

# Behavior of *Eucalyptus urophylla* and *Eucalyptus citriodora* Seedlings Grown in Soil Contaminated by Arsenate

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**ABSTRACT:** Persistent areas of tailings and deposits from coal and gold mining may present high levels of arsenic (As), mainly in the arsenate form, endangering the environment and human health. The establishment of vegetation cover is a key step to reclaiming these environments. Thus, this study aimed to evaluate the potential of *Eucalyptus urophylla* and *E. citriodora* seedlings for use in phytoremediation programs of arsenate-contaminated areas. Soil samples were incubated at increasing rates (0, 50, 100, 200 and 400 mg dm<sup>-3</sup>) of arsenic (arsenate form, using Na<sub>2</sub>HAsO<sub>4</sub>) for 15 days. The seedlings were produced in a substrate (vermiculite + sawdust) and were transplanted to the pots with soil three months after seed germination. The values of plant height and diameter were taken during transplanting and 30, 60 and 90 days after transplanting. In the last evaluation, the total leaf area and biomass of shoots and roots were also determined. The values of available As in soil which caused a 50 % dry matter reduction (TS<sub>50%</sub>), the As translocation index (TI) from the roots to the shoot of the plants, and its bioconcentration factor (BF) were also calculated. Higher levels of arsenate in the soil significantly reduced the dry matter production of roots and shoots and the height of both species, most notably in *E. urophylla* plants. The highest levels of As were found in the root, with higher values for *E. citriodora* (ranging from 253.86 to 400 mg dm<sup>-3</sup>). The TI and BF were also reduced with As doses, but the values found in *E. citriodora* were significantly higher than in *E. urophylla*. *E. citriodora* plants presented a higher capacity to tolerate As and translocate it to the shoot than *E. urophylla*. Although these species cannot be considered as hyperaccumulators of As, *E. citriodora* presented the potential to be used in phytoremediation programs in arsenate-contaminated areas due to the long-term growth period of this species.

**Keywords:** phytoremediation, phytotoxicity, revegetation.

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## INTRODUCTION

Arsenic is a highly toxic, accumulative and carcinogenic element widely distributed in the biosphere (Singh et al., 2015). It usually originates geogenically, but deposition can be intensified by human activities such as the application of pesticides and wood preservatives, mining and smelting operations, and coal combustion (Wang and Mulligan, 2006). In Brazil, soils contaminated with arsenic are commonly found in mining areas of coal, gold, copper and uranium, usually associated to metallic sulfides such as arsenopyrite (Silva, 1997; Cotta et al., 2006). In soils, where there is normally an aerobic environment, arsenates ( $\text{As}^{5+}$ ) are the stable species and are strongly sorbed onto clays, iron and manganese oxides/hydroxides and organic matter (Mandal and Suzuki, 2002; Komárek et al., 2013).

The revegetation of substrates and soils contaminated by As is hindered by the lack of information about tolerant or phytoremediating species. Therefore, the selection of As-tolerant plants for the revegetation of contaminated soils and substrates is very important to the initial process of environmental restoration. Phytoremediation is an eco-friendly technique that uses plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment (Greipsson, 2011; Ali et al., 2013). Naturally occurring heavy metal hyperaccumulator plants that, when grown in metal-enriched habitats, can accumulate 100-1,000  $\text{mg kg}^{-1}$  fold higher levels of metal than normal plants are very susceptible to phytoextraction (Rascio and Navari-Izzo, 2010).

Considering the relevance of As as soil and water contaminant, there are few studies available in the literature evaluating the potential of tree species for the phytoremediation or revegetation of contaminated areas. Most studies in the literature are related the excellent potential of some ferns species to be used as As-hyperaccumulators (Ma et al., 2001; Zhao et al., 2009; Singhi et al., 2010). With regard to tree species, knowledge about the pattern of absorption, translocation and accumulation of metal ions, with the establishment of tolerance limits, the development of techniques for the phytoremediation of areas contaminated by these elements is allowed (Kahle, 1993). In this context, "in situ" application technologies with the use of plants for immobilization and/or stabilization are more attractive in terms of cost, compared to other "ex situ" physical and chemical remediation techniques (Vithanage et al., 2012).

The hypothesis is that some *Eucalyptus* species have potential for use as phytoremediation arsenic-contaminated soils. Although these species are not hyperaccumulators, they produce a large biomass and so can remove large quantities of soil arsenate. The present work aimed to evaluate the behavior of *Eucalyptus urophylla* S.T. Black and *Eucalyptus citriodora* Hill seedlings cultivated in soil contaminated with arsenate and the potential use of these species in phytoremediation programs of arsenic-contaminated soils.

## MATERIALS AND METHODS

Subsurface samples of a Oxisol (*Latossolo Vermelho-Amarelo*) (Santos et al., 2013) (0.20-0.40 m) from the municipality of João Pinheiro, MG (Table 1) were crumbled, passed through sieves (<4 mm) and amended with 0, 50, 100, 200 and 400  $\text{mg dm}^{-3}$  of As (arsenate form, using  $\text{Na}_2\text{HAsO}_4$ ). Fifteen days after incubation with As, 1.94  $\text{dm}^3$  of those samples were placed in plastic pots. The As rates resulted in related solutions were 0.0, 12.8, 26.8, 58.7 and 128.8  $\text{mg dm}^{-3}$  of available As (Mehlich-3), respectively, for the rates applied, according to the equation curve  $\hat{y} = -3.3525 + 0.3520 x$ ,  $R^2 = 0.997$ .

Seedlings of *Eucalyptus urophylla* and *Eucalyptus citriodora* were produced in tubettes in a substrate containing vermiculite + sawdust. When the seedlings presented, on average, two pairs of fully-expanded leaves (two months after sowing), they were transferred to a greenhouse for about one month for acclimatization before being transferred to pots.

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

Property	Value
pH(H <sub>2</sub> O)	5.2
P-rem (mg L <sup>-1</sup> )	26.3
As-rem (mg L <sup>-1</sup> )	27.8
P (mg dm <sup>-3</sup> )	1.1
K (mg dm <sup>-3</sup> )	25.0
As-T mg dm <sup>-3</sup> )	10.7
As (mg dm <sup>-3</sup> )	0.0
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.04
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.01
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	1.3
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> )	4.5
Organic matter (g kg <sup>-1</sup> )	20.0
Gravel (g kg <sup>-1</sup> )	400
Sand (g kg <sup>-1</sup> )	170
Silt (g kg <sup>-1</sup> )	20
Clay (g kg <sup>-1</sup> )	410
Bulk density (Mg m <sup>-3</sup> )	1.29
Textural class	Clay-sandy
Moisture equivalente (kg kg <sup>-1</sup> )	0.13

pH in water, relation 1:2.5, v/v; P-rem: remaining phosphorus (Alvarez V et al., 2000); As-rem: remaining arsenic (Ribeiro Jr., 2000); P and K: extractor Mehlich-1; As-T: total arsenic (EPA, 1996); As: extractor Mehlich-3; Ca<sup>2+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>: extractor 1 mol L<sup>-1</sup> KCl; H+Al: extractor 0.5 mol L<sup>-1</sup> calcium acetate at pH 7.0; Organic matter (Walkley-Blake method); Gravel, sand, silt, clay: pipette method. BD: bulk density, and Moisture equivalent (Donagema et al., 2011).

Since the As source used contained Na, the Na contents in the soil were 15.4, 30.8, 61.6 and 124.4 mg dm<sup>-3</sup>, respectively for the 50, 100, 200 and 400 mg dm<sup>-3</sup> As standards. In a separate test with these species, no toxicity effects were detected in the plants with the equivalent standards of Na, in the form of NaCl. In this test, carried out with the same soil, the soil electrical conductivity (soil-water of 1:1) ranged from 116 to 566 µS cm<sup>-1</sup>.

Three months after germination, the seedlings were standardized for height and vigor and then transplanted to constitute the experimental units (one plant per pot) which were arranged in a randomized complete block design with three replications.

On the day of the transplanting and 30 and 65 days after this procedure, the application of nutrient solution was carried out to attain the following final concentrations: 100 mg dm<sup>-3</sup> N; 150 mg dm<sup>-3</sup> P; 100 mg dm<sup>-3</sup> K; 150 mg dm<sup>-3</sup> Mg; and 16 mg kg<sup>-1</sup> S. The micronutrients were applied only once (during transplanting) in the form of solutions at concentrations of 0.81, 3.66, 4.00, 1.33, 0.15 and 1.56 mg dm<sup>-3</sup> of B, Mn, Zn, Cu, Mo and Fe, respectively.

The values of plant height and diameter were taken during transplanting and 30, 60 and 90 days after this. In the last evaluation, the total leaf area was determined by comparing the contour of leaf blades drawn on a paper sheet with the weight of known areas of the same paper. With these data, the leaf area per plant was estimated from the ratio between the specific leaf area and dry leaf matter total weight. After 90 days of exposure to As, the plants were collected and classified into young leaves (YL), intermediate leaves (IL), basal leaves (BL), stem (S), branches (B) and roots (R). The roots were washed with tap water until complete soil removal. After this, they remained for approximately 1.0 min in a HCl 0.1 mol L<sup>-1</sup> solution for the removal of the As superficially adsorbed to the roots (Tu and Ma, 2003). Then, the roots were washed several times with deionized water. To determine the dry matter weight, the different parts of the plant were dried at 60-70 °C to a constant weight.

To determine the concentration of As in the different parts of the plants, 1.00 g of finely ground dry matter samples were submitted to nitric perchloric digestion 3:1. The determination of the As contents in the plant extracts and the As available in the soil (Mehlich-3) was performed by ICP-OES. The reference standard sample GBW07603 was used to standardize the method of As analysis. That sample was composed of branches and leaves from shrubs grown in Zn and Pb mining areas of China, provided by the Institute of Geophysical and Geochemical Exploration, Langtang, China.

Regression analysis, plotting, shoot and root dry matter against the As concentrations applied, determined the As rates which caused a 50 % dry matter reduction in relation to the control treatment. By replacing these values in the regression equations by the As contents available in the soil, according to the As standards applied, it was possible to estimate the available soil As ( $TS_{50\%}$ ) which caused a 50 % reduction in dry matter.

The As translocation index (TI) from the root to the shoot of the plants and its bioconcentration factor (BF) were also calculated. In the present work, BF was calculated based on the ratio between the As concentration in the plant tissues and the available soil As (Mehlich-3).

## RESULTS AND DISCUSSION

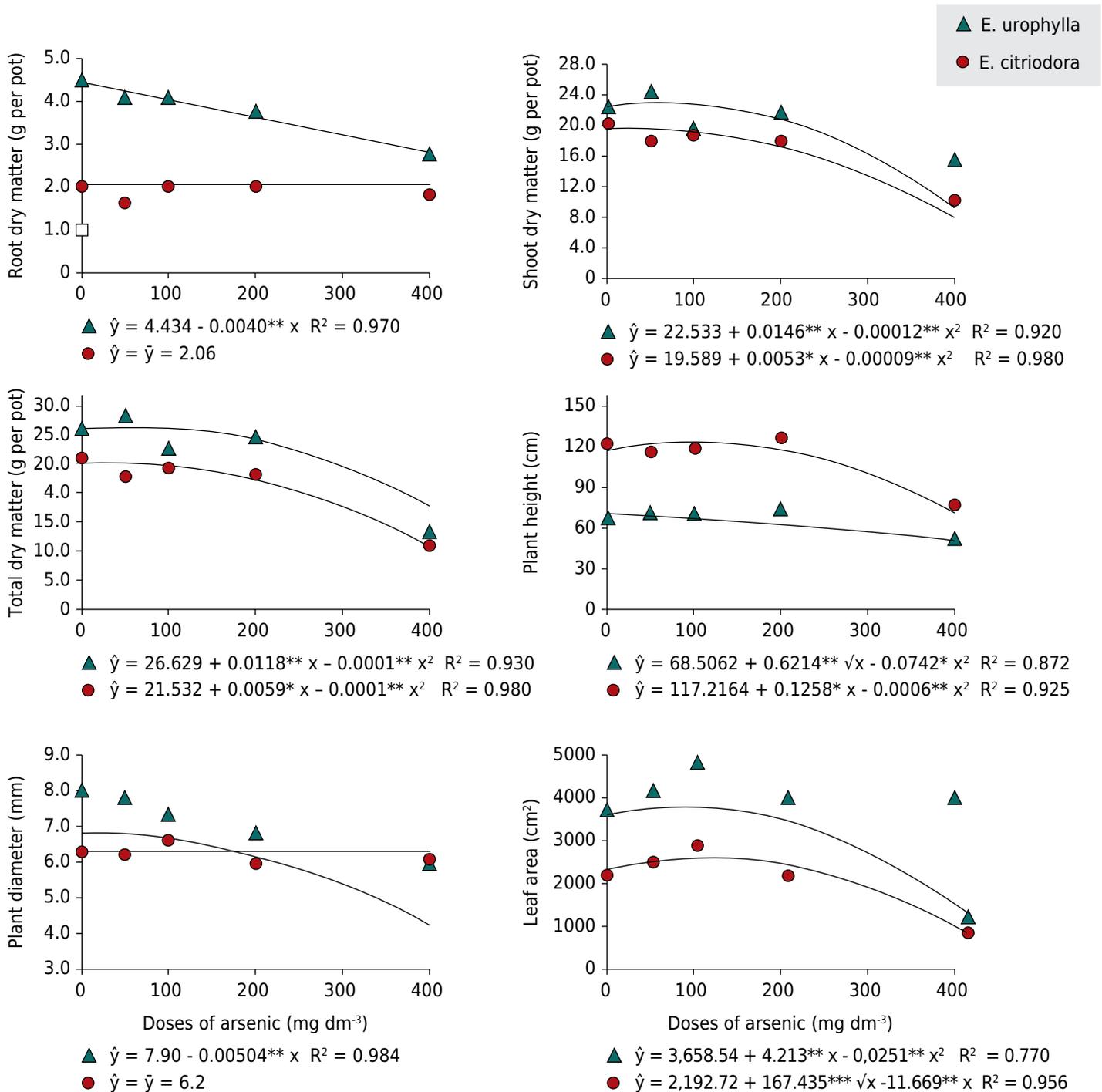
### Toxicity symptoms, dry matter production and plant height

Thirty days after the cultivation in contaminated soil, the plants of *E. urophylla* submitted to the highest As concentration ( $400 \text{ mg dm}^{-3}$ ) began to show symptoms of toxicity in the leaves and death of the apical bud. The symptoms of As toxicity are purplish basal leaves, with internodal chlorosis followed by necrosis (Gardea-Torresdey et al., 2005; Melo et al., 2009). These symptoms were not observed in the plants of *E. urophylla* submitted to As concentrations lower than  $400 \text{ mg dm}^{-3}$ , or in the plants of *E. citriodora* submitted to any of the As concentrations applied to the soil.

Root dry matter varied according to the As concentrations applied to the soil. For *E. urophylla*, the regression analysis allowed the adjustment of a significant model ( $p < 0.01$ ), while for *E. citriodora*, the adjustment could not be performed (Figures 1a and 1b). The effects that heavy metals and metalloids, such as As, exert on root production depend on plant sensitivity and the intensity of the contamination. Root production is an important process for the phytostabilization of As in contaminated areas, since, besides absorbing As, roots protect the soil against erosion, favoring aggregation, reducing leaching and promoting soil microbial activity (Carneiro et al., 2002).

Both species tended to decrease shoot biomass production as the As concentrations applied to the soil increased (Figure 1c). However, specifically for the *E. urophylla* plants submitted to  $50 \text{ mg dm}^{-3}$  As, a 10 % increase in shoot biomass was observed in relation to the control treatment plants. This result corroborates the findings for *Pteris vittata* plants (Tu et al., 2002), which increased their biomass when cultivated in soil amended with  $50 \text{ mg dm}^{-3}$  As. Considering the high adsorption capacity of P by the utilized soil, this increased biomass production may be related to increased P availability in the soil, since arsenate can displace phosphate adsorbed at positively charged sites of minerals in the soil clay fraction (Im et al., 2015).

Plant height of both species also decreased when As was applied to the soil (Figure 1d). Comparing the height of the plants submitted to the highest concentration with those cultivated without As, the reduction was of 22 and 36 %, for *E. urophylla* and *E. citriodora*, respectively. Similarly, the stem diameter and leaf area (Figures 1e and 1f) of both species decreased with increases in the As rate applied to the soil. However, the reduction in stem diameter was higher for *E. urophylla* plants, in comparison to *E. citriodora* plants, while the reduction in leaf area was higher for *E. citriodora* plants (Figures 1e and 1f).



**Figure 1.** Root dry weight (a), shoot dry weight (b), total dry matter (c), plant height (d), plant diameter (e) and leaf area (f) of *E. urophylla* and *E. citriodora* seedlings subjected to various rates of arsenic. \*\*\*, \*\*, \*: at 0.1, 1 and 5 % significant, respectively, by the F test.

### Arsenic concentrations and contents in different plant organs

The As contents in leaves, branches, stem and roots increased with increasing As concentrations (Table 2). The As content in the roots of both species was higher than those observed in the other parts of the plants. The increase in the As concentrations in the different parts according to the concentrations applied to the soil allowed the adjustment of square root, quadratic and linear models by the regression analysis (Table 3).

The As distribution among the different compartments of the plants is related to their capacity to absorb and redistribute internally the metalloid, which in turn depends on internal mechanisms of tolerance to the toxic element, which can vary significantly in different species (Rascio

and Navari-Izzo, 2010) or also in populations of the same species (Deng et al., 2007). This variability among species were also observed in plants such as leucaena, sesbania (Melo, 2006), oats, ryegrass, peanuts and *Stylosanthes* (Melo et al., 2009).

As concentrations from 0.1 to 5.0 mg kg<sup>-1</sup> in shoot dry matter can be considered normal for plants (Wauchope, 1983). Considering the average contents found in the basal leaves of both species, the values remained in the normal range only for the plants submitted to 50 mg dm<sup>-3</sup> As. Among the shoot compartments, the branches

**Table 2.** Contents of arsenic in young leaves (YL), intermediate leaves (IL), baseline leaves (BL), branches (B), stem (S) and roots (R) and arsenic amounts in the roots (Ra), shoot (Sa), and whole (Ta) plants of *E. urophylla* and *E. citriodora*, according to the arsenic rates applied to the soil

Specie	Rate	Content						Amount		
		YL	IL	BL	B	S	R	Ra	Sa	Ta
	mg dm <sup>-3</sup>	mg kg <sup>-1</sup>						mg per plant		
<i>E. urophylla</i>	0	nd <sup>(1)</sup>	nd	nd	nd	nd	nd	nd	nd	nd
	50	1.72	2.88	4.14	2.22	2.15	19.02	0.08	0.32	0.40
	100	2.10	3.86	7.79	3.03	4.27	64.15	0.26	0.41	0.67
	200	2.61	5.59	12.30	3.29	4.32	79.20	0.30	0.61	0.91
	400	3.13	3.80	5.39	3.84	11.05	87.71	0.24	0.23	0.48
	CV (%)	9.6	16.3	6.6	10.4	10.1	4.9	5.33	11.2	8.83
<i>E. citriodora</i>	0	nd	nd	nd	nd	nd	nd	nd	nd	nd
	50	2.97	3.04	4.46	2.66	2.81	27.99	0.05	0.29	0.33
	100	3.46	5.15	8.87	3.37	4.95	98.05	0.20	0.48	0.68
	200	5.72	5.51	12.30	4.27	10.54	143.83	0.29	0.69	0.98
	400	8.45	12.43	10.84	4.38	60.75	253.86	0.47	0.75	1.22
	CV (%)	8.2	12.8	14.2	9.8	4.2	4.8	9.98	5.18	4.63

<sup>(1)</sup> nd: concentrations below the determination method (ICP-OES) detection limit (0.02 mg kg<sup>-1</sup>).

**Table 3.** Regression equations for arsenic content in young leaves (YL), intermediate leaves (IL), baseline leaves (BL), branches (B), stem (S) and roots (R), and for arsenic amounts in shoots (Sa), roots (Ra) and whole plants (Ta) of *E. urophylla* and *E. citriodora*, according to the arsenic rate applied to the soil

Specie/Variable	Equation <sup>(1)</sup>	R <sup>2</sup>
<i>E. urophylla</i>		
YL <sup>(2)</sup>	$\hat{y} = 0.02415 + 0.2667^{**} \sqrt{x} - 0.0056^{**} x$	0.9976
IL	$\hat{y} = 0.3052 + 0.0458^{**} x - 0.000093^{**} x^2$	0.9514
BL	$\hat{y} = -0.3430 + 0.10796^{**} x - 0.00023^{**} x^2$	0.9998
B	$\hat{y} = 0.02055 + 0.3803^{**} \sqrt{x} - 0.0095^{**} x$	0.9928
S	$\hat{y} = 0.5332 + 0.0258^{**} x$	0.9440
R	$\hat{y} = -1.4459 + 0.6209^{**} x - 0.00105^{**} x^2$	0.9581
Sa <sup>(3)</sup>	$\hat{y} = 0.0264 + 0.00537^{**} x - 0.00012^{**} x^2$	0.9769
Ra	$\hat{y} = -0.00535 + 0.00266^{**} x - 0.000005^{**} x^2$	0.9345
Ta	$\hat{y} = 0.02080 + 0.00803^{**} x - 0.000017^{**} x^2$	0.9958
<i>E. citriodora</i>		
YL	$\hat{y} = 0.4861 + 0.0351^{**} x - 0.000038^{**} x^2$	0.9748
IL	$\hat{y} = 0.9934 + 0.02822^{**} x$	0.9467
BL	$\hat{y} = 0.1282 + 0.0991^{**} x - 0.000181^{**} x^2$	0.9938
B	$\hat{y} = -0.02006 + 0.47068^{**} \sqrt{x} - 0.0127^{**} x$	0.9982
S	$\hat{y} = 1.892 - 0.0364^{**} x - 0.00046^{**} x^2$	0.9936
R	$\hat{y} = -2.0847 + 0.8936^{**} x - 0.00064^{**} x^2$	0.9872
Sa	$\hat{y} = 0.0263 + 0.005118^{**} x - 0.000008^{**} x^2$	0.9922
Ra	$\hat{y} = -0.00875 + 0.00193^{**} x - 0.000002^{**} x^2$	0.9804
Ta	$\hat{y} = 0.0166 + 0.00706^{**} x - 0.000010^{**} x^2$	0.9935

\*\* ,\*: significant at 1 and 5 % by the F test, respectively. <sup>(1)</sup> x = rates applied to the soil (mg dm<sup>-3</sup>); <sup>(2)</sup> Content of arsenic (mg kg<sup>-1</sup>); <sup>(3)</sup> Amount (mg per plant).

and young or not completely expanded leaves presented the lowest As contents in relation to basal leaves and stem of the plants. It is especially noteworthy for the 400 mg dm<sup>-3</sup> As rate, in which the concentration in the stem of *E. citriodora* was 5.7 times higher than in the observed plants submitted to 200 mg dm<sup>-3</sup> As (Table 2) and about 12 times higher than the normal limit concentration of arsenic in plants. These results show that these species cannot be considered as hyperaccumulators, but indicate a certain tolerance and capacity to accumulate this metalloid.

Higher As accumulation in the stems of *E. citriodora* plants led to less of a reduction in stem diameter, in comparison to *E. urophylla* plants. The As accumulation in the shoots of the plants was higher than the accumulation in the roots and almost equal for both species up to 200 mg dm<sup>-3</sup> As. At 400 mg dm<sup>-3</sup> As, due to the higher capacity to translocate and accumulate As in the stem, *E. citriodora* plants accumulated 3.2 times more As in the shoot than *E. urophylla* plants (Table 2). Thus, the As accumulation capacity in the shoot and roots can be considered similar for both species up to 62 mg dm<sup>-3</sup> As available in soil As (content available in the soil by the Mehlich-3 extractor after the application of 200 mg dm<sup>-3</sup> As). For higher values, *E. citriodora* plants presented a higher capacity to tolerate and accumulate As, mainly in the roots and stem (Table 2).

With higher As accumulation in the shoot, the As phytoextraction process is easier and transfer to other ecosystem components is prevented, considering that the stem (the wood) has high economic value and can be used by industry for several purposes. However, studies must be carried out on the behavior of these species at higher contamination levels than those used in this assay. Considering that the experimental period was short, the maximum potential for phytoextraction may be higher for longer periods under field conditions than those assessed in this research.

#### **Translocation index and available arsenic in the soil which reduced dry matter by 50 %**

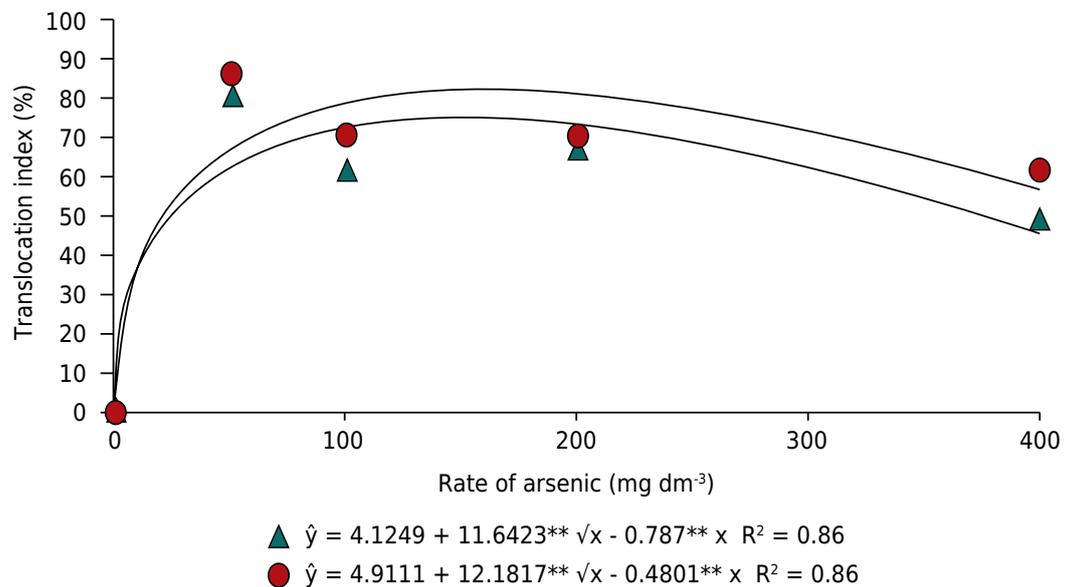
The As translocation index (TI) varied among species; nevertheless the regression analysis was able to adjust quadratic models for all (Figure 2). The variation was significant ( $p \leq 0.01$ ) among species, with higher values for *E. citriodora* plants. Translocation index values <100 % are typical for excluded plants (Nadgórska-Socha et al., 2015). The highest TI value (88 %) was achieved under 50 mg dm<sup>-3</sup> As, while the lowest values were found under 400 mg dm<sup>-3</sup> As (48 % for *E. urophylla* and 61 % for *E. citriodora*). The reduction in TI due to the increased As rates for both species may have resulted from internal control mechanisms, aiming at reducing and/or avoiding its toxic effects on shoot. These mechanisms may work by means of controlling the absorption and/or increasing the efficiency of As translocation control under high levels of contamination, preventing it from reaching more metabolically active tissues of the shoot. Excellent efficiency in terms of detoxification and sequestration is a key property of hyperaccumulators, and allows them to concentrate huge amounts of metals in above-ground organs without suffering any phytotoxic effects. The mechanism of detoxification in the shoot organs of hyperaccumulators consists mainly of metal complexation with ligands and/or in metal removal from the metabolically active cytoplasm by moving it into inactive compartments, mainly vacuoles and cell walls (Rascio and Navari-Izzo, 2010).

The content of As available in the soil that reduced the shoot dry matter by 50 % (TS<sub>50%</sub>) was higher for *E. urophylla*. However, the values observed for both species were well above the As concentration for uncontaminated soils, which is usually below 10 mg kg<sup>-1</sup> (Adriano, 2001), but in contaminated soils may reach 30,000 mg kg<sup>-1</sup> (Vaughan, 1993).

No significant model was adjusted by the regression analysis for *E. citriodora* root dry matter according to the As rates, which prevented the calculation of the TS<sub>50%</sub> value for its root. For *E. urophylla*, the TS<sub>50%</sub> value was 171.7 mg dm<sup>-3</sup>.

The  $TS_{50\%}$  value for the total dry matter of *E. urophylla* plants was about 25 % higher than the value observed for *E. citriodora*, which was  $79.1 \text{ mg dm}^{-3}$ . However, this value is 2.6 times higher than the available As limit to begin taking remediation actions, which is  $12 \text{ mg kg}^{-1}$  (Chen et al., 2001; Davis et al., 2001).

Bioaccumulation factor (BF) values higher than 1.0 indicate the occurrence of As accumulation by the plant compartment evaluated. The BF values calculated for the stem and for the roots decreased as the As rates increased for both species (Table 4). For the roots, the values were equal to 1.0 only for the plants submitted to  $50 \text{ mg dm}^{-3}$  As. In others words, the roots did not perform bioaccumulation above this value. On the other hand, considering the shoot compartment that presented the highest As contents (stem), the BF remained higher than 1.0 up to the  $200 \text{ mg dm}^{-3}$  concentration for *E. urophylla* and up to the highest concentration for *E. citriodora*. At  $400 \text{ mg dm}^{-3}$  As, the stems of *E. citriodora* plants presented the capacity to accumulate three times more As than the plants of *E. urophylla* (Table 4).



**Figure 2.** Translocation index of arsenic in plants of *E. urophylla* and *E. citriodora* according to the arsenic rate applied to the soil. \*\*, \*: at 1 and 5 % significant, respectively, by the F test.

**Table 4.** Soil available arsenic which reduced in 50 % root ( $As\text{-}soil_{50\% \text{ roots}}$ ), shoot ( $As\text{-}soil_{50\% \text{ shoot}}$ ) and total ( $As\text{-}soil_{50\% \text{ total}}$ ) dry matter and As bioconcentration factors (BF) for seedlings of *E. urophylla* and *E. citriodora*, as influenced by arsenic rates applied to the soil

Specie	$As\text{-}soil_{50\% \text{ roots}}^{(1)}$	$As\text{-}soil_{50\% \text{ shoot}}$	$As\text{-}soil_{50\% \text{ total}}$	Rate	BF	
					Stem	Root
	$\text{mg dm}^{-3}$				$\text{mg dm}^{-3}$	
<i>E. urophylla</i>	171.7	88.5	98.0	50	9.1	1.02
				100	6.1	0.40
				200	2.5	0.14
				400	0.9	0.37
<i>E. citriodora</i>	_(2)	69.2	79.1	50	9.7	1.00
				100	7.8	0.39
				200	5.1	0.37
				400	2.9	0.70

<sup>(1)</sup> Soil As concentration to reduce in 50 % dry matter production. <sup>(2)</sup> Not significant.

The BF data are listed, by means of the regression analysis, as the dependent variable of the As concentration applied. Based on the adjusted model, the rate corresponding to a BF value equal to 1.0 was calculated. For *E. urophylla*, this concentration was 350 mg dm<sup>-3</sup>, while for *E. citriodora*, the concentration was 960 mg dm<sup>-3</sup>. These values correspond to 110 and 309 mg dm<sup>-3</sup> soil available As (Mehlich-3) for the former and the latter species, respectively.

A joint interpretation of the As-soil<sub>50% total</sub> values and BF values indicates that, although *E. citriodora* presented a 50 % decrease in the total biomass production with a soil As content lower than previously observed for *E. urophylla* (79.1 vs 98 mg dm<sup>-3</sup>), the capacity to accumulate As by *E. citriodora* would be occur up to 309 mg dm<sup>-3</sup> soil available As, which is about three times higher than the value observed for *E. urophylla*.

## CONCLUSIONS

*Eucalyptus citriodora* and *E. urophylla* cannot be considered as hyperaccumulators of As, but both species present the potential to be used in phytoremediation programs in As-contaminated soils, especially *E. citriodora*.

For the soil arsenic concentration of 309 mg dm<sup>-3</sup>, *E. citriodora* showed three times more capacity to accumulate As than *E. urophylla*. The two species showed decreased shoot and root biomass with increasing As concentrations applied to the soil. *E. citriodora* plants presented a higher capacity to tolerate As, in the arsenate form, and translocate it to the shoot than *E. urophylla*.

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