

BEHAVIOR OF *Eucalyptus grandis* AND *E. cloeziana* SEEDLINGS GROWN IN ARSENIC-CONTAMINATED SOIL⁽¹⁾

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SUMMARY

Arsenic has been considered the most poisonous inorganic soil pollutant to living creatures. For this reason, the interest in phytoremediation species has been increasing in the last years. Particularly for the State of Minas Gerais, where areas of former mining activities are prone to the occurrence of acid drainage, the demand is great for suitable species to be used in the revegetation and “cleaning” of As-polluted areas. This study was carried out to evaluate the potential of seedlings of *Eucalyptus grandis* (Hill) Maiden and *E. cloeziana* F. Muell, for phytoremediation of As-polluted soils. Soil samples were incubated for a period of 15 days with different As (Na_2HAsO_4) doses (0, 50, 100, 200, and 400 mg dm⁻³). After 30 days of exposure the basal leaves of *E. cloeziana* plants exhibited purple spots with interveinal chlorosis, followed by necrosis and death of the apical bud at the 400 mg dm⁻³ dose. Increasing As doses in the soil reduced root and shoot dry matter, plant height and diameter in both species, although the reduction was more pronounced in *E. cloeziana* plants. In both species, As concentrations were highest in the root system; the highest root concentration was found in *E. cloeziana* plants (305.7 mg kg⁻¹) resulting from a dose of 400 mg dm⁻³. The highest As accumulation was observed in *E. grandis* plants, which was confirmed as a species with potential for As phytoextraction, tending to accumulate As in the root system and stem.

Index terms: phytoremediation, arsenate, phytotoxicity.

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RESUMO: *COMPORTAMENTO DE MUDAS DE E. grandis E. Eucalyptus cloeziana CULTIVADAS EM SOLO CONTAMINADO POR ARSÊNIO*

O arsênio (As) tem sido considerado o poluente inorgânico de solo mais tóxico para os seres vivos, razão pela qual o interesse por espécies indicadoras e fitorremediadoras tem aumentado nos últimos anos. Particularmente para o Estado de Minas Gerais, que apresenta áreas remanescentes de atividade mineradora sujeitas à ocorrência de drenagem ácida, existe grande demanda por espécies com potencial para serem utilizadas na revegetação e “limpeza” de substratos contaminados por esse metaloide. Este trabalho teve como objetivo avaliar o potencial de mudas de Eucalyptus grandis (Hill) Maiden e Eucalyptus cloeziana F. Muell para fitorremediação de solos contaminados por arsênio. Antes de receberem as mudas das duas espécies, amostras de solo foram incubadas por um período de 15 dias, com as seguintes doses de arsênio (Na_2HAsO_4): 0, 50, 100, 200 e 400 mg dm^{-3} de solo. Após 30 dias de exposição, as plantas de E. cloeziana submetidas à dose de 400 mg dm^{-3} exibiram pontuações arroxeadas com clorose intervalar de folhas basais, seguida de necrose e morte da gema apical. O aumento do teor de arsênio no solo reduziu significativamente a biomassa de raízes e parte aérea, a altura e o diâmetro de plantas de ambas as espécies, sendo esse efeito mais acentuado nas plantas de E. cloeziana. Os maiores teores de As nas plantas foram observados no sistema radicular, com maiores valores para E. cloeziana (305,7 mg kg^{-1}) na dose de 400 mg dm^{-3} , sendo o maior acúmulo de As observado nas plantas de E. grandis, que demonstrou ser uma espécie com potencial para fitoextração de arsênio, com tendência de acumulação no sistema radicular e no caule.

Termos de indexação: fitorremediação, arsenato, fitotoxidez.

INTRODUCTION

The biogeochemical cycling of trace elements and their natural flow in the biosphere have been affected by the increased impact of human activity on ecosystems (Kabata-Pendias & Pendias, 2001). Some elements such as Cd, Pb, Hg and As have no nutritional or physiological functions in living organisms, but are recognized as environmental pollutants due to the possibility of bioaccumulation in the food chain (Milton & Johnson, 1999).

The destruction of vegetation in contaminated areas increases land degradation due to water and wind erosion and the leaching of contaminants into groundwater. The recovery of these environments requires comprehensive studies of soil, vegetation and water (Accioly & Siqueira, 2000). With a view to reforestation, there is an interest in contamination-tolerant plants. Studies on the fitness of tree species for the rehabilitation of polluted areas, in particular in the case of As contamination, have attracted interest in view of the great contamination disasters in different countries in the world, causing the death of thousands of people and animals and serious health problems in humans (Chatterjee et al., 1995). In this sense, the phytostabilization of contaminated soils has been considered a possibility of reducing contaminant erosion and dispersion in the environment.

The use of As-accumulating plants seems a viable alternative to mitigate the serious problems generally caused by As-absorption and immobilization in plant

tissues. In the case of tree species, knowledge on absorption patterns, translocation and accumulation of metal ions along with the establishment of tolerance limits permit the development of techniques of phytoremediation of soils contaminated by these elements (Kahle, 1993).

This study was conducted with the aim of evaluating growth and As accumulation and distribution patterns in the different parts of *Eucalyptus grandis* (Hill) Maiden and *E. cloeziana* F. Muell seedlings, as well as the potential of these species for use in phytoremediation programs of As-contaminated soils.

MATERIALS AND METHODS

Eucalyptus grandis and *E. Cloeziana* seedlings were grown in tubetes on a substrate of vermiculite and sawdust. When the plantlets presented an average of two sets of two leaves (two months after sowing), they were transferred to greenhouse for another month for acclimatization before planting.

Subsurface samples of a Red Yellow Latosol (20–40 cm) from João Pinheiro-MG (Table 1) were harrowed, sieved ($\varnothing < 4$ mm) and received As doses in the form of Na_2HAsO_4 solutions at concentrations of 0, 50, 100, 200, and 400 mg dm^{-3} of soil. After 15 days of incubation with As, subsamples of 1.94 dm^{-3} of soil were placed in plastic pots.

In the preliminary test, the application of these As doses to samples of the same soil resulted in concentrations of 0.0, 12.8, 26.8 and 58.7 and 128.8 mg dm⁻³ of soil-available As (Mehlich-3), respectively. To control the As analysis method, a standard reference sample (As = 1.25 ± 0.15 mg kg⁻¹, GBW07603) was used, composed of leaves and twigs from shrubs grown in Zn and Pb mining areas in China, provided by the Institute of Geophysical and Geochemical Exploration-Langtang, China. The results obtained with this standard sample were within the reference range.

The addition of Na through the application of Na₂HAsO₄ was, respectively, 15.4, 30.8, 61.6 and 124.4 mg dm⁻³ for doses of 50, 100, 200, and 400 mg dm⁻³ of As. In individual tests with the species, toxicity effects were not detected in plants with equivalent doses of Na in the form of NaCl. In this test, performed with the same soil, the electrical conductivity in soil (soil-water ratio of 1:1, Camargo et al., 1986) ranged from 116 to 566 mS cm⁻¹.

Three months after germination, the seedlings were selected, considering the same pattern in relation to height and strength and transplanted to pots (one plant per pot). The experimental units were arranged in a randomized block design with three replications.

On the day of and 30 and 65 days after transplanting, solutions were applied containing concentrations of 100 mg dm⁻³ of N, 150 of P, 100 of K, 150 of Mg, 16 of S. Micronutrients were all applied at once (at transplanting) in solution, at doses of 0.81, 3.66, 4.00, 1.33, 0.15, and 1.56 mg dm⁻³ of B, Mn, Zn, Cu, Mo, and Fe, respectively (Alvarez V., 1974).

The seedlings were irrigated daily as required by the crop and the plant height and diameter determined on the day of transplanting and 30, 60 and 90 days after. At the end of the experimental period the total leaf area was calculated by comparing the leaf shape

drawn on paper with the weight of known areas of the same paper. With this information, the leaf area per plant was estimated by the ratio of the specific leaf area and total weight of the leaf dry matter (Pereira, 1987). After 90 days of As exposure the plants were collected and separated into young leaves (YL), intermediate leaves (IL), basal leaves (BL), stems (S), branches (Br) and roots (R). Plant roots were washed with tap water until complete removal of soil and then maintained for about 1 min in a 0.1 mol L⁻¹ HCl solution to remove the adsorbed at the root surface (Tu & Ma, 2003). Then the roots were washed several times with deionized water. To determine the dry weight, the different plant parts were dried (60–70 °C) to constant weight.

To determine the As concentration in the different plant parts, 1.00 g sub-samples of dry matter were finely ground and subjected to nitric-perchloric digestion 3:1 (Tedesco et al., 1995). Samples were taken to a digester with controlled temperature: initially 50 °C for about 30 min, 100 °C for 30 min and finally from 160 to 180 °C until complete digestion. The As contents in the plant extracts and available As in soil (Mehlich-3) were determined by atomic emission spectrometry with inductively coupled plasma in argon (ICP/OES).

The As doses which caused a reduction of 50 % of shoot dry matter were obtained by regression analysis, considering the shoot dry matter as function of applied As doses. The concentration of soil-available As that resulted in a reduction of 50 % of shoot dry matter (SAs 50 %) was estimated by the regression equations for soil-available As as a function of the applied As dose.

The root, shoot and total As contents were calculated based on the concentration and dry matter production. From these values, we calculated the As translocation rate (TR), according to the following equation (Abichequer & Bohnen, 1998):

Table 1. Chemical and physical properties of soil samples used in the experiment

Chemical characteristic											
pH H ₂ O	P-rem	As-rem	P ⁽¹⁾	K ⁽¹⁾	As-T ⁽²⁾	As ⁽³⁾	Ca ²⁺ ⁽⁴⁾	Mg ²⁺ ⁽⁴⁾	Al ³⁺ ⁽⁴⁾	H + Al ⁽⁶⁾	MO
	mg L ⁻¹		mg dm ⁻³			cmol _c dm ⁻³			g kg ⁻¹		
5.2	26.3	27.8	1.1	25	10.7	0.0	0.0	0.0	1.3	4.5	20.0
Physical characteristic											
Gravel ⁽⁶⁾	Thin Sand ⁽⁶⁾	Silt ⁽⁶⁾	Clay ⁽⁶⁾	Soil density ⁽⁷⁾		Textural class		Equiv. humidity ⁽⁷⁾			
%				kg dm ⁻³				kg dm ⁻³			
40	17	2	41	1.29		Clay-sandy		0.13			

⁽¹⁾ Mehlich I Extractor (Mehlich, 1984). ⁽²⁾ Total As by wet digestion (Huang et al., 1988). ⁽³⁾ Available As by Mehlich-3. ⁽⁴⁾ KCl 1 mol L⁻¹ Extractor. ⁽⁵⁾ Potential acidity at pH 7,0 extracted with 1 mol L⁻¹ calcium acetate ⁽⁶⁾ Pipette method (Embrapa, 1997). ⁽⁷⁾ Embrapa (1997); LVA: Red yellow Latosol; P-rem: remaining P and As-Rem: remaining As; as Alvarez V. et al. (2001) and Ribeiro Jr (2002), respectively.

$$TR (\%) = \frac{\text{Amount of accumulated As in shoots (leaves + stem)} \times 100}{\text{Amount of accumulated As in plants}}$$

RESULTS AND DISCUSSION

Toxicity symptoms, dry matter production and plant height

After the first week of incubation, the basal leaves of *E. cloeziana* plantlets exposed to doses of 400 mg dm⁻³ turned purple with paling, followed by necrosis (Figure 1), which are typical symptoms of As toxicity, observed in other crops as well (Melo et al., 2009).

On the other hand, *E. grandis* plantlets that appear to have As-absorption and translocation control mechanisms to shoot tissues, as well as not presenting toxicity symptoms of As, responded positively (growth) to lower As doses. That is, in relation to plants grown in soil without As application (control), a dose of 50 mg dm⁻³ of As resulted in an increase of 21 and 5 % shoot and root dry matter, respectively. For a dose of 100 mg dm⁻³ the increase was 8 and 6 % for shoots and roots, respectively. These results corroborate those reported by Tu & Ma (2002), which show an increase of up to 107 % in dry matter production in *Pteris vittata* plants when treated with doses of 0.67 mmol kg⁻¹ of arsenate. Possibly the increase in plant biomass of *E. grandis* is related to P availability, since arsenate competes with phosphate for adsorption sites on soil minerals, especially Fe and Al oxides and hydroxides. The higher As-sensitivity of *E. cloeziana* than of *E. grandis* could explain the absence of this effect for this species.

At the highest As dose shoot dry matter decreased in both species (Figure 2), but this reduction was more intense, when compared with the control treatment plants that had not received As application for *E. cloeziana* (84 %) than for *E. grandis* (31 %). The high reduction in biomass observed for *E. cloeziana* plantlets highlights once again the absence of mechanisms for greater As tolerance of this species.



Figure 1. Visual toxicity symptoms in the basal leaves of *E. cloeziana* seedlings after eight days of growth in soil samples treated with 400 mg dm⁻³ of As.

Root production was significantly reduced ($p \leq 0.01$) by increasing As doses in both *E. grandis* and *E. cloeziana*, adjusting to square root and linear equations, respectively (Figure 2). The impact of the toxic elements on root production depends on the plant sensitivity and intensity of contamination. Root production is an important indicator of phytostabilization in As-contaminated areas, since the root system protects soils against erosion, reduces leaching and

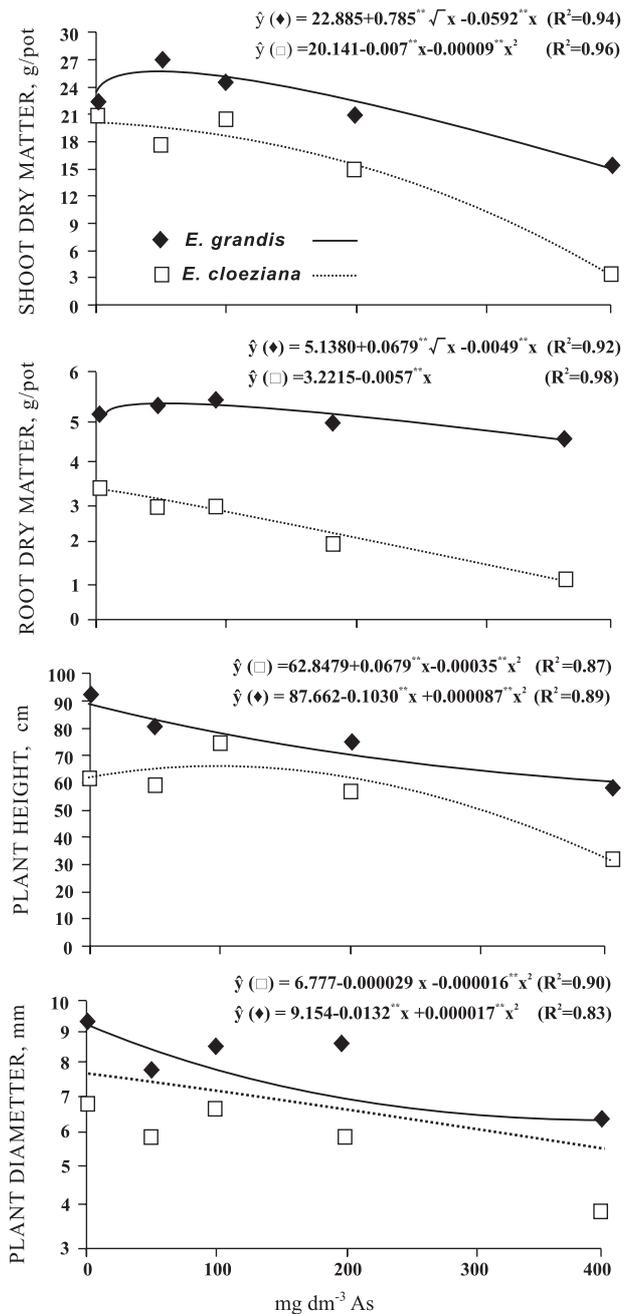


Figure 2. Shoot and root dry weight, height and diameter of *E. grandis* and *E. cloeziana* seedlings treated with As doses. * significant and ** significant effect at 5 and 1 % probability by the F test, respectively.

favors aggregation and microbial activity (Carneiro et al., 2002).

In both species plant height was significantly reduced ($p \leq 0.01$) by the As doses applied to the soil (Figure 2). Considering the plant height in the control treatment compared to those treated with doses of 400 mg dm^{-3} , reductions of 35 and 44 % for *E. grandis* and *E. cloeziana* were observed, respectively.

The increase of As doses in the soil also exerted a different effect on the diameter of the studied plants (Figure 3). In *E. grandis* plants the diameter was reduced by 28 % while in *E. cloeziana* plants it decreased by 72 % at a dose of 400 mg dm^{-3} compared to the control treatment plant. This marked reduction in the diameter of *E. cloeziana* plants indicates sensitivity to As.

For both species the As concentrations in the different analyzed plant structures increased with increasing doses applied to the soil (Table 2). Regression analysis resulted in fitted square root, quadratic and linear models (Table 3) for these increases. The different distribution of toxic elements in the plant root and shoot tissues is directly related to their absorption and translocation, as observed in plants of *Leucaena leucocephala* and *Sesbania virgata* (Melo, 2006) and may be directly related to tolerance mechanisms (Accioli & Smith, 2000).

The highest As concentrations were found in the root system, that is, in the order of $222.37 \text{ mg kg}^{-1}$ for *E. grandis* and $305.72 \text{ mg kg}^{-1}$ for *E. cloeziana*. Despite the higher concentrations found in the root system of *E. cloeziana* plants, the concentration of only 5.75 mg kg^{-1} in the basal leaves was sufficient to promote the appearance of some symptoms. Concentrations between 0.1 and 5.0 mg kg^{-1} shoot dry

matter are considered toxic to plants (Wauchope, 1983).

The calculation of the bioconcentration index (BI) clearly indicates the ability to compartmentalize As in the roots. This index is computed by the ratio of the root As concentrations and As availability in the soil (Tu et al., 2002). The highest BI values for roots were observed in *E. grandis* at a dose of 100 mg kg^{-1} of As (12.31) and of 50 mg kg^{-1} of As (6.00) for *E. cloeziana*. Values above 1.0 indicate As accumulation capacity by the plants.

The *E. grandis* plants tended to translocate and accumulate As in the stem, with concentrations that were up to 13 times higher than As concentrations in non-accumulating plants [considering the limit of 5 mg kg^{-1} suggested by Wauchope (1983)]. Some species admittedly known as As hyperaccumulators can accumulate high concentrations of metals reaching more than 1000 times the normal concentration, accumulated by most species (Wauchope, 1983).

Hyperaccumulator plants tolerate and accumulate large amounts of metals in their tissues (Baker, 1987); they can accumulate concentrations exceeding 1000 mg kg^{-1} of As in the dry matter, such as *Pteris vittata* and *Pityrogramma calomelanos*, in which As concentrations reach, respectively, 23,000 and $8,350 \text{ mg kg}^{-1}$ (Ma et al., 2001; Francesconi et al., 2002).

In *E. grandis* plants the total As accumulation ranged from 0.39 to 2.46 mg/plant , indicating different behavior among species (Table 2). The plants had higher relative As shoot contents, showing the ability of As translocation, immobilized mainly in the stem, as pointed out before. Species with these characteristics can facilitate the process of As phytoextraction, preventing the entry into the food chain, capitalizing

Table 2. As concentrations in young leaves (FJ), intermediate (FI), baseline (FB), branches (Ra), stem (C) and roots (R) and content of the shoot (PA), roots (R) and total (T) plants in *E. grandis* and *E. cloeziana* according to arsenic doses applied to the soil

Species	Doses	Concentrations						Contents		
		FJ	FI	FB	Ra	C	R	PA	R	T
	mg dm^{-3}	mg kg^{-1}						mg/plant		
<i>E. grandis</i>	0	nd	nd	nd	nd	nd	nd	nd	nd	nd
	50	0.55	1.25	1.95	0.59	1.00	21.97	0.14	0.12	0.39
	100	0.99	2.39	1.03	1.34	1.39	99.13	0.26	0.54	0.80
	200	1.23	4.03	11.88	2.42	6.61	159.70	0.55	0.79	1.34
	400	2.69	8.53	11.06	6.73	64.13	222.37	1.44	1.02	2.46
	CV (%)	8.73	8.72	2.77	4.06	4.06	1.38	15.48	5.50	5.39
<i>E. cloeziana</i>	0	nd	nd	nd	nd	nd	nd	nd	nd	nd
	50	0.82	1.78	1.83	1.38	1.07	17.47	0.12	0.05	0.17
	100	1.19	2.44	3.93	1.87	2.64	44.85	0.25	0.13	0.37
	200	2.93	3.30	6.66	3.21	3.62	152.56	0.29	0.29	0.58
	400	2.82	3.01	5.75	0.86	36.20	305.72	0.16	0.30	0.46
	CV (%)	4.62	7.27	5.77	9.27	10.36	2.73	4.65	5.16	3.97

nd: concentrations below the detection limit (0.02 mg kg^{-1}) by the determination method (ICP/AES).

on the fact that the economic value of the stem (wood) is high and can be used in industry for different purposes, allowing the detention. However, studies should be conducted to understand the behavior of this species under higher As doses than used in this test and for a longer exposure time. Although *E. grandis* is no hyperaccumulator, it may have advantages over hyperaccumulator species such as *Pteris vittata*, due to the high economic value of timber and non-metal immobilization in the plant compartment.

The ability of some eucalyptus species to tolerate high As doses in the soil indicates a potential use of these species in phytoremediation programs. Relatively high As concentrations in soil and water did not affect the growth of trees of *Tamarix parviflora* and *Eucalyptus camaldulensis* (Schoebi, 2005).

There was an increase of the ratio root/shoot (R/PA) with increasing doses in both species, indicating that the effect of As on the production is more harmful at higher concentrations (Figure 3). The dry root is not necessarily indicative of the total absorption, because changes in root architecture can occur without causing changes to this feature (Hodge, 2004). The total leaf area varied among species: The doses reduced the leaf area of *E. grandis* to a lesser extent than *E. cloeziana* (Figure 3).

Translocation index and critical arsenic levels in the soil

The translocation index of As varied among the studied species, with highly significant dose effects

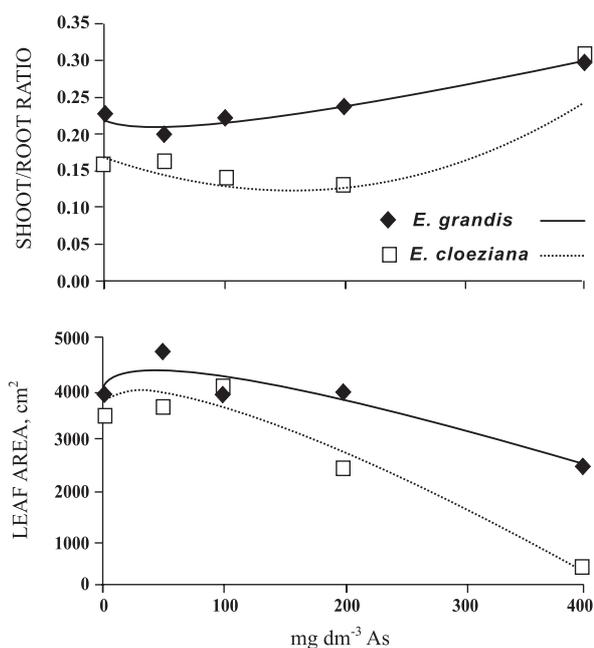


Figure 3 Shoot/root ratio and leaf area of *E. grandis* and *E. cloeziana* seedlings as related to the As dose applied to the soil.

($p \leq 0.01$), adjusting to square root and quadratic models (Figure 4). The highest translocation index (TI) was obtained for *E. cloeziana* at a dose of 50 mg dm⁻³ (70.8 %). The reduction in TI with increasing As doses for this species appears to be due to tolerance

Table 3. Regression equations for As concentrations in young leaves (YL), intermediate (IL), baseline (BL), branches (B), stem (S) roots (R), content of As in shoots (CAsS), and roots (CAsR) and total content (TAsC) in *E. Grandis* and *E. Cloeziana* plants and available As in soil samples (AAs), depending on the arsenic dose applied to the soil

Species	Variables	Regression equation	R ²
<i>E. grandis</i>	YL ⁽²⁾	$\hat{y} = 0.1479 + 0.0063^{**}x^{(1)}$	0.973
	IL ⁽²⁾	$\hat{y} = 0.1059 + 0.0289^{**}x$	0.997
	BL ⁽²⁾	$\hat{y} = -1.1373 + 0.08238^{**}x - 0.00013^{**}x^2$	0.997
	B ⁽²⁾	$\hat{y} = 0.0932 + 0.0084^{**}x + 0.00020^{**}x^2$	0.994
	S ⁽²⁾	$\hat{y} = 2.0844 - 0.0923^{**}x + 0.00064^{**}x^2$	0.994
	R ⁽²⁾	$\hat{y} = -0.5483 + 1.0696^{**}x - 0.00128^{**}x^2$	0.998
	AAs ⁽³⁾	$\hat{y} = -1.8169 + 0.08635^{**}x + 0.000437^{**}x^2$	0.997
	CAs S ⁽⁴⁾	$\hat{y} = -0.06034 + 0.00359^{**}x$	0.984
	CAsR ⁽⁴⁾	$\hat{y} = 0.00449 + 0.0056^{**}x - 0.000008^{**}x^2$	0.994
	TAsC ⁽⁴⁾	$\hat{y} = 0.0951 + 0.0060^{**}x$	0.993
<i>E. cloeziana</i>	YL	$\hat{y} = -0.1143 + 0.02033^{**}x - 0.000032^{**}x^2$	0.962
	IL	$\hat{y} = 0.2061 + 0.0256^{**}x - 0.000047^{**}x^2$	0.964
	BL	$\hat{y} = -0.2339 + 0.02183^{**}x - 0.000092^{**}x^2$	0.993
	B	$\hat{y} = -0.0195 + 0.0284^{**}x - 0.000065^{**}x^2$	0.976
	S	$\hat{y} = 1.4743 - 0.04328^{**}x + 0.00032^{**}x^2$	0.986
	R	$\hat{y} = -10.511 + 0.6841^{**}x + 0.000283^{**}x^2$	0.990
	AAs	$\hat{y} = -9.4941 + 0.2805^{**}x$	0.975
	CAsPA	$\hat{y} = 0.0067 + 0.0027^{**}x - 0.000006^{**}x^2$	0.975
	CAsR	$\hat{y} = -0.0231 + 0.002036^{**}x - 0.00003^{**}x^2$	0.970
	TAsC	$\hat{y} = -0.0165 + 0.0047^{**}x - 0.000009^{**}x^2$	0.995

⁽¹⁾ x: Arsenic doses applied to the soil (mg dm⁻³). ⁽²⁾ Arsenic concentrations (mg kg⁻¹). ⁽³⁾ Soil-available As (Mehlich-3) at the end of the experiment (harvest). ⁽⁴⁾ Arsenic content (mg/pot). ** and *: significant at 1 and 5 % by F test, respectively.

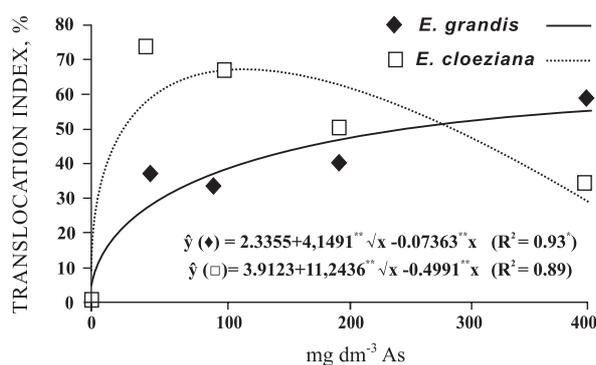


Figure 4. Translocation index of As in plants of *E. grandis*, *E. cloeziana* as a function of the As dose applied to the soil. ** and *: significant at 1 and 5 %, respectively, by the F test.

mechanisms of the species, in order to reduce and/or prevent the toxic effect of this shoot.

For *E. grandis* plants there was an increase in TI, with increasing As doses, by adjusting the square root positive model, reaching a TI of 58 % at the highest As dose applied to the soil. This behavior suggests the existence of an effective control mechanism in the translocation to the shoot, without resulting in toxicity symptoms in leaves. This behavior characterizes the greater tolerance of *E. grandis* than of *E. cloeziana*.

The differential behavior between species can have a genetic or physiological basis, such as high root concentration of phytochelatins, capable of complexing and transporting As to the vacuole and thus avoiding plant toxicity (Ma et al., 2001).

The concentration of soil-available As to reduce 50 % of dry matter (SAs 50 %) varied among species (Table 4). The SAs 50 % shoot differed among species, ranging between 156.15 and 92.44 mg dm⁻³ of soil. These values are well above the As concentration in uncontaminated soil, which is usually below 10 mg kg⁻¹ (Adriano, 2001), but can reach up to 30,000 mg kg⁻¹ in contaminated soils (Vaughan, 1993).

In *E. grandis*, SAs 50 % was higher in all compartments, which reinforces the greater As tolerance than of *E. cloeziana*. The values of SAs 50 % are around 13.01 (shoot), 25.63 (roots) and 14.02 (total) times the critical limit of soil-available As (12 mg kg⁻¹) to start remedial action (Chen et al., 2001; Davis et al., 2001).

CONCLUSIONS

1. *E. grandis* plants showed higher tolerance to As, growing satisfactorily in soil containing 400 mg dm⁻³ of As. This dose resulted in average concentrations of 128.8 mg dm⁻³ of available As in the

Table 4. The concentration of available As (Mehlich-3) in soil (SAs50 %) causing reductions of 50 % in the root, shoot and total biomass of *E. grandis* and *E. cloeziana* seedlings grown in soil samples treated with As doses

SAs50 % ⁽¹⁾	<i>E. grandis</i> <i>E. cloeziana</i>	
	mg dm ⁻³	
Shoot	156.2	92.4
Root	307.6	86.7
Total	168.3	91.5

⁽¹⁾The content in the soil to reduce the dry matter by 50 %.

soil leading to no visual toxicity symptoms on the plant leaves.

2. In both species, As accumulation was higher in the root system than in stems and branches and higher in plants of *E. grandis* than of *E. cloeziana*.

3. The highest translocation capacity of As from roots to shoots observed in the *E. grandis* plants suggests the potential of this species for programs of As phytoextraction.

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LITERATURE CITED

- ABICHEQUER, A.D. & BOHNEN, H. Eficiência de absorção, translocação e utilização de fósforo por variedades de trigo. R. Bras. Ci. Solo, 22:21-26, 1998.
- ACCIOLY, A.M.A. & SIQUEIRA, J.O. Contaminação química e biorremediação do solo. In: NOVAIS, R.F.; ALVAREZ V., V.H.; SCHAEFER, C.E.G.R., eds. Tópicos em ciência do solo. Viçosa, MG, Sociedade Brasileira de Ciência do Solo, 2000. p.299-352.
- ADRIANO, D.C. Trace elements in the terrestrial environment. New York, Springer, 2001. 867p.
- ALVAREZ V., H.V. Equilíbrio de formas disponíveis de fósforo e enxofre em dois Latossolos de Minas Gerais. Viçosa, MG, Universidade Federal de Viçosa, 1974. 125p. (Tese de Mestrado)

- ALVAREZ V., V.H.; DIAS, L.E.; RIBEIRO JR., E.S.; SOUZA, R.B. & FONSECA, C.A. Métodos de análises de enxofre em solos e plantas. Viçosa, MG, Universidade Federal de Viçosa, 2001. 131p.
- BAKER, A.J.M. Metal tolerance. *New Phytol.*, 106:93-111, 1987.
- CAMARGO, O.A.; MONIZ, A.C.; JORGE, J.A. & VALADARES, J.M.A.S. Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas. Campinas, Instituto Agronômico de Campinas, 1986. 93p. (Boletim Técnico, 106)
- CARNEIRO, M.A.C.; SIQUEIRA, J.O. & MOREIRA, F.M.S. Comportamento de espécies herbáceas em misturas de solo com diferentes graus de contaminação com metais pesados. *Pesq. Agropec. Bras.*, 37:1629-1638, 2002.
- CHATTERJEE, A.; DAS, D.; MANDAL, B.K.; CHOWDHURY, T.R.; SAMANTA, G. & CHAKRABORTI, D. Arsenic in groundwater in six districts of West Bengal, India: The biggest arsenic calamity in the world. Part I: Arsenic species in drinking water and urine of affected people. *Analyst*, 120:643-650, 1995.
- CHEN, M.; MA, L.Q.; HOOGEWEG, C.G. & HARRIS, W.G. Arsenic background concentrations in Florida, U.S.A. surface soils: Determination and interpretation. *Environ. Forensics*, 2:117-126, 2001.
- DAVIS, A.; SHERWIN, D.; DITMARS, R. & HOENKE, K.A. An analysis of soil arsenic records of decision. *Environ. Sci. Technol.*, 35:2401-2406, 2001.
- EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Centro Nacional de Pesquisa de Solos. Manual de métodos de análise de solos. 2.ed. Rio de Janeiro, 1997. 212p.
- FRANCESCONI, K.; VISOOTTIVISETH, P.; SRIDOKCHAN, W. & GOESSLER, W. Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: A potential phytoremediator of arsenic-contaminated soils. *Sci. Total Environ.*, 284:27-35, 2002.
- HODGE, A. The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytol.*, 162:9-24, 2004.
- HUANG, J.; GOLTZ, D. & SMITH, F. A microwave dissolution technique for the determination of arsenic in soils. *Talanta*, 35:907-908, 1988.
- KABATA-PENDIAS, A. & PENDIAS, H. Trace elements in soils and plants. Boca Raton, CRC Press, 2001. 413p.
- KAHLE, H. Response of roots of trees to heavy metals. *Environ. Exper. Bot.*, 33:99-119, 1993.
- MA, L.Q.; KOMAR, K.M.; TU, C.; ZHANG, W.; CAI, Y. & KENNELLEY, E.D. A fern that hyperaccumulates arsenic. *Nature*, 409:579, 2001.
- MEHLICH, A. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractants. *Comm. Soil Sci. Plant Anal.*, 15:1409-1416, 1984.
- MELO, R.F. Potencial de espécies vegetais para fitorremediação de um solo contaminado por arsênio. Viçosa, MG, Universidade Federal de Viçosa, 2006. 107p. (Tese de Doutorado)
- MELO, R.F.; DIAS, L.E.; MELLO, J.W.V. & OLIVEIRA, J.A. Potencial de quatro espécies herbáceas forrageiras para fitorremediação de solo contaminado por arsênio. *R. Bras. Ci. Solo*, 33:455-465, 2009.
- MILTON, A. & JOHNSON, M. Arsenic in the food chains of a revegetated metalliferous mine tailings pond. *Chemosphere*, 39:765-779, 1999.
- PEREIRA, A.R. Estimativa da área foliar em milharal. *Bragantia*, 46:147-150, 1987.
- RIBEIRO JR, E.S. Adsorção e dessorção de arsênio em solos e substratos de mineração de ouro e práticas de mitigação de drenagem ácida em colunas de lixiviação. Viçosa, MG, Universidade Federal de Viçosa, 2002. 117p. (Tese de Doutorado)
- TEDESCO, M.J.; GIANELLO, C.; BISSANI, C.A.; BOHNEN, H. & VOLKWEISS, S.J. Análise de solo, plantas e outros materiais. Porto Alegre, Universidade Federal do Rio Grande do Sul, 1995. 174p.
- TU, C. & MA, L.Q. Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. *J. Environ. Qual.*, 31:641-647, 2002.
- TU, C. & MA, L.Q. Effects of arsenate and phosphate on their accumulation by an arsenic-hyperaccumulator *Pteris vittata* L. *Plant Soil*, 249:373-382, 2003.
- SCHOEBI, K. Plants like to be poisoned. Checkbiotech. Thursday, March 10, 2005 Disponível em: <www.checkbiotech.org>. Acesso em 20 jul., 2006.
- VAUGHAN, G.T. The environmental chemistry and fate of arsenical pesticides in cattle tick dip sites and banana land plantations. Melbourne, CSIRO Division of Coal Industry, Center for Advanced Analytical Chemistry, 1993.
- WAUCHOPE. R.D. Uptake, translocation and phytotoxicity of arsenic in plants. In: LEDERER, W.H. & FENSTERHEIM, R.J., eds. Arsenic: Industrial, biomedical, environmental perspectives. New York, Van Nostrand Reinhold, 1983. p.348-375.