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Accumulation of arsenic and nutrients by castor bean plants grown on an As-enriched nutrient solution

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ABSTRACT

Phytoextraction is a remediation technique that consists in using plants to remove contaminants from soils and water. This study evaluated arsenic (As) accumulation in Castor bean (*Ricinus communis* cv. Guarany) grown in nutrient solution in order to assess its phytoextraction ability. Castor bean plants were grown under greenhouse conditions in pots containing a nutrient solution amended with increasing doses of As (0, 10, 50, 100, 250, 500 and 5000 μg L^{-1}) in a completely randomized design with four replications. Shoot and roots dry matter production as well as arsenic and nutrient tissue concentrations were measured at the end of the experiment. The results showed that increasing As concentration in nutrient solution caused a decrease in shoot and root biomass but did not result in severe toxicity symptoms in castor bean growing under a range of As concentration from 0 to 5000 μg L^{-1}. The As doses tested did not affect the accumulation of nutrients by castor bean. Although castor bean did not pose characteristics of a plant suitable for commercial phytoextraction, it could be useful for revegetation of As-contaminated areas while providing an additional income by oil production.

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1. Introduction

Arsenic (As) is a naturally occurring trace element that is distributed thoroughly in the terrestrial crust and is found in over 245 minerals [1]. Arsenic in soils can originate from natural (e.g., rock weathering and volcanic activity), as well as anthropogenic sources (e.g., pesticides, herbicides, fertilizers, mining, fossil fuel burning) [2]. Soil pollution with arsenic can cause loss of the vegetation covering and contamination of water bodies, besides contributing to the entrance of As into the food chain [3].

Due to its toxicity as well as the possibility of exposure – mainly to humans – arsenic is considered a key priority pollutant worldwide [4]. Acceptable concentrations of arsenic in drinking water vary between 10 and 50 μg of As L^{-1} [5,6]. However, values about 10 times higher have been reported in surface waters close to mining areas in Brazil [7] and in several studies conducted in Southern Asia [8] as well, where health problems arising from As water contamination are noteworthy. Likewise water contamination, soil pollution with trace elements might also pose an ecological and/or human health risk. In addition, it may decrease agricultural productivity and affect ecosystems sustainability. Owing to their intrinsic toxicity and high persistence, these elements are an environmental problem requiring urgent and affordable solutions.

Conventional remediation technologies are sometimes cost prohibitive and frequently harmful to soil properties [9]. On the other hand, phytoremediation is a promising technology for soil remediation due to its relatively low cost, which makes it a viable alternative for countries where funds for environmental restoration are scarce [10].

The success of the phytoextraction approach depends on both the biomass production and the ability of plants to accumulate the pollutant in the shoots in concentrations that are sufficiently high to reduce the concentration in the media to acceptable levels [11]. Thus, an appropriate selection of plant species possessing such a phytoextraction potential requires a better understanding of the behavior of such species when submitted to increasing doses of the target pollutant. Hydroponic systems are particularly suitable for assessing plants phytoextraction potential, because they eliminate the interaction between the target pollutant and the media [12,13].

Castor bean (*Ricinus communis* cv. Guarany) is a species belonging to the *Euphorbiaceae* family. It has been shown to possess potential for phytoremediation of heavy metals [14–16] due to its fast growth and high biomass. These traits make castor bean widely adapted to several soil types and climatic conditions. Moreover, this plant species has been recently appointed as a good alternative for biodiesel production in Brazil [17]. The phytoextraction potential

Abbreviations: TF, translocation factor; BF, bioaccumulation factor; $[As]_{shoot}$, concentration of the element in the shoot dry matter; $[As]_{root}$, concentration of the element in the root dry matter; $[As]_{solution}$, concentration of the element in the nutrient solution.

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of As by castor bean aiming to its use in soil remediation can be improved through a better understanding of the tolerance to the element. Such knowledge can lead to strategies to be used on engineering the species for high As accumulation. Otherwise, studies concerning the castor plant development under As-stress conditions are relevant to create an additional option for soil revegetation of As-impacted areas.

This study evaluated the As accumulation and tolerance by castor bean (*Ricinus communis* cv. Guarany) grown in nutrient solution as well as its potential for As phytoextraction.

2. Materials and methods

The experiment was conducted in greenhouse at the Soil Science Department of the Federal University of Lavras (Brazil) from August to December, 2007. Castor bean seedlings (*Ricinus communis* cv. Guarany) were produced in a vermiculite substratum, irrigated with 0.1 mmol L^{-1} calcium sulfate ($CaSO_4 \cdot 2H_2O$). Castor bean seeds were obtained from the Plant Science Department at Federal University of Lavras, Brazil.

Seedlings were transplanted 23 days after sowing into 30-L trays containing a Hoagland and Arnon [18] solution at 20% of its original ionic strength. Plants were kept in this solution for a 10-day adaptation period, under constant aeration and pH adjusted close to 5.5 by addition of either 0.1 mol L^{-1} NaOH or HCl solutions. Seedlings were then transferred to 1.75-L pots containing a solution with 50% of the Hoagland and Arnon original ionic strength. Arsenic was added to each pot 3 days after transplanting. The nutrient solution was renewed whenever the electrolytic conductivity dropped from approximately 1.2 to 0.4 dS m^{-1}.

Arsenic was supplied as $Na_2HAsO_4 \cdot 7H_2O$ (analytical-reagent grade) at 7 concentrations (0, 10, 50, 100, 250, 500, and 5000 $\mu g\,L^{-1}$ of As), in four replicates disposed in a completely randomized experimental design, totaling 28 pots. Such doses were used to represent the range of As concentrations likely to be found in drinking water standards up to As-contaminated areas.

After 30 days of exposure to As, castor bean plants were harvested and separated into shoots and roots. Both roots and shoots were washed and oven-dried at 60 °C. Root and shoot weights were recorded and then the plant material was digested following the USEPA 3051A. The arsenic concentration was determined by graphite furnace atomic absorption spectroscopy. NIST standard reference materials (SRM 1573a Tomato Leaves, SRM 1547 Peach Leaves) were used to check the accuracy of As determination, which was found satisfactory, i.e., less than 10% of variation. The plant nutrient concentrations were determined as follows: P and S by inductively coupled plasma-optical emission spectroscopy; K by photometry; Ca, Mg, Cu, Mn, Fe, and Zn by flame atomic absorption spectroscopy. Total N was determined using the Kjeldahl method [19]. Arsenic speciation in nutrient solution was assessed using Visual Minteq version 2.53 [20] aiming to verify the main forms of As at each solution concentration. Results of such a speciation at the highest As concentration (5000 $\mu g\,L^{-1}$) revealed that 93.41% of the As occurred as $H_2AsO_4^-$, 6.55% as $HAsO_4^{2-}$, and 0.04% as H_3AsO_4. Thus evidencing that 100% of the element remained dissolved in solution, i.e., promptly available for plant uptake.

The phytoextraction ability of castor bean plants was assessed using both the translocation factor (TF) and the bioaccumulation factor (BF) as follows:

$$TF = \frac{[As]_{shoot}}{[As]_{root}}$$

$$BF = \frac{[As]_{shoot}}{[As]_{solution}}$$

Table 1
Root (RDM) and shoot dry matter (SDM) production of castor bean plants exposed to increasing concentrations of arsenic in nutrient solution. Standard errors are shown in parenthesis.

Treatment, As ($\mu g\,L^{-1}$)	Dry matter (g)	
	Shoots	Roots
0	20.59a (1.92)	9.44a (1.06)
10	21.51a (2.27)	9.49a (1.37)
50	19.91a (1.34)	9.39a (0.33)
100	19.39a (0.99)	9.29a (1.29)
250	20.06a (0.88)	9.08a (0.93)
500	20.21a (0.67)	9.69a (0.62)
5000	13.37b (1.28)	7.27b (0.38)

Means followed by the same letters are not significantly different by Scott–Knott Test at $p < 0.05$.

where $[As]_{shoot}$ and $[As]_{root}$ stand for the concentration of the element in the shoot and root, respectively (mg kg^{-1}), while $[As]_{solution}$ is the element concentration in the nutrient solution (mg L^{-1}) [21–25].

The results were submitted to an ANOVA with data transformed to a logarithmic scale in order to reduce the inequality of As concentration intervals used in the nutrient solution, thus contributing for a better graphic representation of the data. Regression equations were chosen based on R^2 obtained with the Sigma Plot software (version 10.0). Pearson correlation coefficients were also used to determine significant relationships between the concentration of As and each nutrient (N, P, K, S, Ca, Mg, Cu, Fe, Mn, and Zn) in roots and shoots.

3. Results and discussion

Except for the highest As concentration (5000 $\mu g\,L^{-1}$), the addition of arsenic had little effect on the root (RDM) and shoot dry matter (SDM) of the castor bean plants (Table 1). Data from SDM demonstrated a 35% decrease in production for the highest dose when compared with the control. Even though we could not find statistical difference for data from RDM, increasing the As dose to 5000 $\mu g\,L^{-1}$ caused a 23% decrease in RDM production.

Arsenic concentration in SDM was smaller than in RDM, [As] shoot < [As] root, and both increased exponentially with increasing As concentration in the nutrient solution (Fig. 1). The estimated value of $[As]_{root}$ at the highest As solution concentra-

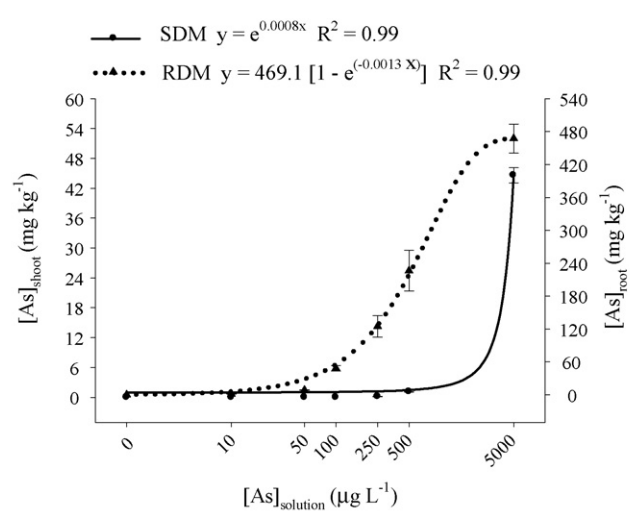

Fig. 1. Arsenic concentration in the shoots – $[As]_{shoot}$ – and in the roots – $[As]_{root}$ – of castor bean plants exposed to increasing concentrations of arsenic in nutrient solutions (As concentration in solution presented in log scale for simplicity. Bars represent the standard error of the mean).

Table 2
Translocation and bioaccumulation factors for arsenic in castor bean plants exposed to increasing concentrations of arsenic in nutrient solutions (mean standard errors in parenthesis).

Treatment, As (μg L^{-1})	Factor	
	Translocation	Bioaccumulation
250	0.001b (0.000)	0.584c (0.098)
500	0.005b (0.001)	2.178b (0.036)
5000	0.096a (0.008)	8.920a (0.307)

Means followed by the same letters are not significantly different by Scott–Knott Test at $p < 0.05$.

tion (5000 μg L^{-1}) was 468.40 mg kg^{-1}, whereas that of [As]$_{shoot}$ was 44.6 mg kg^{-1}. Toxicity symptoms such as dark brown-colored leaves and necrosis in tips and leaves margins, followed by the death of the plant [3], were not observed in this study. It is worthy to point out that only from As concentration close to 5000 μg L^{-1} roots start to transfer substantial amounts of As to shoots (Fig. 1). Taking in account that castor bean plants did not display any toxic symptoms, it is likely that its potential to accumulate As in shoots could be higher than found here.

Anderson and Walsh [26] reported that *Thelypteris palustris* is not a good species for remediating As-contaminated soil and water due to the appearance of toxicity symptoms – necrosis in fronds containing 48.3 mg kg^{-1} – in plants exposed to 500 μg As L^{-1}. Based on our results, castor bean could grow well in As-contaminated areas since it accumulates up to 43.5 mg kg^{-1} in shoots when exposed to As concentrations 10-fold higher in solution (5000 μg As L^{-1}) than the used for *T. palustris*, with no toxicity symptoms.

Rahman et al. [27] studied the phosphate–arsenate interactions in nutrient solutions and reported a 3.18-fold decrease in As concentration in shoots of *Spirodela polyrhiza* L. exposed to 300 μg As L^{-1} when the phosphate concentration in solution was increased from 0.02 to 500 μmol L^{-1}. The P concentration in solution for the present work was 500 μmol L^{-1}. Therefore the P:As ratio varying from 0.75 to 7.5 enhanced the As concentration in shoots from 0.94 to 43.5 mg L^{-1}, respectively. These data are not in line with the results by Tu and Ma [28] who argues that the P:As ratio in solution (mol L^{-1}) should be 1:2 aiming at effective removal of As by plants.

Several works have recently reported a high capacity of As accumulation ([As]$_{shoot}$ > 1000 mg kg^{-1}) by a brake fern (*Pteris vittata* L.) grown either in polluted soils or under greenhouse conditions where As has been added to the soil [3,22,29–31]. In a recent experiment with sunflower (*Helianthus annuus*) exposed to 30 mg As L^{-1} in nutrient solution, January et al. [32] observed As concentrations in the root, stem, and leaves of 1520, 520, and 1040 mg kg^{-1}, respectively. However, the sunflower biomass was as low as 2.13 g per pot.

In order to be regarded as As hyperaccumulator, a species must pose the ability to uptake and transfer effectively the metalloid into the shoots. Yet, there is no scientific consensus over the [As]$_{shoot}$ value that a hyperaccumulator plant should meet, which could be considered either 100 mg kg^{-1} [33] or 1000 mg kg^{-1} [2]. Moreover, one should evaluate also the TFs and BFs, as proposed by several works [21–25]. The TF measures the plant efficiency to transport an element from roots to shoots, while the BF measures the plant effectiveness to accumulate the element comparatively to its concentration in the media. If both factors are higher than the unit (1) the species present hyperaccumulation. However, the effective removal of metals from soils is a combination of sufficiently high metal concentration in shoots and high biomass instead of only one of such factors [10,11].

The translocation and bioaccumulation factors (Table 2) increased as a function of As concentration in the nutrient solution. The estimated values for the highest As solution concentration

Table 3
Macronutrient, micronutrient and arsenic concentrations in castor bean plants exposed to arsenic in nutrient solution.

Treatment, As (μg L^{-1})	Shoots											Roots										
	g kg^{-1}						mg kg^{-1}					g kg^{-1}						mg kg^{-1}				
	N	P	K	S	Ca	Mg	Cu	Fe	Mn	Zn	As	N	P	K	S	Ca	Mg	Cu	Fe	Mn	Zn	As
0	22.30	3.33	3.32	1.16	10.51	3.24	5.20	47.31	26.13	4.62	0.00	20.89	2.39	9.88	1.08	10.15	1.76	4.35	266.06	54.81	5.90	0.00
10	22.73	3.47	4.16	1.70	13.43	3.72	7.65	47.61	27.52	4.83	0.00	21.10	2.45	8.99	1.12	10.20	1.92	4.51	269.64	59.19	5.31	1.54
50	23.07	4.47	4.39	1.88	14.11	4.11	10.30	52.91	30.20	5.19	0.00	21.33	2.57	8.92	1.18	10.90	1.96	5.01	275.18	59.44	6.02	8.62
100	23.43	5.17	4.56	2.43	13.12	4.35	11.98	65.17	31.02	5.22	0.00	21.35	2.83	8.69	1.23	11.14	2.00	6.23	277.80	63.39	6.38	48.54
250	23.47	3.77	4.69	5.13	12.26	4.09	10.52	49.88	32.16	5.49	0.15	21.38	3.27	8.86	1.43	13.34	1.92	8.45	316.05	63.57	6.50	124.85
500	23.49	3.75	4.70	3.09	12.25	4.07	7.33	45.54	32.53	5.60	1.09	23.67	3.46	8.92	1.15	13.53	1.89	10.02	344.18	64.32	7.82	226.37
5000	23.51	4.63	5.02	3.04	12.22	4.02	2.37	32.42	53.39	5.78	44.60	30.63	6.33	9.38	1.07	13.69	1.85	11.68	301.91	54.62	8.30	467.38

Table 4
Pearson correlations between As and nutrient concentrations (N, P, K, S, Ca, and Mg in g kg^{-1}; Cu, Fe, Mn, and Zn in mg kg^{-1}) in root and shoot dry matter of castor bean plants exposed to increasing concentrations of arsenic in nutrient solutions.

Nutrient	Arsenic	
	Root	Shoot
N	0.98^{***}	0.41^{NS}
P	0.98^{***}	0.31^{*}
K	0.28^{NS}	0.31^{NS}
S	-0.36^{NS}	0.20^{NS}
Ca	0.48^{**}	-0.14^{NS}
Mg	-0.27^{NS}	0.13^{NS}
Cu	0.75^{*}	-0.72^{*}
Fe	0.23^{NS}	-0.75^{*}
Mn	-0.45^{**}	0.98^{**}
Zn	0.76^{*}	0.47^{**}

NS—non-significant difference.
* Significant at the $p < 0.05$ level.
** Significant at the $p < 0.01$ level.
*** Significant at the $p < 0.001$ level.

(5000 μg L^{-1}) were TF = 0.096 and BF = 8.920. Translocation factor is far below the reference value (1.0) for hyperaccumulation. This might explain why we could not see toxicity symptoms in the shoots of castor bean plants. According to Schmöger et al. [34], the low capacity of transporting As to the shoots seems to be important mechanisms to minimize As phytotoxicity.

The ability of specific plants to survive in polluted soils has been related to a variety of mechanisms of metal tolerance or detoxification, which includes chelation, compartmentalization, biotransformation and cellular repair [35]. There has been evidence that the ease of reducing arsenate to arsenite is a strategy that hyperaccumulator plants use to accumulate large amounts of As without developing toxicity symptoms [36,37]. Although arsenite is more phytotoxic than arsenate, once reduced arsenite becomes less toxic within the plant due to the formation of arsenite–thiol complexes (–SH) and As-phytochelatins [38,39].

Arsenic uptake by plants is dependent on environmental factors such as soil type, nutrient supply and pH. Thus, a better understanding of the relationships between As uptake and plant nutrition is essential for developing an efficient strategy for plant growth in phytoremediation programs [28]. Increasing As concentrations in the nutrient solution did not affect nutrient accumulation by the castor bean plant (Table 3), since there were not nutrient deficiency symptoms. This implies that nutritional deficiency was not a drawback for As uptake.

The increase of As concentrations in the plant tissue was positively correlated with N, P, Ca, Cu, and Zn concentration in RDM and with P, Mn, and Zn concentration in SDM of the castor bean plant. Inverse correlation was observed for Mn in RDM and with Cu and Fe in SDM (Table 4). These results corroborate those reported by Carbonell-Barrachina et al. [40] who found increasing concentrations of N, P, and Ca in bean plants (*Phaseolus vulgaris* L.) submitted to increasing doses of As. Carbonell-Barrachina et al. [41] showed that As caused a reduction of macronutrients (K, Ca, and Mg) and micronutrients concentration (B, Cu, Mn, and Zn) in tomato plants (*Lycopersicum esculentum* Mill). Increasing nutrient concentrations in As-stressed plants may be related to a “concentration effect”, since shoots and roots biomass decreases for high doses of As in solution.

4. Conclusions

Castor bean can be regarded as a species moderately tolerant to arsenic. Therefore, this species could be used successfully for revegetation of As-contaminated areas. The potential use of castor bean seeds for biodiesel production might be preceded by a much detailed work to be conducted until the stage of fruit production. However, taking into consideration the very low root-to-shoot translocation observed in this present study, one might expect also a small translocation to fruits. Such oil production could reduce the costs associated with revegetation which make the process sustainable on an economic and environmental perspective.

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