Induced changes in the growth of four plant species due to lead toxicity

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Abstract
Lead is a toxic metal that affects plant growth and the ecosystem. This study evaluated the lead (Pb) bioaccumulation potential of vetiver (Vetiveria zizanioides L.), sunflower (Helianthus annuus L.), elephant ear (Alocasia macrorrhiza) and ‘embaúba’ (Cecropia sp.). The plant species were tested in a 50% nutrient solution - Hoagland and Arnon, constantly aerated, containing five Pb concentrations: 0, 50, 100, 200 and 400 mg L⁻¹. The treatments were arranged in a completely randomized design in a 4 x 5 factorial scheme, with four replicates. The Pb contents in the plants increased linearly with the Pb exposure concentration. Vetiver was the species with the highest Pb content in the shoots (260.24 mg kg⁻¹) and sunflower, in the roots (44925.20 mg kg⁻¹). Pb concentration of up to 100 and 50.9 mg L⁻¹ stimulated sunflower biomass and root length, respectively. None of the evaluated species met the hyperaccumulator criterion; however, sunflower and vetiver have the potential to be tested for phytostabilization purposes.

Key words: trace metals remediation phytostabilization

Palavras-chave: metal traço remediação fitoestabilização

Mudanças induzidas no crescimento de quatro espécies vegetais pela toxicidade de chumbo

Resumo
O chumbo é um metal tóxico que impacta o crescimento de plantas e os ecossistemas. Avaliou-se o potencial de bioacumulação de Pb nas espécies vetiver (Vetiveria zizanioides L.), girassol (Helianthus annuus L.), orelha de elefante (Alocasia macrorrhiza) e embaúba (Cecropia sp.) em cinco concentrações de Pb (0, 50, 100, 200 e 400 mg L⁻¹) em solução nutritiva Hoagland e Arnon, meia força, constantemente aerada. Os tratamentos foram dispostos em delineamento experimental inteiramente casualizado em esquema fatorial 4 x 5, com 4 repetições. Durante 21 dias, a concentração do metal nas plantas aumentou linearmente com a concentração de exposição ao Pb. O vetiver foi a espécie com maior concentração de Pb na parte aérea (260.24 mg kg⁻¹) e girassol na raiz (44925.20 mg kg⁻¹). A biomassa e o comprimento radicular de girassol foram estimulados até a concentração de 100 e 50.9 mg L⁻¹ de Pb, respectivamente. Nenhuma das espécies atendeu aos critérios de hiperacumulação; entretanto, girassol e vetiver devem ser testados como espécies fitoestabilizadoras.
**Introduction**

Among the 275 priority substances of the USA, based on frequency, toxicity and exposure potential, lead (Pb) is considered as the second most toxic, according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2015). According to this environmental agency, Pb concentrations between 10 and 40 µg dL\(^{-1}\) in the blood cause adverse effects on human health (neurological, hematologic and renal systems). Despite the effects caused on soils, water, atmosphere and living organisms, Pb continues to be widely used in industrial processes.

Pb-contaminated areas represent danger to living organisms and must be remediated for human benefit and agroecological sustainability. The municipality of Santo Amaro, Bahia, is the example of Pb contamination from galena processing activities. The soil of this area has been contaminated by the direct disposal of residues and deposition of particles emitted by the chimney, in a wide range of Pb concentration, between 2.03 and 12,678 mg kg\(^{-1}\), at the depth of 0-5 cm (Asevedo, 2012), evidencing the need for adopting remediation methods.

Phytoextraction has been indicated as a low-cost alternative to extract the metal present in the soil and water (Bhargava et al., 2012), if the concentration in the soil allows plant growth. This technique is based on the assumption of the existence of plants that are tolerant to the metal. Toxic effects have been observed on plants with Pb concentrations between 30 and 300 mg kg\(^{-1}\) (Shikhova, 2012); this wide range of toxicity reveals different levels of tolerance, combined with the fact that Pb does not have a known biological function (Ali et al., 2013).

The selection of phytoextraction plants aims to identify species that are tolerant to the contaminant, which must exhibit characteristics such as fast growth and sufficient biomass production to accumulate greater contents of metals, especially in the shoots (Paz-Alberto & Sigua, 2013). Plants intended for phytostabilization must be limited to the accumulation of the metal in the roots (Magalhães et al., 2011).

Hydroponics has been used to select plants and determine the efficiency of absorption and tolerance to metals in potentially phytoremediation species (Espinoza-Quinones et al., 2013), but studies under greenhouse and field conditions are necessary to validate the obtained results. This study compared the absorption, potential of distribution, effect on root system and level of tolerance to Pb of four plant species under hydroponic conditions.

**Material and Methods**

The experiment was carried out in a greenhouse of the Soils and Quality of Ecosystems Graduate Program of the Federal University of Recôncavo of Bahia, municipality of Cruz das Almas, BA, Brazil.

The study evaluated the capacity of four plant species: vetiver (Vetiveria zizanioides L.), elephant ear (Alocasia macrorrhiza) and ‘embáuba’ (Cecropia sp.) to absorb, tolerate and distribute Pb in the different plant parts when exposed to the Pb concentrations of 0, 50, 100, 200 and 400 mg L\(^{-1}\), applied in the form of Pb acetate (AL) (Pb(C\(_2\)H\(_3\)O\(_2\))\(_2\)3H\(_2\)O), referred to as C\(_0\), C\(_{50}\), C\(_{100}\), C\(_{200}\) and C\(_{400}\), respectively. The treatments were arranged in completely randomized design in a 4 x 5 factorial scheme, with four replicates, and the experimental unit consisted of one plant per pot (capacity for 3 L), with 2.5 L of nutrient solution (pH 5.0 ± 0.2).

The seedlings were acclimated for 10 days in nutrient solution of Hoagland & Arnon (1950), 50% ionic force, without Pb (C\(_0\)). After this period, the nutrient solution was replaced and added of Pb acetate at the concentrations indicated in the treatments. One control treatment, without Pb (C\(_0\)), was also evaluated. Twenty-one days after the beginning of the exposure to Pb, the plants were harvested and washed in running water, in a solution of 0.1 M HCl and distilled water. Then, the plants were divided into shoots and roots, and their biomasses were determined after drying at 65 ºC for 72 h, in a forced-air oven.

Roots were washed using a fine mesh sieve and air-dried; then, they were digitalized using a scanner (Hp G4050, 100 dpi resolution, shades of gray and bitmap format) and processed in the application GSRoot (Guddanti & Chambers, 1993), to obtain the root length for the diameter classes of < 0.1; 0.1-0.25; 0.25-0.5; 0.5-0.75; 0.75-1.0; 1.0-2.0 and > 2.0 mm.

After drying, the plant material was weighed on a semi-analytical scale (Marte, AC200, 210 g x 0.001 g) and ground in a TE 650 Wiley-type mill (1-mm-mesh screen) for digestion. In summary, 0.500 g of the biomass were weighed and mixed with 5 mL of 65% H\(_2\)NO\(_3\), which was cold-digested for 16 h. Then, temperature was gradually increased to 80 ºC and, after successive additions of 30% H\(_2\)O\(_2\) until the volume of 3 mL, the temperature was increased to 140 ºC, the extracts were filtered and the volume completed to 25 mL with 2% H\(_2\)NO\(_3\). The Pb contents were determined through atomic absorption (Varian AA 240F, Varian Instruments, Australia) and the bioaccumulation (BF) and translocation (TF) factors were calculated through Eqs. 1 and 2.

\[
BF = \frac{\text{Pb content in the shoots or roots}}{\text{Pb concentration in the nutrient solution}} \quad (1)
\]

\[
TF = \frac{\text{Pb content in the shoots}}{\text{Pb content in the roots}} \quad (2)
\]

According to the ability to accumulate Pb, the species were classified as accumulators (BF > 1); indicators (BF = 1) and excluders (BF < 1) (Accioly & Siqueira, 2000), while those with TF > 1 were classified as phytoextractors.

The data was statistically analyzed using the statistical package Sisvar 5.3 (Ferreira, 2011). For the attributes with significant interaction (p < 0.05), the species was fixed to calculate the behavior of the attributes at the different Pb concentrations using regression equation, with minimum determination coefficient of 65%.

**Results and Discussion**

After 21 days of exposure to Pb, its contents in shoot and roots of the species increased linearly (p < 0.01) with the concentration (C) in the solution (Table 1). The low Pb contents found at
Table 1. Pb contents in the shoots and roots (mg kg\(^{-1}\)) of the species, in response to the increasing Pb concentrations (mg L\(^{-1}\)) in the nutrient solution

| Pb mg L\(^{-1}\) | Shoots Vetiver | Shoots Sunflower | Shoots Elephant ear | Shoots 'Embaúba' |
|-----------------|----------------|-----------------|---------------------|-----------------|
|                 | Roots Vetiver  | Roots Sunflower | Roots Elephant ear  | Roots 'Embaúba' |
| 0               | 7.54           | 338.21          | 6.12                | 60.23           |
|                 | 8.85           | 11.310.8        | 13.95               | 1.833.93        |
|                 | 19.95          | 2.276.96        | 35.96               | 2.410.79        |
| 200             | 110.77         | 4.356.25        | 35.79               | 4.063.89        |
|                 | 78.36          | 7265.32         | 35.96               | 2.617.15        |
| 400             | 260.24         | 13.790.44       | 78.36               | 4.063.89        |
| Intercept       | 10.40          | 33.71           | 9.21                | 4089.34         |
| LC              | 0.65**         | 32.13**         | 0.20**              | 106.04**        |
| R²              | 0.98           | 0.96            | 0.95                | 0.97            |

** Significant at 0.01 probability level by F test. Intercept, linear coefficient (LC) and correlation coefficient (R²) of the regression equation

Table 2. Bioaccumulation factor (BF) in shoot and root biomass of the species and translocation factor (TF) of roots, in response to the Pb concentrations

| Pb mg L\(^{-1}\) | Shoots Vetiver | Shoots Sunflower | Shoots Elephant ear | Shoots 'Embaúba' |
|-----------------|----------------|-----------------|---------------------|-----------------|
|                 | Roots Vetiver  | Roots Sunflower | Roots Elephant ear  | Roots 'Embaúba' |
| 50              | 0.467          | 44.783          | 0.011               | 0.654           |
|                 | 0.279          | 33.673          | 0.002               | 0.455           |
| 100             | 0.360          | 35.508          | 0.010               | 0.300           |
|                 | 0.200          | 22.770          | 0.002               | 0.360           |
| 200             | 0.554          | 21.781          | 0.026               | 0.229           |
|                 | 0.197          | 21.163          | 0.001               | -               |
| 400             | 0.651          | 34.476          | 0.019               | -               |
| CV (%)          | 16.84          | 28.47           | 22.46               | 15.40           |

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All evaluated species showed TF < 1, which confirms the low ability to translocate Pb from roots to shoots (Table 2), a desirable factor for phytostabilization species. Evaluating two species of vetiver (Vetiveria zizanioides and Vetiveria nemoralis), Aksorn & Chitsomboon (2013) identified that both can effectively act as phytostabilizers for Pb, due to the high contents of the metal in the roots, with values of TF < 1. The results of this study show that, among the evaluated species and despite the TF < 1, vetiver was the most efficient in the translocation of Pb, followed by elephant ear and 'embaúba', at all evaluated concentrations.

The exposure of the species to Pb negatively influenced the shoot and root biomass production of vetiver, elephant ear and 'embaúba'. This effect increased with the Pb exposure concentration (Table 3), except for sunflower.

In sunflower, the exposure to Pb concentrations of up to 100 mg L\(^{-1}\) stimulated biomass production and, for higher concentrations, there was a reduction in biomass production (Table 3). Senth et al. (2011) also observed increase in shoot biomass (6%) and root biomass (13%) of sunflower when exposed to a Pb concentration (20 µM = 4.1 mg L\(^{-1}\)) in hydroponic system. With a concentration approximately 25 times higher than that of the present study, shoot and root biomasses in sunflower were 22.80 and 30.12%, respectively, higher than that reported by Seth et al. (2011).

The shoot biomasses of vetiver, elephant ear and 'embaúba', in the control treatment (C\(_0\)), were higher in the presence of any Pb concentration (Table 3). At C\(_{90}\), shoot biomass was 90.71% ('embaúba') to 81.05% (elephant ear) lower in relation to the biomass in the control treatment. Pb also limited root biomass production, in lower intensity than in the shoots, varying from 62.17% (elephant ear) to 85.24% (sunflower) in relation to the control. The smaller effect of Pb on the biomass of elephant ear can be attributed to the lower Pb contents in its shoots and roots.

The limitation of plant growth by the presence of Pb, reducing biomass, has been attributed to the negative effect concentration zero (C\(_0\)) must be attributed to the impurities of the reagents used. The Pb content in the shoots at C\(_{400}\) followed a decreasing order: vetiver (260.24 mg kg\(^{-1}\)) > sunflower (91.67 mg kg\(^{-1}\)) > elephant ear (78.36 mg kg\(^{-1}\)). Since the Pb content in shoot biomass was below 1000 mg kg\(^{-1}\), none of the species could be classified as hyperaccumulator of Pb (Accioly & Siqueira, 2000). The decreasing order of Pb contents in the roots was different from that obtained in the shoots: sunflower (44,925.17 mg kg\(^{-1}\)) > vetiver (13,790.44 mg kg\(^{-1}\)) > elephant ear (7,265.32 mg kg\(^{-1}\)). The 'embaúba' at C\(_{400}\) did not produce sufficient biomass for the determination of the Pb contents.

The Pb content in the root of sunflower at C\(_{400}\) was three times higher than that of vetiver (Table 1). The efficiency of vetiver to concentrate Pb was reported by Pidatala et al. (2016), due to its high growth rate, increasing the absorption of pollutants (Sharma et al., 2016).

The highest Pb contents in the roots of the evaluated species were similar to those found by Nascimento et al. (2014), who studied Pb accumulation in forage species in contaminated soils. The accumulation of Pb in the roots has been attributed to its low mobility in the plant, high affinity for the negative charges of the cell wall and to the physiological barriers of Casparian stripes, which restrict its access to the xylem, reducing the translocation (Zhivotovsky et al., 2011).

The Pb bioaccumulation factor in the shoots of the different species varied from 0.179 ('elephant ear') to 0.651 (vetiver). At all evaluated concentrations, except C\(_{400}\), vetiver was the species with highest Pb bioaccumulation in the shoots (Table 2). 'Embaúba' (C\(_{100}\)) and elephant ear (C\(_{200}\)) were the treatments with lowest Pb bioaccumulation in the shoot tissue.

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| 50              | 0.467          | 44.783          | 0.011               | 0.654           |
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| 100             | 0.360          | 35.508          | 0.010               | 0.300           |
|                 | 0.200          | 22.770          | 0.002               | 0.360           |
| 200             | 0.554          | 21.781          | 0.026               | 0.229           |
|                 | 0.197          | 21.163          | 0.001               | -               |
| 400             | 0.651          | 34.476          | 0.019               | -               |
| CV (%)          | 16.84          | 28.47           | 22.46               | 15.40           |
The Pb content of 30 mg kg\(^{-1}\) in shoot biomass was obtained when the Pb concentration in the solution was of 62 mg L\(^{-1}\) (vetiver) to 149 mg L\(^{-1}\) (‘embaúba’). The efficiency of vetiver (62 mg L\(^{-1}\)) to bioaccumulate Pb in the shoots, to achieve 30 mg Pb kg\(^{-1}\) in the biomass, was approximately 2.3 and 2.4 times higher than those of elephant ear (142 mg L\(^{-1}\)) and ‘embaúba’ (149 mg L\(^{-1}\)), respectively. The Pb accumulation of 30 mg kg\(^{-1}\) at a lower concentration, observed in vetiver, contributes to the anatomic modifications of the roots.

The greatest proportion of roots of the species occurred in the diameter range from 0.10 to 0.25 mm (Figure 1A, 1B, 1C and 1D). The exposure of the plant species to Pb limited root growth in all diameter ranges (Figures 1A, 1B, 1C and 1D).

Length reduction and the elongation of finer roots seem to be the most affected by Pb, which results in the decrease in the capacity of the plants to absorb water and nutrients and in biomass production (Castro et al., 2009).

Table 3. Shoot and root biomass production (g pot\(^{-1}\)) of the species, in response to the increasing Pb concentrations in the nutrient solution

Table 4. Root length (cm) of the species in response to the increasing Pb concentrations in the nutrient solution

Table 5. Alteration in the biomass and root length of the species when the Pb concentration in the hydroponic solution results in 30 mg kg\(^{-1}\) of Pb in the shoots
a smaller effect on shoot (-15%) and root (-12%) biomass production and root length (-13%), in comparison to elephant ear and ‘embuába’.

Although vetiver exhibited capacity to accumulate approximately 40% more Pb than sunflower (Table 2), the latter was more tolerant to the Pb concentration, as evidenced by the effect on shoot biomass (increase of 3%), root biomass (reduction of 1%) and root length (no effect) compared with the control. Seth et al. (2011) report that sunflower, for producing proteins that combat stress, is a tolerant species at low concentrations and short periods of exposure to Pb.

**Conclusions**

1. Vetiver showed higher capacity to concentrate Pb in the shoots and sunflower in the roots, compared with ‘embuába’ and elephant ear.

2. The evaluated species showed characteristics of phytostabilizers (TF < 1).

3. In general, Pb reduced the biomass production of the plants, especially of ‘embuába’.

4. Pb negatively interfered with the biomass production of the species, except sunflower, up to the concentrations of 100 mg L\(^{-1}\) of Pb for the shoots and 50.9 mg L\(^{-1}\) of Pb for the roots.

5. The species vetiver, sunflower and elephant ear are the ones with highest potential to be tested under field conditions.

**Acknowledgments**

The authors thank the National Council for Scientific and Technological Development (CNPq) (Protocol 561889/2010-4) and the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

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