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Calotropis procera: A preliminary survey on its phytoextraction capabilities in Brazil

Calotropis procera: um levantamento preliminar sobre as suas capacidades de fitoextração no Brasil

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Abstract

Calotropis procera (Apocynaceae) is a non-native species that has its origin in Africa and Asia, but was introduced into the northeastern region of Brazil in the beginning of last century. In some areas, the dry biomass of *C. procera* is used as animal feed. However, previous studies indicated that the species is capable of accumulating many chemical elements. On the other hand, it could also mean that this species may be used for phytoremediation. Hence, we used neutron activation analysis, k_0-method, to test the capability of the leaves of *C. procera* to accumulate trace elements. The study was carried out in two distinct areas (polluted and non-polluted) in the State of Pernambuco, Brazil. Our results indicate that new elements can be added to the list of elements taken up by *C. procera*, thus contributing to the understanding of the biology of this plant species as an accumulator of trace elements. Some elements, such as Ba and Sr, have higher concentrations in the non-polluted areas than in polluted areas. Thus, the presence of many trace elements in *C. procera* indicates that its use as animal feed should be viewed with extreme caution. However low levels of these trace elements in *C. procera* do not indicate that this species is a hyper-accumulator plant.

Key words: phytoremediation, biological invasion, invasive species, Pernambuco.

Resumo

Calotropis procera (Apocynaceae) é uma espécie exótica originária da África e Ásia tropical, introduzida na região nordeste do Brasil no início do século passado. Em algumas regiões do Brasil e do mundo, a biomassa seca de *C. procera* é utilizada como alimento forrageiro para animais. Entretanto, muitos estudos indicam que essa espécie é capaz de acumular diversos elementos químicos. Isso pode significar que a espécie também pode ser utilizada em processos de fitorremediação. Dessa forma, foi realizada uma análise de ativação neutrônica, método k_0, para testar a capacidade das folhas de *C. procera* acumularem elementos-traço. O estudo foi realizado em duas áreas distintas (poluídas e não poluídas) no estado de Pernambuco, Brasil. Nossos resultados indicam que novos elementos podem ser adicionados à lista de elementos-traço absorvidos por *C. procera*, contribuindo para o entendimento da biologia dessa espécie como acumuladora de elementos-traço. Alguns destes, tais como Ba e Sr, ocorreram em maiores concentrações em áreas não-poluídas em comparação com áreas poluídas. Dessa forma, o uso de *C. procera* como ração animal deve ser vista com extrema cautela. Entretanto, as baixas concentrações desses elementos-traço não sugerem que *C. procera* seja uma hiperacumuladora.

Palavras-chave: fitorremediação, invasão biológica, espécie invasora, Pernambuco.

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Introduction

Calotropis procera (Aiton) W.T. Aiton (Apocynaceae) is an invasive shrub, 2.5 m to 6.0 m tall (Ismail, 1992) adapted to poor soils (Figure 1). The species has a robust root system that may reach a depth from 1.7 m to 3.0 m in sandy desert soils. The species produces fruits all year round, and its seeds germinate with an enormous facility (Barbosa *et al.*, 2007). After pruning, new branches are quickly formed. *Calotropis procera* is native to the arid regions of tropical Africa, India and Middle East (Brandes, 2005). This species has the capacity to successfully establish in arid, degraded, and nutrient poor soils, such as those found along roadsides, pastures, and abandoned areas (Ferreira, 1973; Kissmann and Groth, 1992; Colombo *et al.*, 2007).

Calotropis procera represents a highly invasive species that has reached the status of weed in many regions. The high potential of *C. procera* to invade pristine or economic important areas is of much concern, as it is also very difficult to eradicate (Barreto *et al.*, 1999; Melo *et al.*, 2001). In Brazil, the species has been successfully established in some areas of Caatinga, Tropical Rain Forest, Cerrado, and in the Northeast Seasonally Dry Forest (Ferreira and Gomes, 1976; Barbosa *et al.*, 2007; Oliveira *et al.*, 2009). The origin of *C. procera* in the Brazilian territory is disputed. While Kissmann and Groth (1992) have argued that it was introduced into the Northeastern region of Brazil at the beginning of the 20th Century as an ornamental plant, Barbosa *et al.* (2007) argued that the species was brought from Africa in slavery ships as beds and mattresses.

In spite of its invasiveness and potential impact on the ecosystems, *C. procera* may have the potential to be used in the biomonitoring of polluted areas (Aitaf, 1997; Díaz and Massol-Deyá, 2003; Massol-Deyá *et al.*, 2005; Al-Farraj and Al-Wabel, 2007; Pandey *et al.*, 2010). In addition, mercury was reported in the leaves of *C. procera* by Rao and Dubey (1992), whereas arsenic was reported in another species of this genus (*C. gigantea*) by Nagaraju and Killham (2005). *Calotropis procera* was suggested to be used in the biosorptive removal of Cd from groundwater and effluents (Pandey *et al.*, 2008; Overah, 2011).

In some arid regions of Brazil the use of *C. procera* as animal feed (e.g., Melo *et al.*, 2001) has been encouraged by some research institutions. Hence, it is important to analyse plants of this species for trace elements accumulation, specially the ones whose use is part of the lore in many countries. Plants that present the accumulation of trace elements are also fit for phytoremediation. One of the aims of phytoremediation is the search of plants that are hyperaccumulators, i.e., that are capable of accumulating pollutants and/or contaminants in high concentrations (e.g., Ma *et al.*, 2001). In this study we investigated whether *C. procera* can absorb trace elements from soil, in polluted and non-polluted sites of Pernambuco State, Brazil, applying a non-destructive analytical technique. The aim of this work was to verify which elements *C. procera* is capable of absorbing, allowing its use in phytoremediation activities. In addition, we highlight the need to take care about the use of this species as animal feed.

Figure 1. *Calotropis procera*: (a) latex exuding after a leaf was cut; (b) green fruits (~ 6.0 cm diameter); (c) aspect of the trunk of a mature specimen; (d) inflorescence (~1.5 cm); (e) dehiscent fruit; and (f) mature specimen in Caatinga, northeast Brazil.

Material and methods

Eighteen individuals of *Calotropis procera* were arbitrarily chosen from polluted and non-polluted sites from the state of Pernambuco (nine for each site), in northeast Brazil. We considered polluted sites those near polluting industries and with high traffic. Since much of the air pollution can be found in the soil in the form of sediments, the soil in these areas were also considered contaminated. The polluted site was along the roadside of the highway BR 232 (from 8°04’00” S, 34°56’27” W to 8°15’10” S, 35°46’42” W). The highway is known for its heavy traffic. The non-polluted site was located in a distant rural area (7°58’32” S, 38°17’18” W, Unidade Acadêmica de Serra Talhada), 450 km away from the capital of the state of Pernambuco, Recife, in the Caatinga biome.

In each site, three 50m x 50m plots were randomly chosen and in each of them three mature plants were randomly selected for the study. From each plant, 10 leaves without signs of predation or pathogen attack were sampled. The leaves were washed with distilled water, and frozen. These leaves were weighed, lyophilized, hand ground, and then transferred to irradiation vials and weighed.

Soil samples were collected in order to investigate the soil-plant relationship, in regard to the trace elements content. For this, one composite soil sample was also collected in each plot from the 0-20 cm soil layer for an analysis of the content of trace elements. The soil samples were dried at room temperature, to avoid loss of more volatile compounds, sieved to five granulometric fractions and the finest-grained fractions (< 0.06 mm) were homogenised by quartering, transferred to irradiation vials and weighed.

The k_0-standardization method was applied to determine the arsenic concentration in the samples (e.g., Menezes *et al.*, 2003; Menezes and Jacimovic, 2006; see also Uemura and Menezes, 2007). The irradiation was performed in the carrousel IC-7 of the TRIGA MARK I IPR-R1 reactor, under a thermal neutron flux of 6.35×10^{11} cm^{-2} s^{-1}, 100 kW power. The parameters f and a in the IC-7 are (22.32 ± 0.2) and (-0.0022 ± 0.0002), respectively. The samples were irradiated simultaneously with neutron flux monitor Al-Au (0.1%) IRMM-530RA foil cut into 5 mm diameter and 0.1 mm thick.

The usual neutron activation analysis including the gamma spectroscopy comprised the scheme of 8 hours in an attempt to determine arsenic. The gamma spectroscopy was performed on an HPGe detector with 15% efficiency and for the spectra analysis - peak area evaluation - the HyperLab program (Simonits *et al.*, 2003) was used. For the calculation of elemental concentrations the software KAYZERO/SOLCOI (DSM, 1996) was applied.

The elemental values were statistically analysed using the R software (R Development Core Team, 2007). Independent student’s t-tests were performed to understand the differences between the selected areas (non-polluted and polluted), for soil and leaf samples.

Results and discussion

Higher levels of cobalt, chromium and rubidium were found in non-polluted leaves samples (Table 1, $p<0.001$); while thorium and lanthanum were found in small quantities exclusively in samples of polluted leaves ($p<0.001$). Thorium and lanthanum can form a mineral called monazite sand, along with ytterbium. This mineral is more abundant at the coast, in places where there was regression of the sea (Leonardos, 1974). Also, they are not related to anthropogenic disturbances like vehicular exhausts.

Soil levels of potassium and rubidium were significantly higher on non-polluted soil samples than polluted soil samples (Table 2, $p<0.001$) and calcium was found only in non-polluted soil samples. In addition to these last three elements, gallium was found only in the soil of non-polluted site. On the other hand, six elements were found at higher concentrations in the polluted soil samples: thorium, ytterbium, zinc, uranium, tantalum and sodium (as shown in Table 2, $p<0.001$). Probably, sodium was found due to the proximity of the polluted site with the coast. Among the elements mentioned above, the only element that is considered an inorganic pollutant is Zn, but it has a value less than the prevention index (300 mg/kg) (CONAMA, 2009). Thus, this element cannot be considered a pollutant in this case.

For the first time it has been shown that leaves of *C. procera* can absorb Ba, Br, Cs, La, Sc, Sm, Sr, Ta and Th (Table 1). These elements can now be added to others that have been described to be absorbed by *C. procera*: As, Br, Cd, Cr, Hg, Pb, Rb, Sc and Se (Rao and Dubey, 1992; Aitaf, 1997; Díaz and Massol-Deyá, 2003; Massol-Deyá *et al.*, 2005; Al-Farraj and Al-Wabel, 2007). The presence of rare-earth elements in some samples is not surprising, because they are often absorbed by plants (for a review, see Babula *et al.*, 2008). It is surprising that thorium was absorbed, but U was not, though present in soil, since these elements have a strong association in soil.

The presence of calcium only at non-polluted soil samples are expected, since most soils in the Brazilian semiarid region are moderately weathered and usually present medium to high levels of this element. Moreover, the presence of rubidium could be related with the presence of potassium (Butterman and Reese, 2003), as these elements have nearly the same ionic radius and because potassium is more than 2000 times more abundant than rubidium, which is a substitute in small amounts for potassium in the lattices of the numerous potassium-containing minerals as mica or feldspar (Butterman and Reese, 2003). Some adjacent areas have a history of fertilizer use (J. Almeida-Cortez, personal commu-

Table 1. Element concentration in leaves of *Calotropis procera* along the roadside of the highway BR 232 (polluted sites) and in Serra Talhada (non-polluted sites), northeast Brazil.

Elements	Serra Talhada			Roadsides		
	conc. (mg/kg)*	max. values (mg/kg)	min. values (mg/kg)	conc. (mg/kg)*	max. values (mg/kg)	min. values (mg/kg)
Ba	3371 ± 999	4535	389	2567 ± 524	3490	1401
Br	4.3 ± 0.7	5.9	2.6	5 ± 1	8	3.1
Ca	22436 ± 2340	27050	20810	<LD	<LD	<LD
Ce	136 ± 26	202	90	161 ± 17	205	127
Co	15 ± 4	28	10.4	21 ± 6	39	11
Cr	102 ± 16	150	80	76 ± 3	155	41
Cs	4,3 ± 0.4	5.4	3.5	5 ± 1	7.5	2.3
Eu	2.6 ± 0.6	4.3	1.7	3.0 ± 0.7	4.8	1.5
Fe	34272 ± 5233	49330	26750	48532 ± 1329	81090	25370
Ga	19 ± 1	21	17	< LD	*<LD*	*<LD*
Hf	13 ± 3	21	10.2	20 ± 2	23	15
K	81377 ± 6017	94760	66660	44227 ± 5943	60020	32170
La	15 ± 14	48	5	48 ± 3	104	7
Na	5589 ± 1380	9636	3424	11488 ± 1398	15430	8881
Nd	84 ± 8	141	53	85 ± 8	127	68
Rb	361 ± 33	426	273	173 ± 18	211	127
Sc	14 ± 3	23	9	21 ± 7	39	8.3
Sm	10 ± 2	15	5.8	11 ± 2	18	8.3
Sr	359 ± 29	397	272	420 ± 54	548	298
Ta	0.5 ± 0.1	0.81	0.29	1.8 ± 0.2	2.3	1.12
Th	10 ± 2	15	6.7	21 ± 2	29	17
U	2.1 ± 0.4	3.1	1	4.0 ± 0.5	5.1	2.8
Yb	<LD**	*<LD*	*<LD*	5.0 ± 0,9	8	4
Zn	46 ± 12	79	18	103 ± 14	144	74
Zr	598 ± 134	988	403	942 ± 88	1082	744

Notes: * Standard errors, * Lower than limit of detection

nication, 08/15/2007), which could explain the presence of these elements.

Although some elements were found to be more abundant in the soil at some areas (Table 2), the composition of *C. procera* leaves did not follow the same patterns. For example, the differences for cobalt and chromium levels in soil were not significant between the areas; however, the levels of these elements in leaves were significantly higher on the non-polluted area. Crops cover the surroundings of this area and it is known that the physicochemical status of transition metals such as cobalt in the rhizosphere is entirely different from that in the bulk soil (Talukder and Sharma, 2007). Thus, it is a matter of fact that cultivated soils could show higher amounts of available cobalt for the plants. In addition, the non-polluted soil can probably accumulate at least a minimum amount of water while the polluted soil is very dry due to highway drainage, despite being in a more humid region. Water logging of the soil increases cobalt uptake in some species, as in beans and maize (Iu *et al.*, 1982). Following the same reasoning, the more common stable forms of chromium are the trivalent Cr(III) and the hexavalent Cr(VI) (e.g., Shanker *et al.*, 2005). The last is taken up by plants because their mobile nature on soil (Samantaray *et al.*, 1998) while it is a more toxic form. One possibility is that Cr(VI) is more abundant in the non-polluted area due to some activities related to this element around this area, as leather processing and wood preservating (Shanker *et al.*, 2005).

There are many factors that can influence the uptake of trace elements by *C. procera*. However, it is important to note that *C. procera* is a latex producing species. Latex is an aqueous suspension of extremely variable composition that is exuded for a variable span of time, depending on the species. Figure 1 shows a specimen exuding latex from the stem, after a leaf was cut; that also happens to the leaf, and the amount of latex that is lost is variable; and so is the loss of mass from a sample (consequently some metals and nutrients). Furthermore, many trace elements may be accumulated in other plant parts such

Table 2. Mean element concentrations in soil samples collected along the roadside of the highway BR 232 (polluted sites) and in Serra Talhada (non-polluted sites), northeast Brazil.

Elements	ST Concentration (mg/kg)*									BR Concentration (mg/kg)*								
	random area 1			random area 2			random area 3			random area 1			random area 2			random area 3		
	plant 1	plant 2	plant 3	plant 1	plant 2	plant 3	plant 1	plant 2	plant 3	plant 1	plant 2	plant 3	plant 1	plant 2	plant 3	plant 1	plant 2	plant 3
Ba	<LD**	84	92	1095	157	<LD**	<LD**	102	110	78	<LD**	77	<LD**	<LD**	<LD**	51	43	<LD**
Br	71	131	84	143	146	154	616	575	451	168	193	97	228	187	211	83	126	183
Ca	<LD**	20790	12750	103600	22500	13170	<LD**	27240	19240	18500	9169	21240	16570	19930	10220	18790	<LD**	9924
Co	0.5	<LD**	6.6	<LD**	12.8	<LD**	5	7.6	3.4	0.5	0.37	<LD**	0.7	0.5	0.4	0.3	<LD**	0.26
Cr	5	6	3.8	25	6.7	6	<LD**	3	3	<LD**	<LD**	4	<LD**	<LD**	<LD**	2.3	2.2	1.7
Cs	<LD**	<LD**	0.63	<LD**	<LD**	<LD**	<LD**	<LD**	0.3	0.44	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**
Fe	<LD**	<LD**	<LD**	<LD**	157	<LD**	<LD**	175	<LD**	<LD**	<LD**	<LD**	<LD**	197	<LD**	<LD**	<LD**	91
K	51340	36560	44540	37840	36780	42230	38890	38640	58770	24380	59550	41300	38770	38830	<LD**	31700	31460	28340
La	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	0.6	<LD**	0.51	<LD**	0.35	<LD**	<LD**	<LD**	<LD**
Na	1354	1065	3276	1695	1805	4526	559	2044	7063	446	1495	<LD**	4709	606	<LD**	5695	9391	10320
Rb	26	41	128	131	14	23	210	54	105	57	8	<LD**	42	41	15	<LD**	25	<LD**
Sc	0.04	0.06	0.023	<LD**	0.043	<LD**	0.4	0.022	0.032	0.03	0.02	0.03	0.04	0.03	0.020	0.02	<LD**	<LD**
Se	<LD**	5.1	<LD**	<LD**	<LD**	10.8	93	17	2	1.8	<LD**	<LD**	3.2	<LD**	<LD**	<LD**	2.1	9.2
Sm	<LD**	<LD**	<LD**	1.4	1.8	<LD**	<LD**	1.4	<LD**	<LD**	<LD**	0.05	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**
Sr	210	459	266	2047	341	136	2009	309	228	331	98	265	<LD**	153	<LD**	217	240	152
Ta	<LD**	<LD**	1.25	5	2.0	<LD**	<LD**	1.6	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	0.24	<LD**
Th	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	<LD**	0.37	<LD**	<LD**	<LD**	0.12	<LD**	0.27
Zn	22	<LD**	21	155	21	27	<LD**	36	33	20	32	50	30	62	<LD**	18	27	24

Notes: *Standard errors, **Lower than limit of detection

as stems, roots and fruits (Aitaf, 1997; Díaz and Massol-Deyá, 2003; Massol-Deyá *et al.*, 2005).

The use of *C. procera* for medicinal purposes and as animal feed (e.g., goats) should be viewed with extreme caution due to the presence of several trace elements in its leaves. Although the toxic cardenolides can be neutralized with the drying of the leaves (Melo *et al.*, 2001), the presence of trace elements can be a problem for the consumption of the meat from animals fed with its leaves.

Our results indicate that new elements can be added to the list of elements taken up by *C. procera* (Ba, Br, Cs, La, Sc, Sm, Sr, Ta and Th), thus contributing to the understanding of the biology of this plant species as an accumulator of trace elements. Some elements, such as Ba and Sr, have higher concentrations in non-polluted areas. Regarding Cs, La, Sm, Ta and Th, there are insufficient data for any conclusion (in most cases the data are below the detection limit). In general, the trace element concentration values obtained are low, which leads us to believe that this plant should not be considered a hyper-accumulator of trace elements (e.g., Kabata-Pendias, 2000). Although our results do not show high levels of trace elements linked to pollution, many authors suggest that this plant is able to accumulate elements such as Pb and Cd (e.g., D'Souza *et al.*, 2010). The determination of a certain species as a hyper-accumulator (and its possible use for phytoremediation) should be assured through experiments made under controlled conditions, and not only by samples collected in the field, as in the latter, it is possible to lose data through the loss of latex.

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