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Potential of sunflower, castor bean, common buckwheat and vetiver as lead phytoaccumulators

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A B S T R A C T

Studies concerning the tolerance, absorption and distribution of heavy metals in plants are essential for the success of phytoremediation programs. The present study was carried out in order to evaluate the potential of the sunflower, castor bean, common buckwheat and vetiver as lead phytoaccumulators. The species were grown in nutrient solution containing increasing doses of Pb (0, 50, 100, 200 and 400 mg L^{-1}) during a 30-day exposure period. A completely randomized split-plot design was used, with a 4 x 5 factorial and three replicates. Significant reductions of dry matter of the root, shoot and whole plant were found in the all species under study as a function of the increased Pb doses. Vetiver showed higher tolerance to Pb contamination; sunflower and castor bean had intermediate tolerance and the common buckwheat proved to be the most sensitive species. The concentration and total content of Pb in plant compartments were significantly affected by the increased Pb doses in solution, and higher accumulation of this element was observed, in general, in the roots of the studied species. Common buckwheat proved to be not much promising for Pb-phytoremediation programs; sunflower showed potential for Pb phytoextraction and castor bean and vetiver were the most appropriate for Pb phytostabilization.

Palavras-chave:
poluição
metal pesado
fitotoxidez
tolerância
fitorremediação

Potencial de girassol, mamona, trigo mourisco e vetiver como fitoacumuladoras de chumbo

R E S U M O

Estudos sobre a tolerância, absorção e distribuição de metais pesados em plantas, são essenciais para se obter sucesso em programas de fitorremediação. O objetivo foi avaliar o potencial de girassol, mamona, trigo mourisco e vetiver como fito-acumuladoras de chumbo (Pb). As espécies foram cultivadas em solução nutritiva com doses crescentes de Pb (0, 50, 100, 200 e 400 mg L^{-1}) durante 30 dias. O delineamento empregado foi o inteiramente casualizado em parcela subdividida com o fatorial 4 x 5, com três repetições. Foram constatadas reduções na matéria seca da raiz, parte aérea e planta inteira das espécies estudadas em resposta à elevação das doses de Pb. O vetiver mostrou a maior tolerância ao Pb, a mamona e o girassol apresentaram tolerância intermediária e o trigo mourisco se mostrou a espécie mais sensível. Os teores e conteúdos de Pb nos compartimentos das plantas aumentaram com a elevação das doses de Pb, tendo sido verificado, de forma geral, maior acúmulo deste elemento nas raízes. O trigo mourisco mostrou-se pouco promissor para programas de fitorremediação de Pb, o girassol apresentou potencial para fitoextração de Pb e a mamona e vetiver se mostraram mais adequadas para fitoestabilização desse elemento.

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Introduction

The increase in accumulation rates of heavy metals in the environment, especially in response to human activities, has drawn increasing and pertinent interest from the society, due to the damages that these elements can cause to ecosystems. In Brazil, the occurrence of degraded areas due to the presence of heavy metals has become, in general, increasingly frequent and worrying.

Among the heavy metals, lead (Pb) has stood out as one of the most pollutant, which can be primarily attributed to its large industrial use (industry of extraction, petroleum, accumulators, inks and dyes etc.) (Kabata-Pendias, 2010; Kacálková et al., 2014). Soil contamination with Pb can result in a series of environmental problems, including loss of vegetation, contamination of surface waters and aquifers, besides direct toxicity to microorganisms, animals and humans (Huang & Cunningham, 1996; Gupta et al., 2013). Thus, the rehabilitation of soils contaminated with this element is of great importance, due to its strong pressure on the equilibrium and quality of the ecosystems.

The traditional physical and chemical methods of recovery of areas contaminated with Pb and other heavy metals usually involve excavation and removal of soil layer, physical stabilization and/or washing the soil with strong acids, which, besides being of difficult execution, are costly and aggressive to the environment (Seth et al., 2011; Tangahu et al., 2011). Because of these factors, preference has recently been given to in-situ methods that cause less disturbance in the environment. Phytoremediation, which consists in the use of plants and their associated microbiota to reduce the concentration or toxic effects of the contaminants in the environment (Ali et al., 2013), has drawn great interest from the scientific community, because, compared with traditional remediation methods, this technique has low cost, high decontamination efficiency and is environmentally less impactful (Tangahu et al., 2011; Ali et al., 2013).

Two phytoremediation strategies can be employed in the recovery of Pb-contaminated soils: phytostabilization and phytoextraction (Huang & Cunningham, 1996; Butcher, 2009), and the latter has called the attention of researchers, because it shows better results in the rehabilitation of soils contaminated by heavy metals (Alves et al., 2008; Ali et al., 2013).

Phytoextraction is based on the growth of tolerant plants, with capacity to accumulate high amounts of heavy metals in shoot dry matter (> 100 mg kg^{-1} for Cd, > 1,000 mg kg^{-1} for Co, Cu, Cr, Ni and Pb and > 10,000 mg kg^{-1} for Zn and Mn) (Krämer, 2010; Ent et al., 2013). These species are known as hyperaccumulators and, after cultivation, they are removed from the area (Krämer, 2010; Ent et al., 2013).

Approximately 500 plant species are reported as hyperaccumulators of heavy metals, although just a few are recognized as Pb hyperaccumulators (only 14 plant species) (Krämer, 2010; Ent et al., 2013). Therefore, the selection of plant species with potential to hyperaccumulate Pb is essential for the success of phytoremediation programs of Pb-contaminated areas. This becomes more relevant for tropical conditions like in Brazil, since most plants known to have phytoremediation potential are from temperate climates (Alves et al., 2008). For this selection, it is essential to conduct studies on tolerance, absorption and accumulation of Pb by different plant species.

Given the little knowledge on Pb-hyperaccumulating species, this study aimed to evaluate the potential of sunflower, castor bean, common buckwheat and vetiver as Pb phytoaccumulators.

Material and Methods

The study was conducted in a screened greenhouse of the Department of Soils and Rural Engineering (DSRE) of the Center of Agricultural Sciences (CCA) of the Federal University of Paraíba (UFPB), in Areia-PB, Brazil, using plants of sunflower (*Helianthus annuus* L.), castor bean (*Ricinus communis* L.), common buckwheat (*Fagopyrum esculentum* Moench) and vetiver [*Chrysopogon zizanioides* (L.) Roberty] cultivated in nutrient solution containing increasing doses of Pb. These species were selected due to their adaptation to the conditions of the Northeastern semiarid region, little demanding in soil conditions and tolerant to water stress.

The seedlings of sunflower, castor bean and common buckwheat were produced from seeds, using washed sand as substrate. Ten days after emergence and standardization with respect to size and number of definitive leaves, the seedlings were transplanted to the nutrient solution. Vetiver seedlings were produced through tillering of pre-existing clumps, standardized with respect to the mass (±5 g) and then transplanted to nutrient solution.

After transplanting, the plants were cultivated in static hydroponics, in plastic pots containing 5.5 L of Hoagland's nutrient solution (Hoagland & Arnon, 1938), two plants per pot and receiving doses of Pb (0, 50, 100, 200 and 400 mg L^{-1}) in the form of lead acetate [$Pb(CH_3COO)_2$]. Plants were kept in these solutions for 30 days and pH was daily corrected to ±5.5 with diluted solutions of NaOH or HCl. The volumes of the pots were filled whenever necessary, using deionized water. The nutrient solution was maintained under constant aeration through forced air injection.

After growth for 30 days in the solutions, the plants were cut, divided into roots and shoots, washed with tap water and deionized water and dried in a forced-air oven (65 °C) until constant weight, for the determination of total, shoot and root dry matter.

Dried plant samples were ground and, after nitric-perchloric digestion (Tedesco et al., 1995), analyzed for Pb contents through atomic absorption spectrophotometry. Based on the Pb contents in the plants and dry matter production data, the contents of Pb were determined in the roots and shoots.

The experiment was set in a completely randomized split-plot design, with 4 x 5 factorial and three replicates. The main plots were represented by the 4 species (castor bean, sunflower, vetiver and common buckwheat), while the subplots were represented by 5 doses of Pb (0, 50, 100, 200 and 400 mg L^{-1}). The obtained results were subjected to analysis of variance and a follow-up analysis for the quantitative effect of Pb doses in regressions, at 0.05 probability level. For the dry matter production of the different plant compartments, the logistic dose-response model was used ($\hat{y} = a/[1+(X/b)^c]$) (Streibig et al., 1993). The Pb content necessary to limit by 50% (I_{50}) plant growth (“b” parameter of the model) was determined from the estimation of the logistic model parameters. For the other variables, first-, second- and third-order polynomial models were used. Pb percentages accumulated in the roots and shoots of the evaluated species were compared by Scott-Knott test at 0.05 probability level. All the analyses were performed using the program SAEG, version 9.1 (SAEG, 2007).

Results and Discussion

Considering the low Pb concentrations in the solution, there was different growth of the species in response to the applied doses (Figure 1), confirming the fact that plant species respond differently to the exposure to Pb (Huang & Cunhingham, 1996; Alves et al., 2008; Zhivotovsky et al., 2011; Gupta et al., 2013).

In comparison to the other species, castor bean showed higher dry matter production regardless of the applied doses, followed by sunflower, vetiver and common buckwheat (Figure 1). It should be noted the higher dry matter production of vetiver at the Pb concentrations close to the maximum dose (400 mg L^{-1}), while common buckwheat showed lower growth in all the range of concentrations.

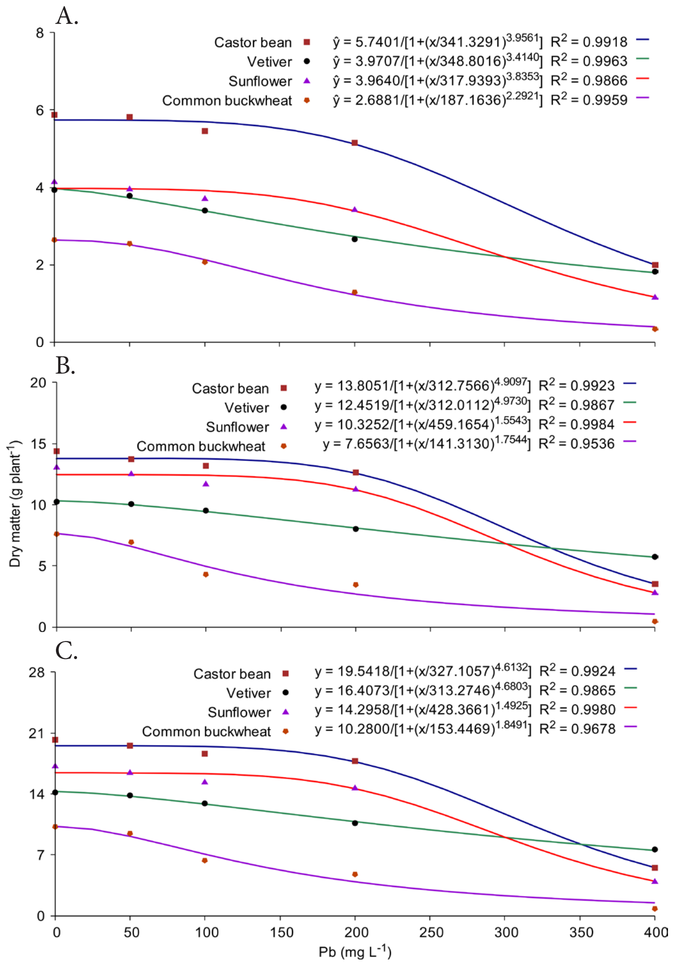

Figure 1. Dry matter production of roots (A), shoots (B) and total (C) of sunflower, castor bean, common buckwheat and vetiver

For sunflower and castor bean, the increase in Pb concentration in the solution to values close to 100 mg L^{-1} did not result in decrease in dry matter production of all the compartments (roots, shoots and the entire plant). From 100 and 200 mg L^{-1} of Pb, there was a slight decrease in production, which started to drop more sharply at Pb concentrations above 200 mg L^{-1}, with minimum production at the Pb dose of 400 mg L^{-1} (Figure 1). In the range of 0.0-150 mg L^{-1} of Pb, the growth of plants from these two species was almost uniform, both in roots (Figure 1A) and shoots (Figure 1B), indicating low toxicity of Pb in the range of concentrations. The maximum Pb concentration (400 mg L^{-1}) caused sharp decreases in total dry matter (75.84% for sunflower and 71.67% for castor bean) (Figure 1C), which shows the toxic effects of Pb at this concentration. Also for common buckwheat, at the maximum Pb concentration (400 mg L^{-1}), there was a sharp decrease in total dry matter (85.47%) (Figure 1C); this crop showed the lowest dry matter production at all the Pb concentrations in the solution (Figure 1).

Common buckwheat showed the lowest growth potential, with and without Pb supply, and was the most sensitive species to this element (Figure 1). On the other hand, vetiver showed higher total dry matter production compared with sunflower, at Pb concentrations higher than 300 mg L^{-1}, and with castor bean at Pb concentrations higher than 350 mg L^{-1} (Figure 1C). Additionally, the effect of the maximum Pb dose (400 mg L^{-1}) was much less drastic (reduction of 47.45% in total dry matter) for the vetiver, in comparison to the other crops (Figure 1C). These results suggest higher tolerance of vetiver to high Pb concentrations, in comparison to the other three species, indicating its potential, among the tested plants, to be used as phytoremediation plant.

Other authors (Paiva et al., 2000; Boonyapookana et al., 2005; Alves et al., 2008; Pereira et al., 2010; Danh et al., 2011; Seth et al., 2011; Zhivotovsky et al., 2011; Hamadouche et al., 2012; Hamvumba et al., 2014) also found reductions of dry matter production in many plant species as a function of the application of increasing doses of Pb in experiments with soil and nutrient solution. The Pb toxicity observed in the present study is due to its action on plants, causing physiological, biochemical and structural disorders, such as disequilibrium in water and hormone balance, nutritional disorders, loss of membrane permeability, reduction of enzymatic activity, inhibition of respiration and photosynthesis, among others (Yadav, 2010; Gupta et al., 2013).

The values of I_{50} (Pb concentrations responsible for the inhibition of 50% of dry matter production), in roots, shoots and in the entire plant, were higher in vetiver plants, followed by castor bean, sunflower and common buckwheat (Table 1), indicating the same order of tolerance to Pb (vetiver > castor bean > sunflower > common buckwheat). The highest values of I_{50} in the roots, compared with the shoots, in plants of sunflower, castor bean and common buckwheat, indicate that in these crops the highest tolerance occurs in the roots, similar to the observations of Paiva et al. (2000) in seedlings of ‘ipê-roxo’. On the other hand, for vetiver, the opposite was

Table 1. Pb concentrations responsible for the inhibition of 50% of dry matter production (I_{50}) in roots, shoots and in the entire plants of sunflower, castor bean, common buckwheat and vetiver

Species	Compartment		
	Roots	Shoots	Entire plant
	I_{50} (mg L^{-1})		
Sunflower	318	312	313
Castor bean	341	322	327
Common buckwheat	187	141	153
Vetiver	349	459	428

observed, with higher tolerance to Pb in the shoots and higher sensitivity in the roots, corroborating the data obtained by Alves et al. (2008).

Recently, Roongtanakiat & Sanoh (2011) verified that vetiver can grow well in soil contaminated with Pb and other metals (Zn and Cd) without any symptom of toxicity, showing capacity to tolerate contamination with heavy metals. In hydroponic cultivation, Alves et al. (2008) showed that vetiver seedlings grown in nutrient solution with 200 mg L^{-1} of Pb, during 45 days, suffered growth reduction lower than 25%.

Such tolerance of vetiver to Pb is probably due to the more efficient mechanisms of defense and detoxification existing in this species (Andra et al., 2009). The compartmentalization in subcellular structures, the decrease in transport through the membrane and formation of chelating peptides rich in proteins, such as phytochelatins, are some of these mechanisms that help, directly or indirectly, plants tolerate the accumulation of Pb and other heavy metals (Yadav, 2010; Hossain et al., 2012; Gupta et al., 2013).

In general, in the studied species, the highest contents of Pb were found in roots, in comparison to the shoots (Figures 2 and 3).

Castor bean and vetiver showed high accumulation of Pb in the roots (85% or more) and lower presence in the shoots (15% or less); in sunflower, the Pb retained in the roots decreased (66%), while it increased in the shoots (34%); for common buckwheat, there was a balance between roots (48%) and shoots (52%) (Table 2). Except for hyperaccumulator species, the tendency of low translocation and mobility of Pb, with intense accumulation in the roots, has been largely documented in the literature for many plant species (Huang & Cunhingham, 1996; Alves et al., 2008; Danh et al., 2011; Zhivotovsky et al., 2011; Hamadouche et al., 2012; Hamvumba et al., 2014; Kacálková et al., 2014; Selamat et al., 2014). The intense retention of Pb in the roots is related to its high affinity to the negative charges of the cell wall, especially in external tissues (rhizodermis and cortex), and to physiological barrier of Casparian strips and plasmalemma of endodermal cells, which restrict the access of Pb to the xylem, reducing its translocation to the shoots (Zhivotovsky et al., 2011). The higher retention of heavy metal ions in the roots and the low translocation to the shoots combat the toxicity of these elements in plant species (Hossain et al., 2012; Gupta et al., 2013).

The contents of Pb in the different compartments of the studied species increased quadratically as a function of the increment in Pb doses in the solution; in most cases, maximum points were observed within the range of the studied doses (Figures 2 and 3). These results corroborate those observed by other authors (Alves et al., 2008; Roongtanakiat & Sanoh, 2011), who also reported increase in the accumulation of Pb in various plant species as a function of the application of increasing Pb doses in experiments with soil and nutrient solution.

In the roots, there were maximum Pb contents of 24,762 mg kg^{-1} (Pb dose of 303 mg L^{-1}) in vetiver, 12,897 mg kg^{-1} (Pb dose of 400 mg L^{-1}) in castor bean, 9,517 mg kg^{-1} (Pb dose of 400 mg L^{-1}) in sunflower and 2,734 mg kg^{-1} (Pb dose of 299 mg L^{-1}) in common buckwheat (Figure 2A).

The highest Pb contents in the shoots were observed in common buckwheat (1,628 mg kg^{-1} at the Pb dose of 384 mg L^{-1}) and sunflower (1,341 mg kg^{-1} at the Pb dose of 308 mg L^{-1}), while for castor bean the maximum content was 898 mg kg^{-1} and for vetiver 920 mg kg^{-1} (both at the Pb dose of 400 mg L^{-1}) (Figure 2B). The Pb contents observed in the shoots of common buckwheat and sunflower are higher than the limit

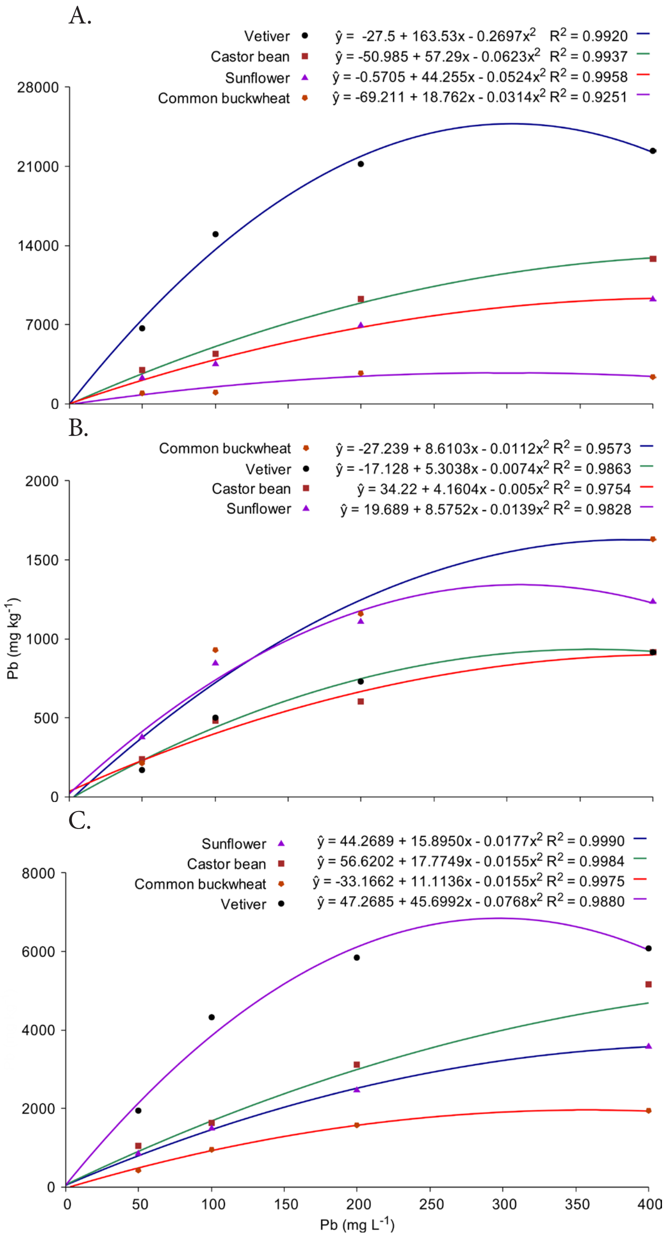

Figure 2. Pb contents (mg kg^{-1}) in dry matter of roots (A), shoots (B) and the entire plant (C) of sunflower, castor bean, common buckwheat and vetiver

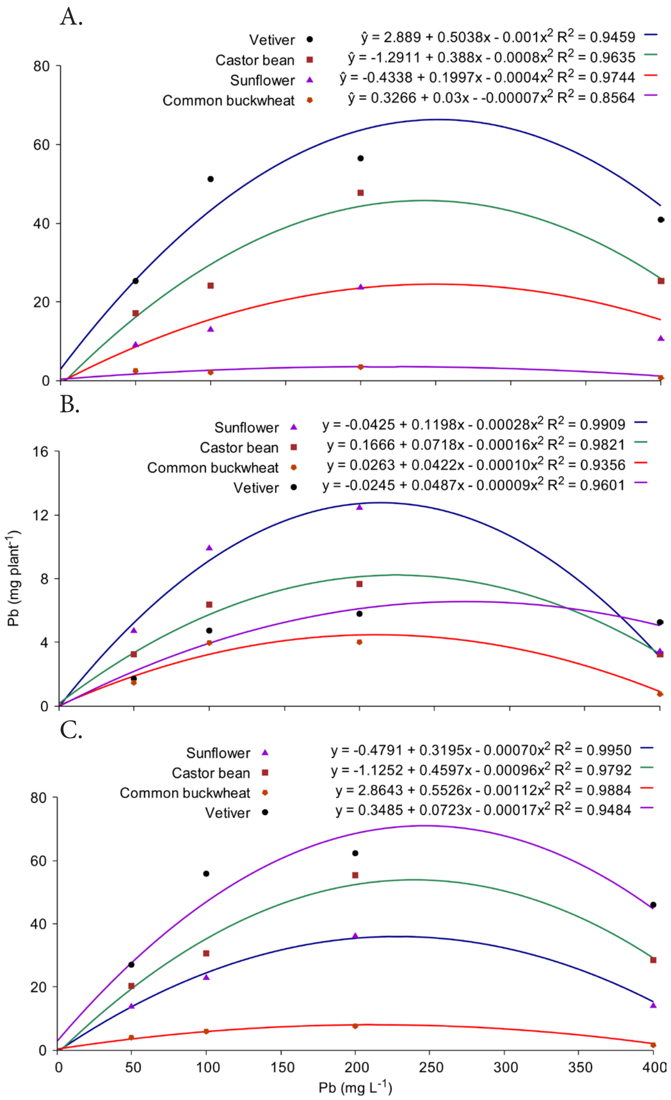

Figure 3. Pb contents (mg plant^{-1}) in roots (A), shoots (B) and in the entire plant (C) of sunflower, castor bean, common buckwheat and vetiver

that characterizes hyperaccumulator species (1,000 mg kg^{-1} of Pb in shoot dry matter) (Krämer, 2010; Ent et al., 2013). Tamura et al. (2005), in common buckwheat cultivated in soil contaminated with Pb, observed 8,000 mg kg^{-1} of Pb in the leaves and 3,300 mg kg^{-1} of Pb in the roots, showing that the plant is hyperaccumulator of Pb. For sunflower cultivated in nutrient solution with Pb, Boonyapookana et al. (2005) found 3,611 mg kg^{-1} of Pb in the leaves and concluded that this species is hyperaccumulator of Pb.

The maximum Pb contents in the roots were 60.6 mg plant^{-1} (Pb dose of 252 mg L^{-1}) for vetiver, 45.8 mg plant^{-1} (Pb dose of 243 mg L^{-1}) for castor bean, 22.8 mg plant^{-1} (Pb dose of 232 mg L^{-1}) for sunflower and 3.56 mg plant^{-1} (Pb dose of 214 mg L^{-1}) for common buckwheat (Figure 3A).

In the shoots, the correspondent values of Pb contents were 12.8 mg plant^{-1} (Pb dose of 271 mg L^{-1}) for sunflower, 8.22 mg plant^{-1} (Pb dose of 224 mg L^{-1}) for castor bean, 6.56 mg plant^{-1} (Pb dose of 214 mg L^{-1}) for vetiver and 4.48 mg plant^{-1} (Pb dose of 211 mg L^{-1}) for common buckwheat (Figure 3B).

Sunflower showed the highest Pb contents in the shoots, with maximum absorption efficiency in the range between 200 and 250 mg L^{-1} of Pb (Figure 3B). Considering the potential for Pb phytoextraction, sunflower seems to have good characteristics, corroborating what has been proposed by other authors (Boonyapookana et al., 2005; Seth et al., 2011; Kacálková et al., 2014; Sewalem et al., 2014). However, it should be pointed out that the data refer to the absorption in nutrient solution and the results must be confirmed with cultivation in soils. On the other hand, although the common buckwheat showed the highest Pb contents in the shoots (Figure 2B), the low Pb contents found in the plant (Figure 3) reflect the low dry matter production of the crop. Despite having characteristics of Pb hyperaccumulator, the low dry matter production of common buckwheat does not favor its use for the technique of phytoremediation. Plants of hyperaccumulator species are characterized, in general, by the low production of biomass, which limits its use for phytoextraction (Alves et al., 2008; Zhivotovsky et al., 2011).

The great capacity of Pb accumulation in the roots and the low translocation of this element to the shoots in castor bean and especially in vetiver (Figures 2 and 3; Table 2) suggest that these species have great potential for phytostabilization of Pb in the soil (Butcher, 2009; Roongtanakiat & Sanoh, 2011). Such accumulation occurred with maximum efficiency in the range from 200 to 250 mg L^{-1} of Pb (Figures 2 and 3). Romeiro et al. (2006) observed that castor bean plants cultivated under Pb concentrations from 0 and 400 µmol L^{-1}, in nutrient solution, accumulated ≈ 25,000 g kg^{-1} of Pb in root dry matter. In vetiver, great capacity of Pb accumulation in the roots and low translocation of this element to the shoots were shown by other authors (Alves et al., 2008; Andra et al., 2009; Danh et al., 2011), highlighting its great potential for Pb phytostabilization.

Table 2. Percentage distribution of Pb accumulated in roots and shoots of plants of sunflower, castor bean, common buckwheat and vetiver

Pb (mg L^{-1})	Sunflower		Castor bean		Common buckwheat		Vetiver	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
	(%)							
0	-	-	-	-	-	-	-	-
50	66	34	84	16	62	38	94	6
100	57	43	80	20	34	66	92	8
200	66	34	86	14	47	53	91	9
400	76	24	89	11	50	50	88	12
Mean	66 Ab	34 Bb	85 Aa	15 Bc	48 Ac	52 Aa	91 Aa	9 Bc

Means followed by different letters are statistically different by the Scott-Knott test at 0.05 probability level. Upper case letters compare the compartments within each species. Lowercase letters compare each compartment between species

For vetiver, the higher accumulation of Pb in the roots compared with the shoots at the maximum dose (400 mg L^{-1} of Pb) (Table 2) coincided with the lower content of this element in the shoots (Figure 2) and with the higher dry matter production in both roots and shoots (Figure 1A and 1B). As Pb doses increased, vetiver showed increment in the percentage of Pb in the shoots, with simultaneous decrease in the roots (Table 2). Additionally, among the studied species, vetiver showed the highest Pb content in the shoots of plants exposed to the maximum Pb dose (Figure 3B). Therefore, it is possible that, when grown in environments with high Pb concentrations, vetiver has some capacity to mobilize the element from the roots to the shoots, which would indicate the possibility of its utilization in the phytoextraction of Pb under these conditions.

Conclusions

1. Sunflower, castor bean, vetiver and common buckwheat respond differently with respect to tolerance, absorption, distribution and accumulation of Pb in the plants.

2. Among the tested crops, vetiver is the most tolerant to Pb, followed by castor bean, sunflower and common buckwheat.

3. Common buckwheat is not much promising for Pb phytoremediation programs. Sunflower showed potential for phytoextraction of Pb and castor bean and vetiver are more indicated for Pb phytostabilization.

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