POTENTIAL OF THREE LEGUME SPECIES FOR PHYTOREMEDIATION OF ARSENIC CONTAMINATED SOILS[1]

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Abstract. Phytoremediation strategies utilize plants to decontaminate or immobilize soil pollutants. Among soil pollutants the metalloid arsenic (As) is the one of primary concern. Elevated soil As results from anthropogenic activities such as use of pesticides (herbicides and fungicides), use of certain fertilizers, metal mining, iron and steel production, coal combustion, and from co-production during natural gas extraction. This study evaluated the potential of pigeon pea (*Cajanus cajan*), wand riverhemp (*Sesbania virgata*), and lead tree (*Leucaena leucocephala*) for phytoremediation of soils polluted by As. Soil samples were placed in plastic pots, incubated with different As doses (0, 50, 100 and 200 mg dm^{-3}) and then sown with seeds of these three species. Ninety days after sowing, the plants were evaluated for height, collar diameter and dry matter of young, intermediate and basal leaves, stems and roots. Arsenic concentration was determined in different aged leaves, stems and roots to establish the translocation index (TI) between plant roots and aerial plant components. The evaluated species showed distinctly different characteristics with respect to As tolerance, since the lead tree and wand riverhemp were significantly more tolerant than pigeon pea. High As levels found in wand riverhemp roots suggest the existence of an effective mechanism of accumulation and compartmentalization in order to reduce As translocation to aboveground tissues. Pigeon pea is a sensitive species and could serve as a potential bioindicator plant, whereas the other two species have potential for phytoremediation programs in As polluted areas. However, further studies are needed with longer exposure times in actual field conditions to reach definitive conclusions on the relative phytoremediation potentials on these species.

Additional Key Words: remediation, decontamination, *Cajanus cajan, Sesbania virgata, Leucaena leucocephala.*

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Introduction

The metalloid arsenic (As) is highly toxic. Concentrations below 10 mg kg^{-1} are common in non-polluted soils (Adriano, 2001), whereas in polluted areas, soil As concentrations can achieve values 3000x higher (Vaughan, 1993). High As concentrations in the soil result from anthropogenic activities such as use of pesticides and fertilizers, mining activities, iron and steel production, and coal combustion and extraction (Smith et al., 1998). Phytoremediation has been considered as an emerging low-cost technique to reclaim areas polluted by toxic elements. Few species, however, have been reported as capable of accumulating significant levels of As.

Despite the existence of many sources of soil contamination, there are few studies related to tolerance, absorption and translocation of As in shrubs. However, shrubby species have been widely studied for other metals in polluted environments. For this reason, the knowledge of patterns of absorption, translocation and accumulation of ions such as As along with associated tolerance limits and phytotoxicity symptoms for species with phytoremediation potential has gained importance (Huang & Cunningham, 1996; Visoottiviseth et al., 2002). The *in-situ* application of technologies using plants for immobilization and/or stabilization of toxic metals appears to be more economically viable than other *ex-situ* non-biological remediation techniques (Glass, 1997; Susarla et al., 2002).

The strategies for selection of suitable plant species for managing high metal and metalloid concentrations in the rhizosphere can be divided into two groups: 1. Plants that use exclusion mechanisms, restricting the absorption and/or transport to aerial parts; and 2. development of internal immobilization mechanisms, compartmentalization or detoxification via production of compounds that promote stable bonds with metals and metalloids (Marschner, 1995; Küpper et al., 1999). Among the several species used to reclaim mined areas in Brazil, those belonging to the family Leguminosae have shown good vegetative growth, even in substrates with very un-favorable physical and chemical conditions (Franco et al., 2000; Dias et al., 2008).

The objective of this study was to determine the potential of pigeon pea (*Cajanus cajan*), wand riverhemp (*Sesbania virgata*), and lead tree (*Leucaena leucocephala*) for the potential phytoremediation and/or revegetation of As-polluted areas.

Material and methods

Subsurface samples (20-40 cm) of a Typic Haplustox soil (Table 1) were collected from the region of João Pinheiro, Minas Gerais State, Brazil. The soil samples were ground and sieved

(4 mm) and incubated for 15 days with different As (V) (Na_2HAsO_4) doses, i.e. 0, 50 (40), 100 (80), and 200 (160) mg dm^{-3} (mg kg^{-1}). According to previous tests carried out with the same soil samples, the addition of these doses resulted in 0, 12.7, 27.4 and 58.9 mg dm^{-3} of available As respectively, by Mehlich 3 extraction.

Table 1. Chemical and physical characteristics of Typic Haplustox soil used in the experiment.

pH H_20	P-rem	As-rem	P§	K§	Ca^{2+}¥	Mg^{2+} ¥	Al^{3+} ¥	H+Al ¥	O.M.
	mg L^{-1}		--- mg kg^{-1} ---		-------------- $cmol_c dm^{-3}$-------------				g kg^{-1}
5.2	26.3	27.8	0.87	19.8	0.0	0.0	1.3	2.0	45

Coarse sand	Fine sand	Silt	Clay	Bulk density	Soil Moisture Equival.	Textural Class
-------------------- % --------------------				----- Mg m^{-3} ----	--------- kg kg^{-1} -------	
40	17	2	41	1.29	0.13	Sandy-Clay

§ Extractor Mehlich I (Mehlich, 1978); ¥ Extractor KCl mol/L; ¥ CEC a pH 7,0 with calcium acetate 1 mol L^{-1}; P-rem – Soil remaining phosphorus and As-Rem- Soil remaining arsenic, according Alvarez et al. (2001) and Ribeiro et al. (2004), respectively.

The use of sodium arsenate as the As source originally was a concern due to potential Na availability in the system, which was 12.2, 24.4, 48.8 mg kg^{-1} for 40; 80, and 160 mg kg^{-1} of As (Na_2HAsO_4) respectively. However, a preliminary test carried out with pigeon pea and the equivalent doses of Na did not show toxic effects in the plants. The soil solution electrical conductivity (1:1 soil-water ratio) ranged from 0.1 to 0.3 dS m^{-1}.

After incubation, 1.75 kg of each amended soil was placed into plastic pots and seeds of *Cajanus cajan* L. Millsp (pigeon pea), *Sesbania virgata* Cav. (wand riverhemp) and *Leucaena leucocephala* L. (lead tree) were sown. Thinning was carried out eight days after emergence, leaving three plants per container.

Macronutrient fertilization was applied at nine days after emergence, with 47.6 mg kg^{-1} of N, 79.4 mg kg^{-1} of P and K and 39.7 mg kg^{-1} of Mg, in the form of solutions of ammonium phosphate, ammonium nitrate, calcium phosphate, potassium phosphate and magnesium sulphate. To guarantee adequate N supply to the plants, we chose not to inoculate the seeds with N_2-fixing bacteria. N fertilization was divided in two applications, at nine and 35 days after emergence, for all species. Micronutrient fertilization was applied in the form of solutions equally divided in four applications (15, 30, 45 and 60 days after emergence), with total doses of 0.64; 2.90, 3.17; 1.06; 0.12 and 1.24 mg kg^{-1} of B, Mn, Zn, Cu, Mo and Fe respectively.

The experiment was conducted in a greenhouse, in a randomized complete block design, with three replications. During the experimental period the temperature in the greenhouse ranged from 20.4 to 31.3 ^{o}C, and the relative humidity from 50 to 69%.

At 30 days after sowing, the pigeon pea plants were evaluated for height and collar diameter (just above the soil surface) and then cut at ground level. Afterwards, the plants were separated into young (YL), mature (ML) and old (OL) leaves, stem (C) and roots (R). For the other two species, the evaluation was conducted 90 days after sowing. Roots were washed with tap water to remove soil, clipped, and then placed into 0.1 mol L^{-1} HCl solution for approximately 1 minute, which was followed by several rinses with deionized water. Soil samples were also collected from each pot to determine the concentration of available As using the Mehlich-3 extraction technique (Ribeiro et al., 2004).

To separate the different types of leaves, we considered the tender and not completely expanded leaves along with their shoots, as "young", whereas the basal leaves plus those that were mature and at senescence stage (yellowish) and all others as "mature". The plant parts were dried in a forced-air oven at 60-70 °C to a constant weight to determine dry mass.

Arsenic concentration in the different plant parts was determined using 1-g samples of dried finely-ground material that was digested with in HNO_3:$HClO_4$ concentrated solution (3:1 v/v). The samples were digested in a block digester under controlled temperatures: initially at 50 ºC for approximately 30 minutes; then 100 ºC for an additional 30 minutes; and finally between 160 and 180 ºC to complete the digestion.

Arsenic concentration in the plant extracts and available As in the soil (Mehlich-3) were determined by inductive coupled plasma atomic emission spectrometry (ICP/AES) at 193.697 nm. A standard reference material (GBW07603 bush branches and leaves, provided by of Geophysical and Geochemical Exploration-Langtang-China Institute) was analysed to validate the method used in this research. The average As recovery rate of the standard sample was 94 ± 0.5%.

The amount of accumulated As was used to calculate the As translocation index (TI), according to Abichequer & Bohnen (1998), with the following equation:

TI (%) = [Amount of As accumulated in the aerial parts (leaves + stem)/Amount of As accumulated in the plant] x 100

Equations to represent the behavior of plants as a function of different As doses were obtained by regression analyses for the following variables: shoots (SDM), roots (RDM) and total (TDM) dry matter (g pot^{-1}); As concentration in young leaves (YL), mature leaves (ML), old leaves (OL), stems (S) and roots (R); soil available As at the end of the experiment (SAA); shoot As content (SAC), roots As content (RAC) and total plant As content (TPAC). By regression analysis we also estimated the critical values of soil available As (TC) that reduced biomass production by 50%. The coefficients from obtained equations were then "F-tested" at 1% and 5% probability levels.

Results and Discussion

Phytotoxicity Symptoms, Dry Matter Production, Plant Diameter and Plant Height

Pigeon pea plants from treatment four (160 mg kg^{-1} of As) showed a marked decrease in growth and strong As toxicity symptoms, which resulted in their harvest after 30 days of As exposure. The symptoms were characterized by interveinal chlorosis followed by wilting and tissue necrosis (Adriano, 1986). These symptoms were only observed in the pigeon pea plants. Arsenic toxicity can arise from the inhibition of several mitochondrial enzymes and deactivation of oxidative phosphorylation, impairing cellular respiration. A major part of As toxicity results from its capacity to interact with sulphydryl groups of proteins and enzymes, and also the capacity to replace phosphorus in several reactions (Goyer, 1996). The pigeon pea sensitivity to As was confirmed by reductions (Fig. 1) in dry matter production of the roots, shoots, height and collar diameter by 75%, 74%, 55% and 8%, respectively, at treatment level of 80 mg kg^{-1} As, in comparison with the control plants.

Lead tree and wand riverhemp were more tolerant of soil As. The lead tree shoots, roots and total dry matter production (Fig. 1) and height were reduced by 36%, 27%, 33%, and 29%, respectively, at the highest dose (160 mg kg^{-1}) when compared with the control (dose 0). For wand riverhemp, the biomass was reduced more than observed with the lead tree, to about 47%, 34%, 45% and 23%, respectively, in comparison with the control plants.

The highest root and total dry matter production of lead trees was observed at the 50 mg dm^{-3} As dose (Fig. 1). The stimulatory behaviour of this species agree with findings reported by Ma et al. (2001) where they have shown that some species exhibit enhanced growth at low concentrations of available As. When exposed to 50 mg dm^{-3} As, the riverhemp formed nodules on the root system, but we did not examine their capacity for N-fixation activity.

Distribution of As in Different Plant Compartments

The increasing As doses significantly affected ($p = 0.01$) its concentration into different plant parts of all species (Table 2 and 3). Overall, the As concentration in the plant parts occurred in the acropetal direction (from base to apex). The highest root As concentrations were found in lead trees and wand riverhemp plants (Table 2). These results agree with those observed for mustard (Pickering et al., 2000); however, they diverge from those found for hyperaccumulative plants such as *Pteris vittata* (Ma et al., 2001) and *Pityrogramma calomelano* (Vissottiviseth et al., 2002) which showed larger accumulations in their leaves. However, the capacity for As translocation to aerial plant parts is a function of tolerance mechanisms that vary among species.

The As content in young and mature leaves of pigeon pea showed similar values for the highest dose (160 mg kg^{-1}), but was significantly higher in basal (old) leaves and stems. Pigeon pea roots had As content (87.1 mg kg^{-1}) lower than those found in roots of lead tree (284.9 mg kg^{-1}) and wand riverhemp (772. mg kg^{-1}).

The As content in the shoots compartments of pigeon pea was relatively high compared with normal limits in plants (5 mg kg^{-1}; Wauchope, 1983). These high levels associated with the large decrease in biomass suggests the lack of more effective mechanisms of tolerance to the metalloid, with reduced retention in roots and resulting translocation and damage to aerial parts. This also suggests the lack of an effective mechanism of internal control to avoid the metalloid translocation to tissues of aerial parts, reducing dry matter production and therefore increasing As concentration in the aboveground compartment.

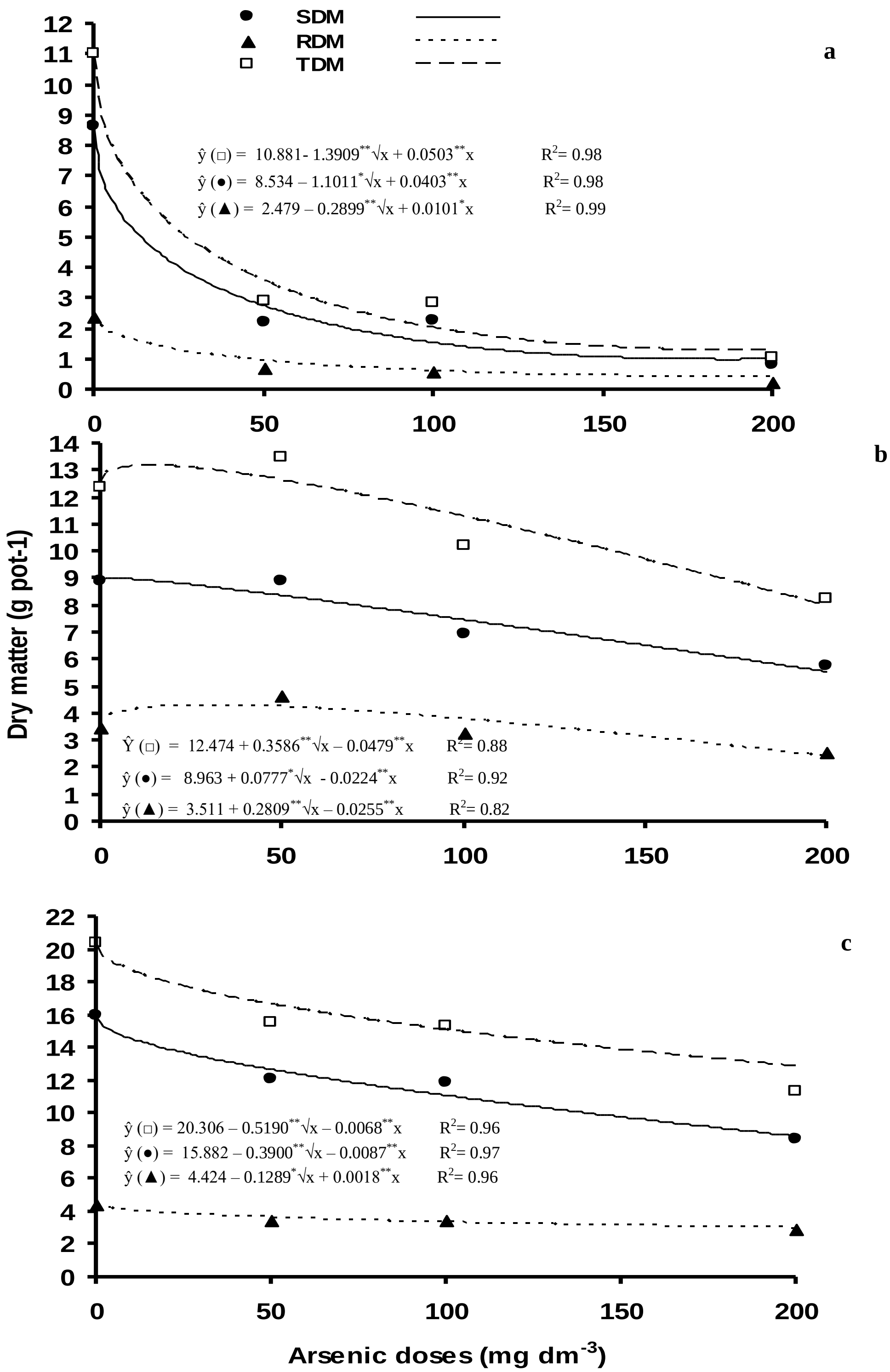

Figure 1. Shoot (SDM), Roots (RDM) and Total (TDM) dry matter (g pot^{-1}) of pigeon pea (a), lead tree (b) and wand riverhemp (c) as function of different arsenic doses. **and * means that the coefficients of the equations are significant at 1 and 5% probability, respectively, and consequently the effect of doses is also significant.

Table 2. Arsenic concentration in young leaves (YL), mature leaves (ML), old leaves (OL), stems (S) and roots (R) of pigeon pea, lead tree and wand riverhemp as a function of different As doses

SPECIES	DOSE	YL	ML	OL	S	R
	mg kg^{-1}	....		mg kg^{-1}		
Pigeon pea	0	#	#	#	#	#
	40	7.3	7.3	12.2	26.2	14.1
	80	7.2	7.2	23.8	38.8	56.4
	160	12.1	13.6	55.1	83.1	87.1
	CV (%)†	11.0	9.9	2.9	6.0	5.9
Lead tree	0	#	#	#	#	#
	40	#	2.5	5.6	0.6	92.6
	80	0.5	2.8	8.4	1.3	267.5
	160	0.6	3.0	5. 8	1.0	284.9
	CV (%)	18.2	13.1	5.6	9.6	3.4
Wand riverhemp	0	#	#	#	#	#
	40	3.4	7.2	6.8	1.6	254.8
	80	5.0	12.7	17.1	2.9	466.2
	160	4.5	17.2	29.7	3.0	772.2
	CV (%)	4.0	2.5	4.6	7.4	2.6

Concentration below to detection limited by used method (ICP/AES).
† Coefficient of variation across the treatments.

Leaves of lead tree had relatively low As contents, particularly the young leaves (Table 2). The highest tissue As levels (8.4 mg kg^{-1}) were found with the 80 mg kg^{-1} dose in old leaves (OL). The stems had the highest content at the 80 mg kg^{-1} dose. For aboveground parts, roots and total plant, tissue As content response fitted both square root and quadratic models (Table 3).

Arsenic content in leaves of wand riverhemp ranged from 5.0 mg kg^{-1} for young leaves (YL) to 29.7 mg kg^{-1} for old leaves (OL), with the 80 mg kg^{-1} and 160 mg kg^{-1} treatments, respectively (Table 2). Arsenic content in stems exhibited a square root response to treatment (Table 3).

Roots of wand riverhemp had relatively high As contents, reaching 772 mg kg^{-1} at the 160 mg dm^{-3} As soil treatment level. This value is 13.1 times higher than the concentration of As available in the soil and 154 times higher than the phytotoxic threshold for normal plants (5 mg kg^{-1}). Some plants can accumulate high levels of metals, ranging from 100-fold to 1000-fold higher than the normal level accumulated by most species (typically 0.1-5 mg kg^{-1}; Wauchope, 1983). Wand riverhemp exhibited a quadratic response for As content in shoots, roots and total plant (Table 3), which was a function of the As applied. Overall, the lead trees and wand riverhemp plants were more tolerant to As in the soil. These two species showed high accumulative capacity, mainly in their root systems, which indicates significant potential for As phytostabilization.

Table 3. Regression equations for As concentration in young leaves (YL), mature leaves (ML), old leaves (OL), stems (S) and roots (R), soil available As at the end of the experiment (SAA), shoot As content (SAC), root As content (RAC) and total plant As content (TPAC) of pigeon pea, lead tree and wand riverhemp as function of different As doses.

SPECIES	COMPARTMENTS	EQUATION	R^2
Pigeon pea	YL[1)]	$\hat{y} = 0.163 + 0.8910^{**}\sqrt{x} - 0.0054^{**}x$	0.96
	ML[1)]	$\hat{y} = 0.194 + 0.7183^{**}\sqrt{x} - 0.0140^{**}x$	0.95
	OL[1)]	$\hat{y} = 0.269 + 0.2079^{**}x + 0.0003^{**}x^2$	0.99
	S[1)]	$\hat{y} = 1.634 + 0.4049^{**}x$	0.99
	R[1)]	$\hat{y} = -3.778 + 0.5925^{**}x - 0.0007^{**}x^2$	0.96
	SAA[2)]	$\hat{y} = -4.189 + 0.3075^{**}x$	0.98
	SAC[3)]	$\hat{y} = 0.001 + 0.0028^{**}x - 0.00001^{**}x^2$	0.99
	RAC[3)]	$\hat{y} = -0.003 + 0.0005^{**}x - 0.000002^{**}x^2$	0.85
	TPAC[3)]	$\hat{y} = -0.002 + 0.0033^{**}x - 0.000013^{**}x^2$	0.99
Lead tree	YL	$\hat{y} = -0.017 + 0.0032^{**}x$	0.80
	ML	$\hat{y} = 0.003 + 0.4719^{**}\sqrt{x} - 0.0189^{**}x$	0.99
	OL	$\hat{y} = 0.123 + 0.1267x^{**} - 0.0005^{**}x^2$	0.99
	S	$\hat{y} = - 0.037 + 0.0192^{**}x - 0.00007^{**}x^2$	0.98
	R	$\hat{y} = -15.696 + 3.4891^{**}x - 0.0097^{**}x^2$	0.95
	SAA	$\hat{y} = -0.082 + 0.0745^{**}x + 0.00046^{**}x^2$	0.99
	SAC	$\hat{y} = -0.0003 + 0.0149^{**}\sqrt{x} - 0.0007^{**}x$	0.99
	RAC	$\hat{y} = -0.037 + 0.0121^{**}x - 0.00004^{**}x^2$	0.96
	TPAC	$\hat{y} = -0.034 + 0.0135^{**}x - 0.000046^{**}x^2$	0.97
Wand revierhemp	YL	$\hat{y} = -0.018 + 0.7565^{**}\sqrt{x} - 0.0286^{**}x$	0.99
	ML	$\hat{y} = -0.043 + 0.1671^{**}x - 0.0004^{**}x^2$	0.99
	OL	$\hat{y} = -0.519 + 0.1804^{**}x - 0.00014^{*}x^2$	0.99
	S	$\hat{y} = -0.042 + 0.0430^{**}x - 0.00014^{**}x^2$	0.99
	R	$\hat{y} = -1.965 + 213433^{**}\sqrt{x} + 2.3851^{**}x$	0.99
	SAA	$\hat{y} = 0.051 + 0.0743^{**}x + 0.0006^{**}x^2$	0.99
	SAC	$\hat{y} = -0.010 + 0.00638^{**}x - 0.00002^{**}x^2$	0.98
	RAC	$\hat{y} = -0.016 + 0.0209^{**}x - 0.00005^{**}x^2$	0.99
	TPAC	$\hat{y} = -0.021 + 0.0273^{**}x - 0.00007^{**}x^2$	0.99

** and * means that the coefficients of the equations are significant at 1 and 5 % of probability, respectively, and consequently the effect of dose is also significant.

[1)] Arsenic concentration (mg kg^{-1}).

[2)] Available arsenic by Mehlich-3.

[3)] Arsenic content (mg pot^{-1}).

Root system volume is an important characteristic related to a higher capacity for absorption and accumulation of As between accumulating and non-accumulating plants. For example, the As hyperaccumulating species Chinese brake fern (*Pteris vitatta*), has a root system volume that is 4-fold larger and accumulated 29-fold more As than *Nephrolepis exaltata* L., a non-accumulating species (Gonzaga et al., 2006). This behavior is somewhat expected, as arsenate shows low mobility in the soil, particularly in acidic high Fe-oxide soils like Oxisols. Therefore, the greater the root system volume, the larger the amount of As that may be assimilated by the plant (Geng et al., 2006).

<u>Translocation index</u>

The application of different As doses to the soil resulted in significant differences ($p = 0.01$) in As translocation indexes (TI) from roots to shoot parts. The TIs found for pigeon pea were considerably high, and the highest index (91.9%) was found with 50 mg dm^{-3}, followed by 83.8 and 87.0% for the doses 80 and 160 mg kg^{-1} respectively (Fig. 2). Contrary to this behavior, lead trees and wand riverhemp plants had relatively low TI values, and the highest indexes were found for 40 and 80 mg kg^{-1} doses with percent translocation of 16.2 and 9.6% for lead tree, and 20.9 and 21.6% for wand riverhemp, respectively (Fig. 2).

A larger accumulation of As in the root system compared with aboveground parts may indicate a low capacity for controlling As absorption and/or a higher efficiency in restraining translocation, avoiding As reaching the metabolically more active tissues of the shoots. This mechanism may be related to phytochelatin synthesis. These peptides are synthesized from glutathione and play a fundamental role in As detoxification by the formation of stable bonds with the As (Sneller et al., 1999; Meharg & Hartley-Whitaker, 2002; Inohue, 2005). In this context, the knowledge of As distribution in the whole plant allows a better evaluation of these species' potential for use in phytoremediation and/or revegetation programs. This also allows evaluation of their ecosystem risk, potentially the risk of mobilizing soil As into the trophic chain.

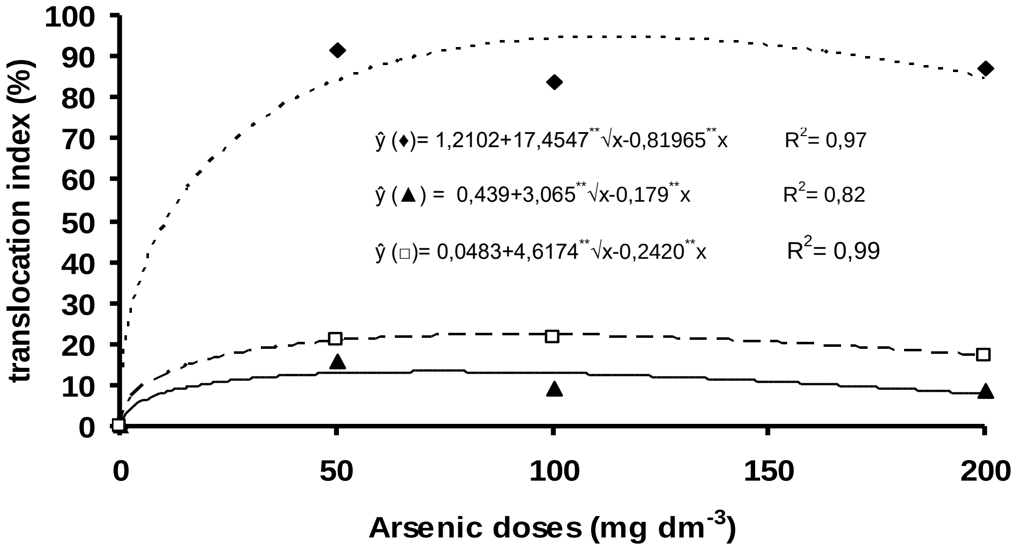

Figure 2. Arsenic translocation index in plants of pigeon pea (...♦), lead tree (__▲) and wand riverhemp (_ _□) as function of different As doses. **and * means that the coefficients of the equations are significant at 1 and 5 % probability levels, respectively, and consequently the effect of dose is also significant.

Conclusions

1 – These three species presented differential responses as a function of As dose applied to the soil. Lead tree and wand riverhemp showed greater tolerance, while pigeon pea was more sensitive to As.

2 - The As concentration in wand riverhemp roots was considerably high than lead tree and pigeon pea, suggesting an efficient and effective mechanism of compartmentalization that reduces metalloid translocation to the young tissues of the aboveground plant.

3 – The low As tolerance of pigeon pea characterizes this species as a potential bioindicator, whereas the other two species show potential for phytostabilization programs.

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