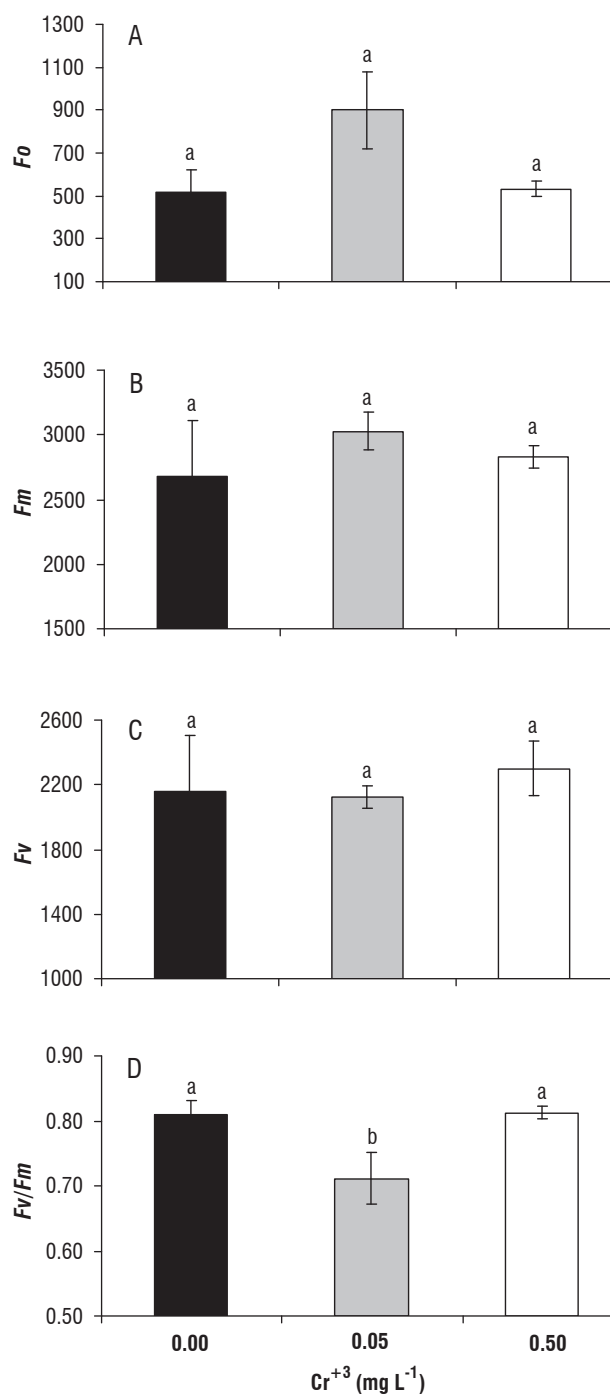
**Analysis of growth:** There were no significant differences in growth measurements, as well as the ratio root: shoot and the LAR, SLW and SLA in plants of *L. racemosa* treated with and without  $\text{Cr}^{+3}$  (Table 1). The pattern of dry weight partition also showed no variation. The occurrence of toxicity in plants is rare due to the low mobility of  $\text{Cr}^{+3}$  through the cell membrane (Panda and Choudhury, 2005). This lack of effect on growth can be associated with the extent chromium concentration used, since inhibitory effects on growth were only found in concentrations higher than 100 mM of chromium (Panda and Choudhury, 2005) highly toxic even for plants that live in a anoxic environment as *L. racemosa*.

**Table 1** - Growth measurements of *L. racemosa* cultivated in hydroponic solution with 20% of ionic strength of Hoagland and Arnon (1938) supplemented with 0.00, 0.05 and 0.50  $\text{mg L}^{-1} \text{Cr}^{+3}$ . Letters compare the different values between the concentrations of  $\text{Cr}^{+3}$ , and  $\pm$  represents standard error ( $P < 0.05$  and  $n=9$ ).

Growth Measurements	$\text{Cr}^{+3}$ ( $\text{mg L}^{-1}$ )		
	0.00	0.05	0.50
Root dry weight (g)	0.184 $\pm$ 0.02a	0.128 $\pm$ 0.08a	0.172 $\pm$ 0.05a
Stem dry weight (g)	0.192 $\pm$ 0.06a	0.140 $\pm$ 0.05a	0.176 $\pm$ 0.09a
Blade dry weight (g)	0.245 $\pm$ 0.02a	0.183 $\pm$ 0.09a	0.238 $\pm$ 0.07a
Petiole dry weight (g)	0.014 $\pm$ 0.00a	0.008 $\pm$ 0.00a	0.010 $\pm$ 0.00a
Shoot dry weight (g)	0.431 $\pm$ 0.05a	0.322 $\pm$ 0.08a	0.325 $\pm$ 0.08a
Total dry weight (g)	0.638 $\pm$ 0.02a	0.443 $\pm$ 0.08a	0.596 $\pm$ 0.07a
Stem length (cm)	16.3 $\pm$ 1.5a	15.1 $\pm$ 2.8a	14.7 $\pm$ 3.2a
Stem diameter (mm)	2.08 $\pm$ 0.1a	1.82 $\pm$ 0.1a	2.20 $\pm$ 0.1a
Leaves number	8 $\pm$ 1.5a	7 $\pm$ 1.6a	7 $\pm$ 0.8a
Leaf area ( $\text{cm}^2$ )	37.1 $\pm$ 5.8a	28.3 $\pm$ 9.4a	37.1 $\pm$ 6.8a
Root:shoot ratio	0.38 $\pm$ 0.0a	0.39 $\pm$ 0.0a	0.42 $\pm$ 0.0a
SLW ( $\text{g cm}^{-2}$ )	6.69 $\pm$ 0.5a	6.41 $\pm$ 0.4a	6.21 $\pm$ 0.8a
SLA ( $\text{cm}^2 \text{g}^{-1}$ )	150 $\pm$ 9.5a	159 $\pm$ 8.7a	162 $\pm$ 7.3a
LAR ( $\text{cm}^2 \text{g}^{-1}$ )	60 $\pm$ 3.5a	64 $\pm$ 5.8a	60 $\pm$ 6.5a

**Pigments, gas exchange and potential photochemical efficiency:** Reduction in concentration of photosynthetic pigments in plants under the influence of heavy metals has been reported in some genotypes (Panda and Parta, 2000; Panda, 2003; Baker and Rosenqvist, 2004). However, in native plants such as the ones from mangrove ecosystem, heavy metals accumulated in leaf tissues did not affect the content of chlorophyll and carotenoids showing no visible signs of toxicity (Macfarlane et al., 2003). Similarly, the pigments in *L.*

*racemosa* were not affected by  $\text{Cr}^{+3}$  (Figure 1, Table 2), both by the method of extraction as the SPAD, suggesting that the species in hydroponic cultivation is tolerant and well adapted to the levels of  $\text{Cr}^{+3}$  tested. The values of *A*, *gs*, *E* and  $\text{DPV}_{\text{leaf-air}}$  (Table 3) did not vary between treatments, thus *L. racemosa* did not show any variation in gas exchange. Perhaps, as this species accumulates  $\text{Cr}^{+3}$  to its roots, there was no negative influence on gas exchange, since the deleterious effects of heavy metal would be more intense in the leaves.



**Figure 1.** Values of (A) initial fluorescence ( $F_0$ ), (B) maximum fluorescence ( $F_m$ ), (C) variable fluorescence ( $F_v$ ) and (D) PSII photochemical efficiency ( $F_v/F_m$ ) in *L. racemosa* seedlings cultivated in hydroponic solution with 20% of ionic strength solution of Hoagland and Arnon (1938) supplemented with 0.00, 0.05 and 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup>. Letters compare the different values between the concentrations of Cr<sup>+3</sup>, and ± represents standard error ( $P < 0.05$  and  $n = 6$ ).

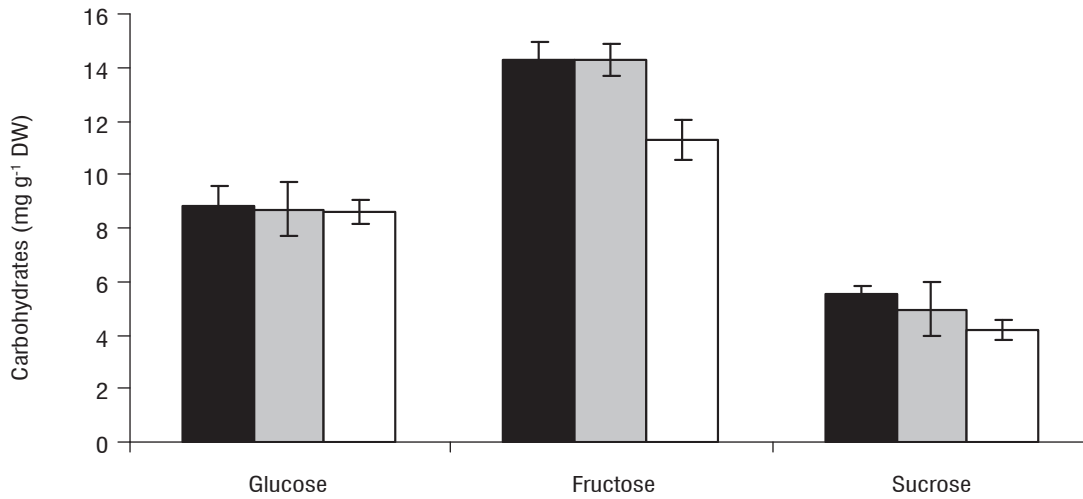
**Table 2 -** Average values of chlorophyll a, chlorophyll b, total chlorophyll (mg.g<sup>-1</sup>), the chlorophyll a/chlorophyll b ratio, carotenoids (μmol.g<sup>-1</sup>) and chlorophyll analyzed with SPAD in *L. racemosa* seedlings cultivated in hydroponic solution with 20% of ionic strength solution of Hoagland and Arnon (1938) supplemented with 0.00, 0.05 and 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup>. Letters compare the different values between the concentrations of Cr<sup>+3</sup>, and ± represents standard error ( $P < 0.05$  and  $n = 6$ ).

Pigments	Cr <sup>+3</sup> (mg L <sup>-1</sup> )		
	0.00	0.05	0.50
Chlorophyll a	0.64 ± 0.05a	0.60 ± 0.05a	0.60 ± 0.05a
Chlorophyll b	0.76 ± 0.06a	0.70 ± 0.03a	0.73 ± 0.05a
Total chlorophyll	1.40 ± 0.11a	1.30 ± 0.11a	1.33 ± 0.11a
Chlorophyll a/b	0.85 ± 0.01a	0.85 ± 0.00a	0.83 ± 0.02a
Carotenoids	0.14 ± 0.01a	0.14 ± 0.01a	0.15 ± 0.01a
SPAD	55.8 ± 2.2a	55.3 ± 2.9a	59.6 ± 3.7a

**Table 3 -** Average values of CO<sub>2</sub> photosynthetic assimilation (A), stomatal conductance ( $g_s$ ), transpiration (E) and leaf to air vapor pressure deficit ( $DPV_{leaf-air}$ ) in *L. racemosa* seedlings cultivated in hydroponic solution with 20% of ionic strength solution of Hoagland and Arnon (1938) supplemented with 0.00, 0.05 and 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup>. Letters compare the different values between the concentrations of Cr<sup>+3</sup>, and ± represents standard error ( $P < 0.05$  and  $n = 6$ ).

Gas exchange	Cr <sup>+3</sup> (mg L <sup>-1</sup> )		
	0.00	0.05	0.50
A (μmol m <sup>-2</sup> s <sup>-1</sup> )	8.50 ± 0.5a	7.20 ± 0.8a	8.00 ± 0.6a
$g_s$ (mol m <sup>-2</sup> s <sup>-1</sup> )	0.31 ± 0.0a	0.31 ± 0.0a	0.27 ± 0.0a
E (mmol m <sup>-2</sup> s <sup>-1</sup> )	8.05 ± 0.8a	8.13 ± 0.5a	7.84 ± 0.9a
$DPV_{leaf-air}$ (kPa)	3.23 ± 0.3a	3.25 ± 0.5a	3.55 ± 0.8a

The low value of  $F_v/F_m$  at 0.05 mg.L<sup>-1</sup> of Cr<sup>+3</sup> in relation to the other doses tested (Figure 2) shows slight decrease in photochemical efficiency due to a low inhibiting in absorption and transfer of light energy to the centers of reaction and low reoxidation electrons acceptors from plastoquinones. The slight reduction in photosynthetic efficiency ( $F_v/F_m$ ) at 0.05 mg.L<sup>-1</sup> of Cr<sup>+3</sup> may be caused by the translocation factor (TF) of chromium which is defined as the ratio of the chemical element concentration between the shoot and root (Yu and Gu, 2007). In low concentrations, the chromium shows more mobility from the root to the leaves (Han et al., 2004). Analyzing the phytotoxic effects of trivalent and hexavalent Cr, Han et al. (2004) found out that, at lower concentration, the Cr<sup>+3</sup> focuses less on the root with a higher translocation to the leaves. The *L. racemosa* plants, exposed to higher doses of chromium, showed greater accumulation of that element in the roots (Figure 3). In concentration of 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup> the TF was 0.07 while in 0.05 mg L<sup>-1</sup> this amount was 0.10.



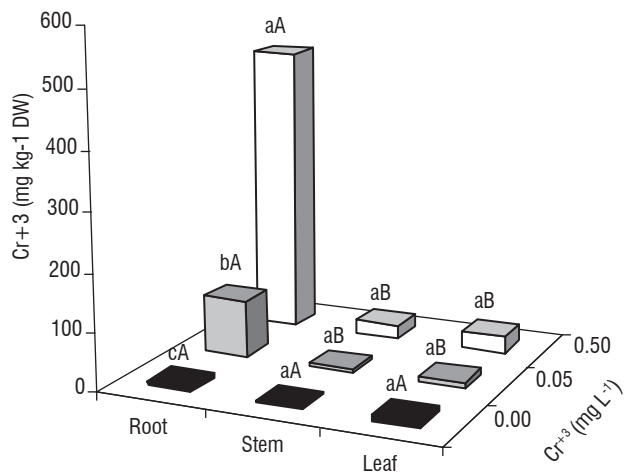
**Figure 2.** Average values of Cr<sup>+3</sup> in root, stem and leaf of *L. racemosa* plants cultivated in hydroponic solution with 20% of ionic strength solution of Hoagland and Arnon (1938) supplemented with 0.00 (■), 0.05 (▒) and 0.50 (□) mg L<sup>-1</sup> of Cr<sup>+3</sup>. Capital letters compare the average concentrations of Cr between plant organs, and lowercase letters compare averages between concentrations of Cr within each plant organ ( $P < 0.05$  and  $n = 18$ ).

According to Shanker et al. (2005), the disorganization of the chloroplasts ultra-structure, the electrons transport inhibition and the changes in enzymatic activities of the carbon sequestration are the likely effects of the photosystem II inhibition affected by chromium. Those effects, reflected in the chlorophyll fluorescence, were not observed in *L. racemosa* plants exposed to the dose of Cr<sup>+3</sup> considered toxic by Brazilian legislation (CONAMA, 2005). According to this document the concentration 0.05 mg L<sup>-1</sup> of total Cr is determined for brackish and saline water of class 1 (water with primary contact). The mangrove is usually in estuarine areas (presence of brackish water) which are usually practiced fishing activities, with primary contact of people (water for direct and prolonged contact) which do these activities that would characterize as brackish water of class 1.

However, studies do not state that the *L. racemosa* photosynthetic activity is not affected by the impact of chromium in the environment since some species only showed photosynthetic inhibitory effects when exposed to chromium levels higher than 50 mg L<sup>-1</sup> (Dhir et al., 2008).

**Soluble non-structural carbohydrates:** Similarly to gas exchange and potential photochemical efficiency, chromium had little influence on the carbohydrates metabolism in *L. racemosa* plants (Figure 3). The sucrose and fructose concentration in leaves showed a slight decrease under higher levels of chromium. Sucrose represented the smallest fraction

of soluble carbohydrates analyzed. That could mean higher translocation of sucrose from the source to the drain organ, feeding the energetic demands of chromium active transport in the vacuole of the cortical cells in root system (Mangabeira et al., 2006; Panda and Choudhury, 2005).



**Figure 3.** Glucose, fructose e sucrose concentrations in *L. racemosa* leaves cultivated in hydroponic solution with 20% of ionic strength solution of Hoagland and Arnon (1938) supplemented with 0.00 (■), 0.05 (▒) and 0.50 (□) mg L<sup>-1</sup> of Cr<sup>+3</sup>. The bars represent standard error ( $P < 0.05$  and  $n = 6$ ).

**Internal concentration of chromium:** The roots in 0.05 and 0.50 mg L<sup>-1</sup> of chromium accumulated high levels of this metal, followed by low concentrations in leaves and stems (Figure 3). In 0.50 mg L<sup>-1</sup> of Cr<sup>+3</sup>, the roots accumulated almost

seven times the concentration of chromium in relation to the plants treated with  $0.05 \text{ mg L}^{-1}$  of  $\text{Cr}^{+3}$ , with no significant differences of this element content in leaves. According to Ramos and Geraldo (2007), values of chromium ranging from  $2.05$  to  $8.2 \text{ mg kg}^{-1}$  in leaves of mangrove species can indicate environmental pollution. However, even in control, that amount was  $6.9 \text{ mg kg}^{-1}$  in *L. racemosa* plants, suggesting a revision of the reference values in internal concentration of chromium that are considered critical and indicative of environmental pollution in mangrove.

The low concentration of chromium in leaves of *L. racemosa* in all treatments, including in  $0.50 \text{ mg L}^{-1}$  whose value is ten times higher than the limit permitted by federal law to brackish waters as Brazilian legislation (CONAMA 2005), may be due to low efficiency of  $\text{Cr}^{+3}$  transfer from root to leaves (Pandey and Sharma, 2003; Maine et al., 2004). According to Mangabeira et al. (2006), this phenomenon characterizes plants under high concentration of chromium. The greatest accumulation of  $\text{Cr}^{+3}$  in roots in relation to air organs is common in mangrove species and other biomes (Zengh et al., 1997; MacFarlane et al., 2003; Maine et al., 2004; Yu and Gu, 2007) as well as some agricultural cultivars (Mei et al., 2002; Scoccianti et al., 2006).

In relation to plants developed *in situ*, the  $\text{Cr}^{+3}$  concentration in *L. racemosa* plants grown in hydroponic system was higher. Ong Che (1999) detected values ranging between  $2$  and  $6 \text{ mg kg}^{-1}$  of dry weigh in *Kandelia candel* roots, a typical mangrove tree. This probably occurred due to the higher  $\text{Cr}^{+3}$  availability in hydroponic solution and the lack of reducing processes that normally occur in sediment (Tam and Wong, 1993). Sediment properties as texture, organic matter (OM) and redox potential are involved in the process of metal immobilization in sediments of mangrove ecosystem (Tam and Wong, 1993). Moreover, under natural conditions, the presence of vegetation can be very important in determining the concentration of metals in the sediment (Ong Che, 1999). Areas covered by vegetation have a redox potential higher than those without vegetation due to oxidative processes in roots (Chiu and Chou, 1991).

In *L. racemosa*, the metabolic tolerance to  $0.50 \text{ mg L}^{-1}$  of chromium may be due to partitioning of heavy metals in vacuoles of root cells associated with their low mobility to the leaves (Panda and Choudhury, 2005; Dhir et al., 2008). This behavior is itself an efficient mechanism of tolerance in

environments contaminated by heavy metals. The formation of iron plaque on roots that can diminish the loss of oxygen is common in aquatic and wetland plants (Møller and Sand-Jensen, 2008) and may influence the availability and mobility of metals in the rhizosphere of aquatic plants (Hansel et al., 2001). Machado et al. (2005) observed exclusion of metals by formation of iron plaque on roots of *L. racemosa*, what possibly can contribute to the adaptability of plants to flooded environments.

Considering the initial growth phase and the lack of influence of chromium in photosynthetic activity and growth of *L. racemosa*, this tree owns physiological characteristics of phytoremediate species, since *L. racemosa* tolerates chromium levels ten times higher than those permitted by Brazilian legislation (CONAMA, 2005). However, studies about *L. racemosa* tolerance to chromium could reveal important information about the physiological mechanism of plants in coastal environments facing the environmental impact caused by heavy metals.

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