CHROMIUM TOXICITY IN HYBRID EUCLYPTUS (EUCALYPTUS UROPHYLLA S. T. BLAKE X GRANDIS W. HILL EX. MAIDEN) CUTTINGS

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CHROMIUM TOXICITY IN HYBRID EUCALYPTUS (EUCALYPTUS UROPHYLLA S. T. BLAKE X GRANDIS W. HILL EX. MAIDEN) CUTTINGS

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A greenhouse experiment was carried out to evaluate chromium (Cr) toxicity in urograndis (Eucalyptus urophylla S. T. Blake x grandis W. Hill ex. Maiden). Chromium nitrate was amended to Clark’s nutrient solution (0.00, 0.04, 0.08, 0.16, 0.32 and 0.64 mmol L\(^{-1}\) Cr), which was used to breed one urograndis cutting per pot (four replications). Regression analysis revealed that Cr rates of 0.08 mmol L\(^{-1}\) or higher significantly decreased (\(P < 0.01\)) dry matter yield of shoot and root. Chromium accumulation order in plant was: root > stem > leaves. Critical Cr toxicity level in leaves was 0.74 mg kg\(^{-1}\). Uptake and translocation of nutrients were affected differently by Cr. Mostly have their uptake reduced due to root damage. Magnesium, iron and manganese translocation increases, supposedly to maintain normal photosynthetic activity. Wilting is the main visual symptom of Cr toxicity. Urograndis may be suitable for contaminated sites phytostabilization and receive industrial waste fertilization.

Keywords: heavy metals, nutrient translocation, nutrient uptake, phytoremediation, urograndis

INTRODUCTION

The intensification of industrial and agricultural activities made heavy metals one of the greatest environmental problems nowadays. Indiscriminate disposal of fertilizers, pesticides, sewage sludge and industrial and urban waste can make terrestrial and aquatic environments unsuitable for the development of plants and animals (He et al., 2005).
Due to its potential as preservative, chromium may be present in every home, coating cutlery, clothes, wood, taps and baths. The main responsible for chromium (Cr) influx in the biosphere are the electroplating and leather tanning activities (Shanker et al., 2005). Chromium is also present in the air, soil and water. Therefore, humans are constantly exposed to this heavy metal. Although required in small quantities to humans and animals, there is no record of chromium being essential to plants.

Chromium occurs in nature in several oxidation states, being Cr(VI) and Cr(III) the most stable and common in the environment. In soils, the latter is more abundant than the former, which is more soluble and toxic (Barlett, 1991; Panda and Choudhury, 2005). Although its uptake mechanism and metabolism in plants are poorly understood, its great phytotoxic potential is well recognized (Shanker et al., 2005).

Despite its phytotoxicity, it is necessary to investigate the behavior of plants exposed to high Cr concentrations due to the increasing use of municipal and industrial waste as fertilizers on forest plantations (Nolasco et al., 2005). Since such wastes might contain considerable amounts of Cr (Nolasco et al., 2005; Aguilera et al., 2007) such a practice may lead to a progressive increase in the levels of Cr in the soil. Moreover, many studies on phytoremediation of Cr-contaminated soils have been conducted, but they focus mainly on herbaceous and temperate climate species, despite the benefits of using trees for phytoremediation and the higher growth rates of tropical species (Pulford and Watson, 2003; Shanker et al., 2005).

The Eucalyptus genus is the most cultivated worldwide. In Brazil the \( E. \) \textit{urophylla} S. T. Blake x \textit{grandis} W. Hill ex. Maiden hybrid (known as urograndis) is the preferred choice for companies and forest farmers due to its growth potential and pathology resistance. In addition, its high economic value and consolidated silvicultural practices indicate that eucalyptus plantations may have great potential for phytoremediation and waste disposal. Still, there is much to discover about eucalyptus as phytoremediator and its tolerance to the presence of chromium in the soil.

The objectives of this study were to evaluate the growth of urograndis cuttings exposed to different levels of chromium, visually diagnose toxicity symptoms, investigate nutritional changes and assess Cr accumulation in the plant organs.

**MATERIALS AND METHODS**

The experiment was conducted in a greenhouse of Departamento de Ciência do Solo - Universidade Federal de Lavras (DCS-UFLA) with urograndis cuttings produced at the same University’s nursery, from September to December 2007.
A completely randomized design with six treatments and four replications was used. The treatments consisted of complete Clark’s nutrient solution (Clark, 1975) with the following chromium levels: 0.00 (control), 0.04, 0.08, 0.16, 0.32, and 0.64 mmol L$^{-1}$. Chromium nitrate (III) nonahydrate [Cr(NO$_3$)$_3$.9H$_2$O] was used as the Cr source. The Cr rates used were chosen based on preliminary trials (unpublished results).

Eight-month cuttings were transferred to a tray with constant aeration containing Clark’s nutrient solution with 50% of its ionic strength for acclimatization. After 14 days, it was replaced by the solution with full ionic strength. After two more weeks, the plants were selected by height, to minimize cutting heterogeneity among treatments, and transplanted into 3 L pots (one plant per pot) containing the solution corresponding to its treatment. When necessary, the pot volume was replenished with deionized water. The solutions were completely changed every two weeks.

The plants were collected as toxicity symptoms became apparent to reduce loss of material for analysis. The symptoms were described and photographed. Height and stem diameter were measured before collecting. All remaining plants were collected 60 days after the exposure to the treatments.

The plants were divided into root, stem and leaves, washed with distilled water and dried in a forced-air oven to constant weight. After obtaining dry matter data, the plant material was ground in a Wiley-type mill equipped with a 0.38 mm sieve to be chemically analyzed using methods described in Malavolta et al. (1997) for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) determination. Atomic absorption spectrophotometry following nitro-perchloric digestion was used for Cr analysis.

The critical toxicity leaf concentration (leaf Cr concentration required to inhibit shoot dry matter production by 10%) and the critical toxicity level in nutrient solution that reduces by 10 and 50% dry matter production were estimated through regression equations. The total Cr content in one organ (mg) was determined by multiplying the total Cr concentration (mg g$^{-1}$) in that organ times the organ dry mass (g). Chromium translocation index (TI) was calculated using the following formula (Abichequer and Bohnen, 1998):

\[
TI = \frac{\text{Shoot Cr content}}{\text{Whole plant Cr content}} \times 100
\]

The data were subjected to analysis of variance and regression using SISVAR and Table Curve 2D software (Systat Inc., Evanston, IL, USA).
RESULTS

Visual Diagnosis

Plants supplied with Cr rates of 0.08 mmol L\(^{-1}\) or higher showed clear symptoms of water deficiency (Figure 1). Leaves wilted, curled outwards and were extremely dry and easily loosened from the stem. This effect was even more intense under 0.32 and 0.64 mmol L\(^{-1}\) Cr, leading to the premature collection of the respective plants by the end of the first month of breeding. It was also possible to observe reduction on the number of leaves and root volume under Cr rates superior to 0.04 mmol L\(^{-1}\) (Figure 1).

Growth and Critical Toxicity Levels

Chromium affected significantly \((P < 0.01)\) all growth variables measured, which were inversely related to Cr level in solution (Figure 2 and Figure 3). Control root dry matter (RDM) was 3.38 \((\pm 0.30)\) g, which was not affected at 0.04 mmol L\(^{-1}\) Cr. However, this value was reduced by 38.76, 48.22, 64.50 and 70.41\% at concentrations of 0.08, 0.16, 0.32 and 0.64 mmol L\(^{-1}\) of chromium, respectively.

Stem dry matter (SDM) and leaf dry matter (LDM) were also severely affected at Cr levels superior to 0.04 mmol L\(^{-1}\) (Figure 2). Reductions were of 62.11, 75.63, 81.97 and 88.45\% on SDM and 61.66, 68.78, 77.77 and 86.31\% on LDM under 0.08, 0.16, 0.32 and 0.64 mmol L\(^{-1}\) Cr, respectively. Control SDM and LDM production were 7.10 \((\pm 2.43)\) g and 9.13 \((\pm 1.81)\) g, respectively, similar to those observed for 0.04 mmol L\(^{-1}\) Cr.

Control shoot dry matter (SDM + LDM) was equal to 16.23 \((\pm 4.21)\) g, which reduced as the Cr level in solution increased, starting with a 7.27\%
reduction under 0.04 mmol L\(^{-1}\) and reaching 87.25% under 0.64 mmol L\(^{-1}\) (Figure 2). Height reduction reached 38.64% under 0.32 mmol L\(^{-1}\) Cr, while lap diameter decreased by 53.32% under 0.64 mmol L\(^{-1}\) (Figure 3).

Critical toxicity levels in solution, determined on the basis of dry matter yield (10% reduction over control–CTL\(_{10}\)), were 0.01 and 0.02 mmol L\(^{-1}\) Cr for shoot and roots, respectively (Table 1). Eight times the CTL\(_{10}\) were needed to reduce by 50% (CTL\(_{50}\)) shoot and root dry matter (Table 1), reflecting the exponential relation between dry matter production and Cr rate (Figure 2). The critical toxicity levels calculated for the shoot are half of those found for the roots (Table 1).
Chromium Toxicity in Urograndis Cuttings

Chromium Toxicity in Urograndis Cuttings

Chromium Uptake and Translocation

Chromium rate in the solution affected significantly Cr concentration in every organ at $P < 0.01$. The metal accumulated mainly in the roots, followed by the stem and leaves, with the lowest values of Cr concentration (Figure 4). In the leaves, Cr concentration was quadratically related to Cr levels in solution, reaching its maximum at 22.17 mg kg$^{-1}$ under 0.36 mmol L$^{-1}$ Cr, estimated through regression analysis (Figure 4). The quadratic model also represented satisfactorily the relationship of Cr concentration in shoot and Cr rate, with the highest value equal to 146.07 (±11.76) mg kg$^{-1}$, observed under 0.64 mmol L$^{-1}$ (Figure 4).

Stem Cr concentration was directly related to Cr rate, adjusting to a simple linear model and reaching 137.29 (±14.69) mg kg$^{-1}$ under 0.64 mmol L$^{-1}$ Cr (Figure 4). Although resisting higher leaf concentrations, 0.74 mg kg$^{-1}$ Cr, which corresponds to a Cr rate of 0.02 mmol L$^{-1}$, was enough to inhibit by 10% shoot dry matter production.

The relationship between root Cr concentration and Cr rate could be adjusted only to the cubic model, rising from 0.00 to 0.16 mmol L$^{-1}$ Cr

### Table 1

Critical Cr toxicity levels in solution needed to reduce by 10 (CTL$_{10}$) and 50% (CTL$_{50}$) root and shoot dry matter

<table>
<thead>
<tr>
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<th>Shoot (mmol L$^{-1}$)</th>
<th>Root (mmol L$^{-1}$)</th>
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<tbody>
<tr>
<td>CTL$_{10}$</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>CTL$_{50}$</td>
<td>0.08</td>
<td>0.16</td>
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</table>

FIGURE 3 A) Diameter and B) height of urograndis cuttings’ response to Cr rate. Vertical bars represent standard errors. Regression analysis: A) $y = 33.513 + 21.6194\exp(-x/0.1920)$, $R^2 = 0.979$; B) $y = 22.927x^2 - 21.061x + 7.9103$, $R^2 = 0.992$. Both interactions were significant at $P < 0.01$ by the F test.
FIGURE 4 Chromium concentration in A) leaf, B) stem, C) root, and D) shoot tissues of urograndis cuttings’ response to Cr rate. Vertical bars represent standard errors. Regression analysis: A) \( y = -179.079x^2 + 129.757x - 1.3288 \), \( R^2 = 0.853 \); B) \( y = 195.971x + 7.0691 \), \( R^2 = 0.816 \); C) \( y = 508295.57x^3 - 528349.44x^2 + 14928.38x - 757.2 \), \( R^2 = 0.978 \); D) \( y = -172.973x^2 + 321.785x + 6.0001 \), \( R^2 = 0.801 \). All interactions were significant at \( P < 0.01 \) by the F test.

(reaching 12,591.08 ± 2,491.68 mg kg\(^{-1}\) Cr in the root tissue), decaying at 0.32 mmol L\(^{-1}\) and rising again at 0.64 mmol L\(^{-1}\) (Figure 4).

The Chromium translocation index (TI) relationship with Cr rate could be fitted with a logarithmic model (Figure 5) and there were significant differences between treatments (\( P < 0.01 \)). Although remaining at very low levels, Cr TI increased along with Cr rate starting at 0.26 (±0.14)% under 0.04 mmol L\(^{-1}\) and reaching 2.53 (±0.16)% under 0.064 mmol L\(^{-1}\).

**Nutrition**

**Macronutrients**

Chromium affected differently the uptake and the translocation of mineral nutrients by the urograndis cuttings. Overall nitrogen concentration in
the shoot did not seem to respond to Cr rate ($P > 0.05$), remaining relatively stable at 45.80 ($\pm 1.98$) dag kg$^{-1}$ (Figure 6). However, N concentrations in stem and leaves were significantly affected by chromium ($P < 0.01$). In stem, it increased from 12.33 ($\pm 1.21$; control) to 17.03 ($\pm 0.40$) dag kg$^{-1}$ under 0.16 mmol L$^{-1}$ Cr, stabilizing from this point on (Figure 6). Conversely, leaf N concentration decreased from 34.53 ($\pm 1.99$; control) to 26.20 ($\pm 0.36$) dag kg$^{-1}$ at 0.16 mmol L$^{-1}$ Cr and stabilized from 0.32 to 0.064 mmol L$^{-1}$ Cr at approximately 29.43 ($\pm 0.55$) dag kg$^{-1}$ N, below leaf N concentration value in control (Figure 6). Nitrogen concentration in roots was also negatively affected by chromium ($P < 0.05$), decreasing from 27.90 ($\pm 6.22$; control) to 19.23 ($\pm 0.91$) dag kg$^{-1}$ under 0.16 mmol L$^{-1}$ Cr, falling again under 0.64 mmol L$^{-1}$ Cr, reaching 16.40 ($\pm 2.29$) dag kg$^{-1}$ (Figure 6).

Phosphorus concentration was significantly affected ($P < 0.01$) by chromium in all organs. In the roots, it consistently increased from 1.69 ($\pm 0.30$; control) to 2.49 ($\pm 0.35$) g kg$^{-1}$ under 0.08 mmol L$^{-1}$ Cr (Figure 6). A similar value was observed for 0.16 mmol L$^{-1}$ (2.51 $\pm 0.17$ g kg$^{-1}$), but under higher Cr rates root P concentration fell to approximately 1.81 ($\pm 0.04$) g kg$^{-1}$. In the shoot, P concentration could be fitted with the quadratic model (Figure 6), starting with 2.21 ($\pm 0.22$) g kg$^{-1}$ (control) and reaching a maximum of 3.67 ($\pm 0.27$) g kg$^{-1}$ under 0.32 mmol L$^{-1}$ Cr, approximately. Considering P concentrations in stem and leaves separately, although the pattern was not as clear as for shoot P, the highest values were also found at Cr rates superior to 0.00 mmol L$^{-1}$. Control stem P concentration was 0.86 ($\pm 0.04$) g kg$^{-1}$, which increased from 0.04 to 0.08 mmol L$^{-1}$ Cr reaching 1.89 ($\pm 0.12$) g kg$^{-1}$ and falling significantly only under 0.64 mmol L$^{-1}$ Cr to 1.68 ($\pm 0.09$) g kg$^{-1}$ (Figure 6). Leaf P remained relatively unaltered from 0.00 to 0.16 mmol L$^{-1}$ Cr at 1.35 ($\pm 0.18$) g kg$^{-1}$ increasing to 1.86 ($\pm 0.20$) g kg$^{-1}$ under 0.32 mmol L$^{-1}$ Cr (Figure 6).
Potassium concentration was significantly influenced ($P < 0.01$) by Cr rate only in the roots (Figure 6). It decreased from 9.40 ($\pm$ 0.92) g kg$^{-1}$ under 0.04 mmol L$^{-1}$ Cr to 4.20 ($\pm$ 1.20) g kg$^{-1}$ under 0.16 mmol L$^{-1}$ Cr. Under 0.64 mmol L$^{-1}$ Cr, K concentration was 4.00 $\pm$ 0.92 g kg$^{-1}$. Potassium
Concentrations in the other organs were 22.00 (±1.91), 15.17 (±1.82) and 6.83 (±0.68) g kg\(^{-1}\) for shoot, leaves and stem, respectively.

Chromium affected significantly \((P < 0.01)\) Ca concentration in all organs (Figure 7). Root Ca concentration response to Cr rate could be fitted with a quadratic model, where the highest value was observed for 0.00 mmol L\(^{-1}\) Cr \((11.36 ± 1.29\) g kg\(^{-1}\)) and the minimum \((4.10 ± 1.05\) g kg\(^{-1}\)) for 0.32 mmol L\(^{-1}\) Cr. In the other organs, Ca concentration could be fitted only with the cubic model (Figure 7), with inconsistent behavior from 0.00 to 0.16 mmol L\(^{-1}\) Cr and maximum value under 0.32 mmol L\(^{-1}\) Cr. Control Ca concentrations were 15.98 \((±0.95)\), 7.80 \((±0.84)\) and 8.19 \((±0.27)\) g kg\(^{-1}\) for shoot, leaves and stem, respectively. Accordingly, the maximum values were 25.48 \((±1.72)\), 12.94 \((±1.64)\) and 12.54 \((±1.04)\) g kg\(^{-1}\). Ca.

Overall shoot Mg concentration was not significantly affected by Cr \((P > 0.05)\), remaining at 2.60 \((±0.22)\) g kg\(^{-1}\) (Figure 7). However, considering stem and leaves separately, Mg concentration responded to Cr rate \((P < 0.05)\), increasing linearly in the latter from 1.99 \((±0.25)\) g kg\(^{-1}\), under 0.00 mmol L\(^{-1}\) Cr to 2.46 \((±0.08)\) g kg\(^{-1}\), under 0.64 mmol L\(^{-1}\) Cr. Stem Mg concentration could not be satisfactorily modeled, however it is possible to notice a smooth but significant decrease of approximately 0.09 g kg\(^{-1}\) over control \((0.47 ± 0.04\) g kg\(^{-1}\)) under 0.08 mmol L\(^{-1}\) Cr (Figure 7). Root Mg concentration response to Cr rate was significant \((P < 0.01)\) and could be fitted with the quadratic model, with minimum point at approximately 0.32 mmol L\(^{-1}\) Cr. The highest root Mg value \((1.57 ± 0.12\) g kg\(^{-1}\)) was observed for 0.00 mmol L\(^{-1}\) Cr.

Sulfur concentrations in shoot, stem and leaves were significantly influenced \((P < 0.01)\) by Cr exposure (Figure 7). Stem S response to Cr rate could be fitted with a quadratic model, increasing from 0.00 (corresponding to 0.74 ± 0.14 g kg\(^{-1}\) S) to the maximum point at approximately 0.32 mmol L\(^{-1}\) Cr, falling to 0.04 \((±0.02)\) mg kg\(^{-1}\) under 0.64 mmol L\(^{-1}\) Cr. Shoot and leaf S did not show such a defined pattern (Figure 7). The latter could be fitted only with the cubic model \((1.80 ± 0.17\) g kg\(^{-1}\) S under 0.00 mmol L\(^{-1}\) Cr, and maximum at 0.64 mmol L\(^{-1}\) Cr, with 2.36 ± 0.06 g kg\(^{-1}\) S) and the former did not fit satisfactorily any model tested, showing unpredictable behavior. Control shoot S concentration was 2.55 ± 0.30 g kg\(^{-1}\). Control root S concentration was 0.15 \((±0.02)\) g kg\(^{-1}\) increasing to unrealistically high values from 0.04 mmol L\(^{-1}\) Cr and above (data not shown), compromising regression analysis.

**Micronutrients**

Boron concentration in roots did not respond to Cr exposure \((P > 0.05)\), presenting a mean value of 35.09 \((±3.49)\) mg kg\(^{-1}\) among treatments (Figure 8). However, B concentration in the other organs was significantly affected by Cr \((P < 0.01)\). Shoot B followed a cubic model, decreasing from
**FIGURE 7** Tissue Ca, Mg and S concentration response to Cr rate. Vertical bars represent standard errors. * Significant at $P < 0.05$. ** Significant at $P < 0.01$. Ns: non-significant at $P < 0.05$. Nf: non-fitted.
70.42 (±1.46) to 58.12 (±6.53) mg kg⁻¹, through the 0.00 to 0.08 mmol L⁻¹ Cr interval, raising to 65.54 (±2.67) mg kg⁻¹ under 0.32 mmol L⁻¹ Cr and falling to 61.31 (±3.69) mg kg⁻¹ under the highest Cr rate (Figure 8). Leaf B did not fit any tested model, but it is possible to observe a consistent decrease...
through the 0.00 to 0.08 mmol L\textsuperscript{−1} Cr interval from 50.68 (±1.39) to 39.18 (±5.37) mg kg\textsuperscript{−1} (Figure 8). Subsequently, it increased at 0.16 mmol L\textsuperscript{−1} Cr, which was followed by another trend of decrease. Boron concentration in stem responded unpredictably to Cr rate, starting at 19.73 (±0.47) mg kg\textsuperscript{−1} (control) and reaching 21.14 (±2.47) mg kg\textsuperscript{−1} under 0.64 mmol L\textsuperscript{−1} Cr (Figure 8).

Chromium affected significantly \(P < 0.01\) Cu concentration in roots, which decreased steeply from 40.21 (±11.89) mg kg\textsuperscript{−1} under 0.00 mmol L\textsuperscript{−1} Cr to 20.76 (±2.92) mg kg\textsuperscript{−1} under 0.08 mmol L\textsuperscript{−1} Cr (Figure 8). Shoot Cu also responded to Cr rate, although less intensively \(P < 0.05\), with the highest value (18.14 ± 0.88 mg kg\textsuperscript{−1}) observed under 0.00 mmol L\textsuperscript{−1} Cr (Figure 8). Leaf Cu concentration response was very significant \(P < 0.01\), falling remarkably under 0.04 mmol L\textsuperscript{−1} Cr, from 11.66 (±1.19) to 7.89 (±2.29) mg kg\textsuperscript{−1}, but oscillating around this value as the Cr rate increased (Figure 8). Stem Cu was not significantly affect by chromium \(P > 0.05\), being 6.52 (±1.18) mg kg\textsuperscript{−1} the mean value among treatments (Figure 8).

Iron concentration in roots interacted significantly \(P < 0.01\) with Cr rate, but its behavior could not be modeled (Figure 8). It began at 629.37 (±13.62) mg kg\textsuperscript{−1} (control), fell to 579.50 (±2.89) mg kg\textsuperscript{−1} (0.04 mmol L\textsuperscript{−1} Cr) and raised to 610.59 (±15.52) mg kg\textsuperscript{−1} under 0.08 mmol L\textsuperscript{−1} Cr, which was followed by another decrease under 0.16 mmol L\textsuperscript{−1} Cr, remaining relatively regardless of the increase in the Cr rate. Leaf Fe was also influenced \(P < 0.05\) by Cr in the solution, but did not fit any tested model (Figure 8). It started with a steep increase from 80.71 (±10.20), under 0.00 mmol L\textsuperscript{−1} Cr, to 117.71 (±13.42) mg kg\textsuperscript{−1} Fe, under 0.08 mmol L\textsuperscript{−1} Cr, decreasing and increasing repeatedly with the increase in the Cr rate. Leaf Fe concentration under 0.064 mmol L\textsuperscript{−1} Cr was 94.77 (±14.48) mg kg\textsuperscript{−1}. Conversely, shoot and stem Fe concentration was not affect by Cr \(P > 0.05\), being 195.02 (±36.82) and 96.48 (±35.95) mg kg\textsuperscript{−1} Fe the mean values observed in shoot and stem, respectively (Figure 8).

Chromium influenced significantly \(P < 0.01\) manganese concentration in roots, but the interaction could not be modeled (Figure 9). Control root Mn was 69.85 (±14.26) mg kg\textsuperscript{−1}, which, after an increase to 80.79 (±26.04) mg kg\textsuperscript{−1} at 0.08 mmol L\textsuperscript{−1} Cr, fell to 28.19 (±9.55) mg kg\textsuperscript{−1} under 0.16 mmol L\textsuperscript{−1} Cr, remaining relatively stable as the Cr rate increased. Shoot and stem Mn responses to Cr rate were both significant, although at different significance levels \(P < 0.05\) for shoot Mn and \(p < 0.01\) for stem Mn), and could not be modeled either (Figure 9). After an increase from 0.00 (359.17 ± 26.42 mg kg\textsuperscript{−1} Mn in shoot and 83.28 ± 10.38 mg kg\textsuperscript{−1} Mn in stem) to 0.08 mmol L\textsuperscript{−1} Cr (484.95 ± 71.01 mg kg\textsuperscript{−1} Mn in shoot and 162.98 ± 41.35 mg kg\textsuperscript{−1} Mn in stem), they fell to the minimum values under 0.16 mmol L\textsuperscript{−1} Cr (428.66 ± 17.63 mg kg\textsuperscript{−1} Mn in shoot and 35.15 ± 11.51 Mn in stem), and raised again to intermediate values under 0.64 mmol L\textsuperscript{−1} Cr (462.12 ± 3.03 mg kg\textsuperscript{−1} Mn in shoot and 122.47 ± 5.46 mg kg\textsuperscript{−1} Mn
Chromium Toxicity in Urograndis Cuttings

FIGURE 9 Tissue Mn and Zn concentration response to Cr rate. Vertical bars represent standard errors. * Significant at $P < 0.05$. ** Significant at $P < 0.01$. Ns: non-significant at $P < 0.05$. Nf: non-fitted.

in stem). Leaf Mn interacted significantly with Cr ($P < 0.05$) and its behavior could be fitted with the quadratic model, increasing from 275.88 ($\pm$17.04) mg kg$^{-1}$ (control) to the maximum at approximately 0.32 mmol L$^{-1}$ Cr, corresponding to 346.13 ($\pm$10.64) mg kg$^{-1}$ Mn (Figure 9).

Cr remarkably affected zinc concentration in all organs at $P < 0.01$ (Figure 9). Leaf Zn relation with Cr could be modeled only by the cubic model (Figure 9), remaining relatively constant between 0.00 (29.82 $\pm$ 2.65 mg kg$^{-1}$ Zn) and 0.16 mmol L$^{-1}$ Cr (28.99 $\pm$ 1.54 mg kg$^{-1}$ Zn), increasing under 0.32 mmol L$^{-1}$ Cr (38.10 $\pm$ 3.39 mg kg$^{-1}$ Zn) and decreasing under 0.64 mmol L$^{-1}$ Cr (33.46 $\pm$ 2.35 mg kg$^{-1}$ Zn). Stem an shoot Zn response curves were similar, with a smooth decrease from 0.00 (12.29 $\pm$ 1.62 mg kg$^{-1}$ Zn in stem and 42.12 $\pm$ 4.22 mg kg$^{-1}$ Zn in shoot) to 0.04 mmol L$^{-1}$ Cr (11.58 $\pm$ 1.62 mg kg$^{-1}$ Zn in stem and 35.48 $\pm$ 3.91 mg kg$^{-1}$ Zn in shoot), followed by a steep increase under 0.08 mmol L$^{-1}$ Cr (25.21 $\pm$ 4.04 mg kg$^{-1}$ Zn in stem and 54.85 $\pm$ 7.62 mg kg$^{-1}$ Zn in shoot) and another decrease
under 0.16 mmol L\(^{-1}\) Cr (16.68 ± 3.94 mg kg\(^{-1}\) Zn in stem and 45.67 ± 2.61 mg kg\(^{-1}\) Zn in shoot), subsequently raising again to intermediate levels. Root Zn response to Cr could be fitted with the quadratic model, decreasing from 108.31 (±7.04) mg kg\(^{-1}\) (control) to a minimum of 45.77 (±4.93) mg kg\(^{-1}\) under 0.32 mmol L\(^{-1}\) (Figure 9). Under Cr rates superior to 0.16 mmol L\(^{-1}\), Zn concentration values were higher in shoots than in roots, inverting the translocation factor for this nutrient (Figure 9).

**DISCUSSION**

Wilting is a very characteristic symptom of chromium toxicity, occurring on a wide range of herbaceous and tree species (Turner and Rust, 1971; Poschenrieder and Barceló, 2004; Dube et al., 2009). Although little is known about the real effect of this metal on the water relations of higher plants, reduction of water potential and transpiration rate with increasing diffusive resistance have been frequently observed in Cr-intoxicated plants (Chatterjee and Chatterjee, 2000; Davies Jr. et al., 2002; Pandey and Sharma, 2003; Vernay et al., 2007; Gopal et al., 2009).

Plant growth inhibition by chromium is commonly reported as well. Toppi et al. (2002) observed growth inhibition by Cr in tomato, maize and cauliflower. Shanker et al. (2005) also cite papers reporting detrimental effects of Cr on the growth of oat, cucumber, lettuce, proso millet, white mustard and some tree species. This has been attributed to detrimental changes induced by Cr in root cell cycle, damaging DNA and inducing apoptosis (Le Bouffant et al., 2008), as well as oxidative damages caused by Cr toxicity to the photosynthetic and mitochondrial processes and related structures (Panda and Choudhury, 2005; Pandey et al., 2009).

Although shoot growth is also reduced in the presence of Cr, the effects on roots are usually more pronounced (Shah et al., 2008). This is due to the preferential accumulation of the element in the roots, with little translocation (see below). In addition, roots are more exposed to Cr than the other organs. The direct contact with Cr damages the root cell surface and induces plasmolysis (McGrath, 1995). Hence, isolating the exact causes of some effects caused by Cr toxicity is difficult, since root damages reduce plant ability to absorb water. The induced water stress, therefore, brings forth various problems, such as growth reduction and nutritional disorders. As revealed by the visual diagnosis, severe drought symptoms can be observed in cases of extreme exposure to Cr.

The critical toxicity levels (CTLs), which are higher for roots (Table 1), indicate that shoot growth is relatively more reduced than root growth under Cr toxicity. The CTLs are in accordance to Hossner et al. (1998), who mentioned that Cr is toxic to most agronomic plant species in the range of 0.5 to 5.0 \(\mu\)g mL\(^{-1}\) (0.01 to 0.10 mmol L\(^{-1}\)) in nutrient solution. Shah et al.
(2008), working with Cd and Cr(VI), observed growth reduction in *Dalbergia sissoo* seedlings under 0.20 mg L$^{-1}$ Cr (0.004 mmol L$^{-1}$) and suggested that this element might be more toxic than Cd to that species. Notice the higher toxic potential of Cr(VI) in comparison to Cr(III), used in this study.

Conversely, Soares et al. (2005) found Cd CTLs for *Eucalyptus maculata* and *E. urophylla* much lower than the Cr(III) CTLs estimated in this study, indicating that Cr(III) might not be as toxic to eucalyptus plantlets as Cd. The same might be inferred for Cu (Soares et al., 2000). Zinc, on the contrary, is much less toxic and has CTLs values higher for shoot than roots (Soares et al., 2001). In fact, the relationship between roots and shoot vulnerability to heavy metals is highly variable among plant species and element interactions. This makes species selection for contaminated sites remediation an arduous task, since there is no species suitable for every condition.

As observed for most non-hyperaccumulating plants (Shanker et al., 2005; Yu and Gu, 2007; Yu et al., 2008; Tiwari et al., 2009), Cr was poorly translocated to the aboveground organs of urograndis cuttings. The preferential order of accumulation was: root > stem > leaves. Chromium translocation index was even lower than those of Cd, Cu and Zn, observed in *E. maculata* and *E. urophylla* (Soares et al., 2000, 2001, 2005). This is due to Cr compartmentalization in the root vacuoles, as a defense against its toxicity, or its retention in the cation exchange sites of the vessel walls of xylem parenchyma cells in roots (Shanker et al., 2005). The portion that cannot be compartmentalized is eventually translocated to the shoot, but this process is poorly understood. It might be related to Cr chelation by organic acids, which would reduce its affinity for the binding sites in the root cell walls, making translocation possible (Jean et al., 2008).

The low Cr translocation within urograndis plants suggests that this hybrid might not be suitable for phytoextraction of chromium. But, since a considerable amount of Cr concentrated in stem (Figure 4), and considering the high biomass production of this tree, large amounts of Cr might be removed from the soil in the long-term. If the soil contamination level is not too prohibitive urograndis plantings might be used for the stabilization or even extraction of Cr and receive industrial waste fertilization. However, this assumption must be tested in field.

Chromium toxicity also affected urograndis nutrition. Most of the effects were, at least in part, result of root damage and inhibition of H$^+$-ATPase, which hampers nutrient and water uptake. In fact, with exception of P, S, B and Fe, all other nutrients analyzed had their concentrations in the root reduced as the Cr rate increased (Figures 6–9). However, the decrease in the concentrations of nutrients in the root was not always followed by a decrease in shoot nutrients, which showed a more unpredictable behavior.

Nitrogen concentration in shoot, for instance, was not affected by Cr (Figure 6). However, this happened because of the opposite behavior observed
between stem and leaf N: the latter reduced, while the former increased. Chromium hinders photosynthesis (Vernay et al., 2007; Liu et al., 2008) and affects N metabolism in plants, reducing the activity of key enzymes (Kumar and Joshi, 2008). Hence the higher N accumulation in the stem observed in the plants under the highest Cr rates might be due to decrease of leaf activity, consequently reducing the requirement for this nutrient. As no chlorosis was observed, the plants probably were not N-deficient, and the cause of early leaf senescence under the highest Cr rates can be attributed to water stress.

Phosphorus interaction with chromium requires further investigation. Although most studies report lower P concentration under Cr exposure (Shanker et al., 2005; Fozia et al., 2008); Dahiya et al. (2003) and Dube et al. (2003) found higher P content in wheat and citrullus, respectively, in the presence of Cr. This suggests that P plays an important role in plant tolerance to Cr and there may be some relationship between their uptake mechanisms. The great reduction in Zn uptake (Figure 9) might have affected P translocation as well (Marschner, 1995).

Although K concentration in roots was reduced in the presence of Cr, it was not affected in stem, leaf or shoot (Figure 6). This might be due to the high mobility of this nutrient within plant organs and tissues and its high specificity in its functions (Marschner, 1995). This did not happen with Ca, in which its concentration in shoot, stem and leaves increased remarkably under 0.32 and 0.64 mmol L\(^{-1}\) Cr (Figure 7). Since Ca, unlike K, cannot be easily remobilized within plants, this increase might be due to a concentration effect, i.e., the severe decrease in dry matter (Figure 2) artificially increased Ca concentration values in those organs. However, there is evidence that supports Cr effects in Ca distribution within organs and tissues (Shanker et al., 2005; Vernay et al., 2007; Zeng et al., 2010). The linear increase observed in Mg concentration in leaves (Figure 7) might be due to higher translocation of this nutrient from roots to leaves, in order to sustain the normal function of leaves under reduced Mg uptake resulted from Cr exposure (Zeng et al., 2010). The fact that no chlorosis, a typical symptom of Mg deficiency, was detected supports this assumption.

Sulfur uptake and translocation was strongly affected by Cr (Figure 7). During root material digestion, the extract color turned into deep yellow, which led the spectrophotometer to show unrealistically high values. Therefore, S in roots could not be satisfactorily determined by the method used in this study (turbidimetry). Nonetheless, there are strong evidences to infer that S concentration in root really increased along with Cr rate, as observed by Dube et al. (2003) in citrullus. This increase might be result of stimulated production of cysteine and GSH compounds in the presence of chromium (Schiavon et al., 2008). Therefore, sulfur would preferentially be retained in roots to alleviate Cr toxicity, as observed for other heavy metals (Santos et al., 2006). The increase in leaf S under 0.32 and 0.64 mmol L\(^{-1}\) Cr is
probably result of concentration effect, since S is not promptly remobilized among plant organs.

Boron does not seem to have competitive relationship with chromium regarding their uptake by plants. Nonetheless, Cr does interfere with B translocation (Figure 8). Moral et al. (1996) also found antagonistic effect between B and Cr in leaves of tomato, but synergistic relation in root. This might be due to lower B demand by leaves and, given the great role of this element in root integrity (Marschner, 1995), higher accumulation of B in roots may also be a means to reduce the negative effects of Cr in this organ.

It was possible to observe a competitive interaction between Cr and Cu uptake, since the concentrations of the latter in roots and leaves was remarkably reduced even in the lowest Cr rate tested (Figure 8). Liu et al. (2008) found similar results in green amaranth, but attributed this effect to accelerated efflux of Cu (along with Mn, Fe and Zn) from the plant. Competitive interaction was also found in tomato (Moral et al., 1996). Probably, these elements interact more deeply in plant metabolism, since both are involved in oxidative stress (Marschner, 1995; Panda, 2007). Perhaps, they also interact in lignin metabolism, given Cr affinity to this material (Dupont et al., 2004; Wu et al., 2008). Studies focused on Cr and Cu interaction, besides helping elucidating many aspects of their uptake and metabolism, would improve knowledge on phytoremediation of chromium-copper-arsenate-contaminated sites, in which these elements are often associated (Lebow and Foster, 2005).

The detrimental effects of Cr in chlorophyll content and photosynthesis (Panda and Choudhury, 2005) might explain its effect on Fe translocation (Figure 8). The increase in leaf Fe from control to 0.08 mmol L\(^{-1}\) cuttings may be due to higher Fe transfer to leaves in order to maintain their functions. However, as previously discussed, higher Cr rates might have impaired urograndis metabolism in many ways, contributing to variations in leaf Fe content. Lower Fe concentration in roots might be due to competition between Cr and Fe for transport binding or higher Fe efflux from the plants. In fact, Fe interaction with Cr seems to be dependent on the Cr source, i.e., Cr(VI) or Cr(III), since Cr might influence Fe nutrition from its uptake (affecting Fe reductase activity) to its role in photosynthesis (Dube et al., 2003; Gardea-Torresdey et al., 2004; Panda and Choudhury, 2005; Shanker et al., 2005; Liu et al., 2008; Aguilera et al., 2007). The way and the extent in which Cr interferes in those processes depend on its valence. Therefore, variations may occur between experimental conditions.

Like Fe and Mg, manganese concentration in the leaves of urograndis also increased in the presence of Cr (Figure 9), reinforcing the assumption that higher translocation of these nutrients occurs in order to maintain normal photosynthetic activity. This was also observed in citrullus (Dube et al., 2003) and green amaranth (Liu et al., 2008), but the effects of Cr on root Mn concentration were different: no effect on citrullus root Mn and reduction
on green amaranth root Mn. On the contrary, Vernay et al. (2007) observed increase of Mn content in both roots and leaves of perennial ryegrass. All of these results were obtained with Cr(VI). In the present study, reduction was observed only under 0.16 mmol L$^{-1}$ Cr or higher rates. Probably, there was no competition between Mn and Cr during uptake, and this reduction might be result of root damage.

Chromium effect on Zn nutrition is highly controversial (Dube et al., 2003; Shanker et al., 2005; Liu et al., 2008; Zeng et al., 2010). In urograndis, the steep decrease in root Zn, together with higher shoot Zn (Figure 9), is probably due to higher translocation of this nutrient even with lower Zn uptake. The reason why this occurred, however, cannot be affirmed, but it is possibly related to the Zn role in oxidative stress control (Marschner, 1995).

**CONCLUSIONS**

The main visual symptom of Cr toxicity in urograndis is wilting, with early leaf fall in the most severe cases. Both shoot and root growths are significantly impaired under certain levels of contamination, being 0.74 mg Cr kg$^{-1}$ dry leaf matter enough to cause a 10% reduction in shoot dry matter. The order of Cr accumulation in the different organs of the plants was: root > stem > leaves. Chromium concentration values in roots were about 100-fold higher than in shoot.

Chromium differently affects the uptake and the translocation of each nutrient. Most nutrients have their uptake reduced due to root damage. Nutrients mainly associated to photosynthesis processes (Mg, Fe and Mn) have their concentrations increased in the leaves of urograndis, which, in part, explains the absence of chlorosis in its leaves.

Given the low accumulation of Cr in urograndis shoot, it is unlikely that this hybrid will be successful in phytoextraction of Cr. But, since this species tolerates Cr to a certain level, and accumulates high amounts of the heavy metal in its roots, it might be useful in phytostabilization and may receive fertilization with industrial wastes containing Cr. A long-term field study is needed to confirm this assumption.

**REFERENCES**


Chromium Toxicity in Urograndis Cuttings


