

Original Research Article

LEAD NITRATE IN THE GERMINATION AND INITIAL DEVELOPMENT OF *Leucaena leucocephala* AND *Peltophorum dubium*

ABSTRACT

The objective of this study was to determine the influence of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) on the germination and initial development of leucaena (*Leucaena leucocephala*) and canafistula (*Peltophorum dubium*). The experimental design was a randomized complete block design in a 2 x 6 factorial scheme [two forest species and six $\text{Pb}(\text{NO}_3)_2$ doses], with 4 replicates per treatment, totaling 24 sample units for each species. The experiment was carried out in August 2015, in the botany laboratory of the Pontifical Catholic University of Paraná (PUCPR), Toledo, Paraná, Brazil. The seeds used were scarified and conditioned to germinate for 14 days in the presence of $\text{Pb}(\text{NO}_3)_2$ treatments in a germination chamber. At the end of 14 days were determined: total germination, chlorophyll content (a, b, total and chlorophyll a/b ratio), lead (Pb) contents in roots and leaves, as well as morphometric variables: shoot length, radicular and collecting diameter. *L. leucocephala* was the species that obtained the highest germination index and higher contents of chlorophyll a, b and total. In the species of *P. dubium* it was found higher levels of lead in the leaves, in the root system and higher chlorophyll a/b ratio. It was concluded that the doses of $\text{Pb}(\text{NO}_3)_2$ influenced the germination of the initial development of *L. leucocephala* and *P. dubium*, *L. leucocephala* being the species that best tolerated the doses administered. Increasing doses of $\text{Pb}(\text{NO}_3)_2$ influenced germination rates and initial development of *L. leucocephala* and *P. dubium* at the end of 14 days after sowing. According to the morphometric evaluations and analysis of lead content in roots and leaves, *P. dubium* was the species that showed the highest sensitivity in the presence of the doses, indicating the higher resistance of *L. leucocephala*, affirming its use in recovery of contaminated areas.

Keywords: Heavy metal; seedling development; recovery of degraded areas; germination test.

1. INTRODUCTION

The soil is the primary means of mineral nutrition of terrestrial plants, in which there is of course the occurrence of heavy metals, which vary in consequence of the source material of the soil [1]–[2]. The absorption of heavy metals by plants is directly related to soil pH, being one of the most representative [3].

Heavy metals generate difficulties for plants, since the same elements at low levels are essential, in high concentrations, may become harmful [1]. The lead (Pb) is the one of the heavy metals of greater importance, demonstrates the physiological, biochemical and structural funds in the plant, triggering symptoms in the reduction of the growth and germination [4].

The use of woody is an advantageous for the recovery of contaminated areas, once the metals absorbed were detained for a longer time in their plant tissues, causing the return of this element to the soil to be postponed [5]. Woody species usually employed in restoration of degraded areas are *Leucaena leucocephala* (Lam.) de Wit. and *Peltophorum dubium* (Spreng.) Taub., belonging to the family Fabaceae, known as leucaena and canafistula, respectively [6] [7] [8].

In this sense, the objective of this work was to determine the influence of the interaction of doses of $Pb(NO_3)_2$ on the germinative behavior and the development of seedlings of *L. leucocephala* and *P. dubium*, in order to characterize the sensitivity of species in the treatments.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The experiment was carried out in August 2015, in the botany laboratory of the Pontifical Catholic University of Paraná (PUCPR), Toledo, Paraná, Brazil, located in geographic coordinates Latitude 24° 42' 49" South, longitude 53° 44' 35" West and altitude of 560 m.

Seeds of *L. leucocephala* and *P. dubium* obtained from headquarters located at geographic coordinates Latitude 24° 42' 49" South, longitude 53° 44' 35" West, altitude of 560 m; latitude 24° 51' 51" South, longitude 54° 19' 49" West, altitude of 264 m, respectively.

The experimental design consisted of randomized blocks, in a 2x6 factorial scheme [two forest species and six doses of $Pb(NO_3)_2$, with 4 repetitions per treatment, totaling 24 sample units for each species.

The seeds were benefited and scarified using sandpaper nº 80 and placed to germinate in the gerbox-type boxes with blotter paper moistened with doses of $Pb(NO_3)_2$ (Table 1). The seeds were maintained in a germination chamber (25 ± 2 °C) for 14 days after sowing.

Table 1. Concentrations of $Pb(NO_3)_2$ applied in germination test of seeds of *L. leucocephala* and *P. dubium*, cultivated in a germination chamber (25 °C $\pm$ 2)

Treatments	$Pb(NO_3)_2$ g L ⁻¹
T1	0.000
T2	0.017
T3	0.038
T4	0.073
T5	0.148
T6	0.289

At the end of 14 days after the sowing was determined the total germination, and subsequently, randomly selected 10 seedlings of each species, which were evaluated shoot length, root and stem diameter, which was quantified by means of a caliper analog.

The determination of chlorophyll content was performed from 100 mg of fresh material of leaves of seedlings incubated with 7.0 mL of dimethyl sulfoxide (DMSO) for 30 minutes in a water bath at 65 °C. Subsequently, the material was subjected to simple filtration, being the volume supplemented with DMSO for 10 mL. Analysis was performed in a spectrophotometer (645 and 663 nm) and the values were submitted to the formula of Arnon [9]. From the analysis, were quantified levels of chlorophyll *a*, chlorophyll *b*, contents contents of total chlorophyll and chlorophyll *a/b* ratio.

The determination of Pb levels in the plant tissues (Pb concentration in the leaves and Pb concentration in the roots) was carried out in a specialized laboratory at the Universidade Estadual do Oeste do Paraná (UNIOESTE), Marechal Cândido Rondon, Paraná, Brazil localized in geographic coordinates Latitude 24° 33' 22" South, longitude 54° 03' 24" W and altitude 410 m, by means of a nitro perclórica digestion [10] and by atomic absorption spectrometry, modality flame [11].

It was performed an analysis of variance, and data whose F test was considered significant at 5% probability, regression analysis was performed in the statistical software Sisvar [12].

3. RESULTS AND DISCUSSION

In accordance with the data obtained at 14 days after sowing, the largest percentage of germination was observed in *L. leucocephala* (96%), while *P. dubium* presented only 31.83% germination at the end of the experiment.

The mean values of the percentage of the length of the aerial part of *L. leucocephala* feature first, increase, and the concentrations between 0.10 and 0.16 mg L⁻¹ Pb(NO₃)₂ those that resulted in higher values of shoot, and subsequently fall, demonstrating the harmful action of increasing doses of treatments (Figure 1a).

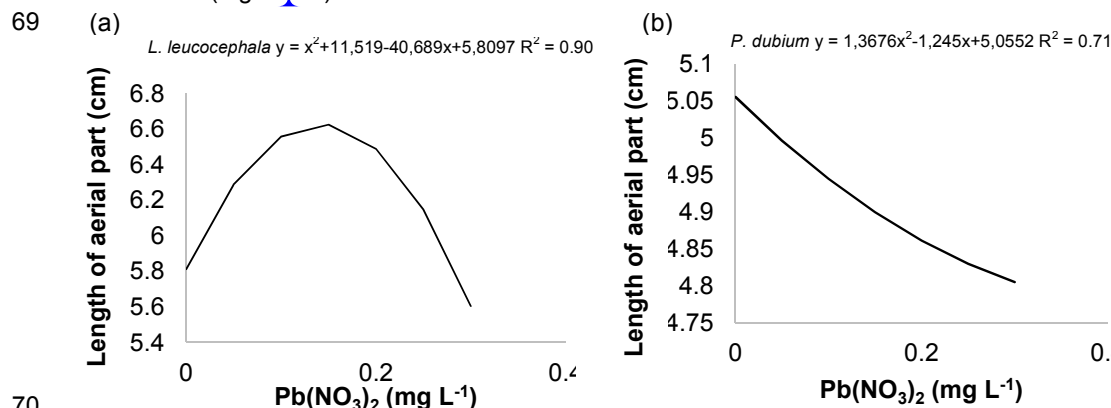


Fig. 1. Length of aerial part (cm) of seedlings of *L. leucocephala* (a) and *P. dubium* (b) cultivated in a germination chamber (25 °C ± 2) as a function of doses of Pb(NO₃)₂

The initial increase presented in the length of the aerial part in *L. leucocephala* demonstrates that the species has a tolerance in the presence of doses of Pb(NO₃)₂, being able to initially support the presence of heavy metal, showing to be a species rustic and well adapted, even when subjected to adverse situations, confirming its use in rehabilitation of degraded areas and reforestation [13].

In a study using sunflower seeds in soil contaminated with Pb, they observed variation in plant height [14], and in another study using tree species, seedling height was also influenced by Pb contamination [15], corroborating the results obtained in this search. The average values obtained for the length of the root system of *L. leucocephala*, there was a linear decrease, presenting the lowest values between the concentrations of 0.27 and 0.29 mg L⁻¹ Pb(NO₃)₂, (Figure 2a). To the extent that concentrations of Pb(NO₃)₂ increased, there was a decrease in the length of the root system, this being the most affected region in relation to the carrier.

In relation to the length of the root system of *P. dubium*, the mean values of the percentage obtained showed a curve to the minimum point at a concentration of 0.25 mg L⁻¹ Pb(NO₃)₂ (Figure 2b). The decrease of the figures was higher for the root system in relation to the carrier and in relation to the values obtained in the length of the root system of *L. leucocephala*, demonstrating that the root of this species was more susceptible to the damaging action of Pb.

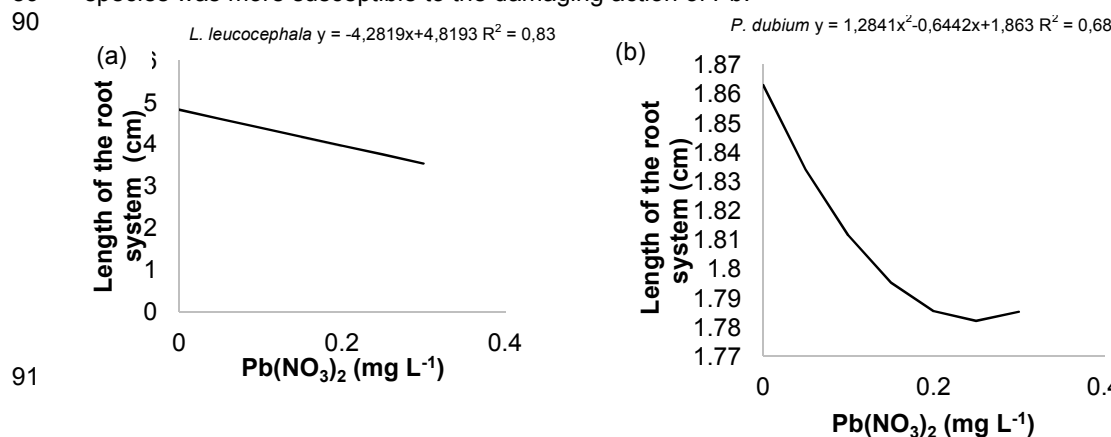


Fig. 2. Length of the root system (cm) of seedlings of *L. leucocephala* (a) and *P. dubium* (b) cultivated in a germination chamber ($25 \pm 2^\circ\text{C}$), as a function of doses of $\text{Pb}(\text{NO}_3)_2$

When present in cationic form, the Pb may be accidentally absorbed by plants because of its similarity with essential minerals. These when present in high concentrations can cause interference in cell division and inhibit the growth of the root system, due to the reduction of CO_2 assimilation caused by Pb. The Pb can also acts on the reduction of the concentration of calcium and magnesium in plants, causing thick roots and short. [16].

In stem diameter, the mean values of the percentage obtained in *L. leucocephala* showed maximum point at a concentration of $0.29 \text{ mg L}^{-1} \text{ Pb}(\text{NO}_3)_2$, with 0.21 mm (Figure 3), while the values of *P. dubium* proved indifferent to the doses, did not differ statistically.

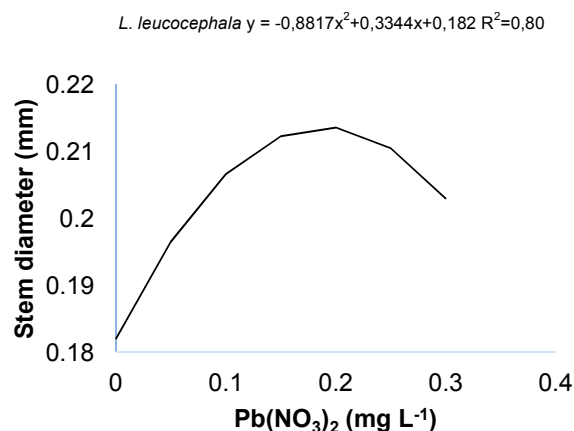


Fig. 3. Stem diameter (mm) of seedlings of *L. leucocephala* grown in a germination chamber ($25 \pm 2^\circ\text{C}$), as a function of doses of $\text{Pb}(\text{NO}_3)_2$

In addition to the morphometric variables, were also quantified the levels of Pb in dry matter of aerial part and roots of seedlings of *L. leucocephala* and *P. dubium*.

The content of Pb in roots in *P. dubium* presented maximum concentration of $317.94 \text{ mg kg}^{-1}$ of Pb in roots, while *L. leucocephala* obtained lower concentration in its root system, regardless of the concentration used, presenting a maximum value of 76.32 mg kg^{-1} of Pb in the root system (Figure 4).

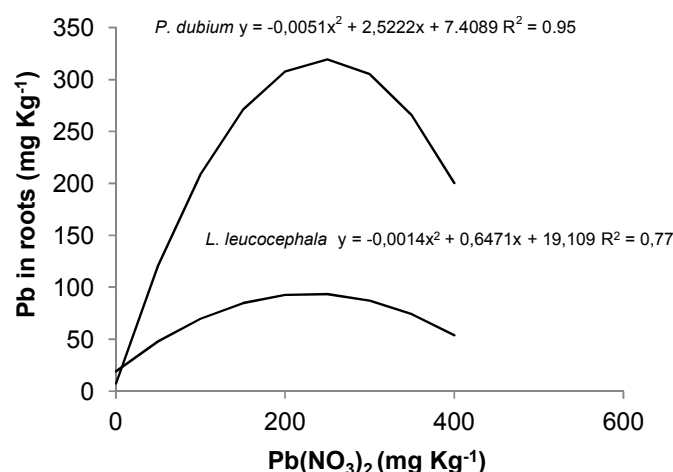


Fig. 4. Average values of the content of Pb in roots of seedlings of *L. leucocephala* and *P. dubium* cultivated in a germination chamber ($25^\circ\text{C} \pm 2$) as a function of doses of $\text{Pb}(\text{NO}_3)_2$

The updraft present in the xylem of plants is the mechanism responsible for the transportation of ions from the roots to the aerial part [17]. These results indicate that *L. leucocephala* may contain specialized internal mechanisms capable of reducing the entry of Pb by the root system, which is not as visible and efficient for *P. dubium*.

The levels of Pb in the leaves in *P. dubium* showed increased while that was provided higher doses of $\text{Pb}(\text{NO}_3)_2$ seed, presenting a maximum value of 81.20 mg kg^{-1} of Pb in the leaf tissue (Figure 5), however, in *L. leucocephala*, the doses of Pb in leaves did not show significant difference according to the increase of the doses applied.

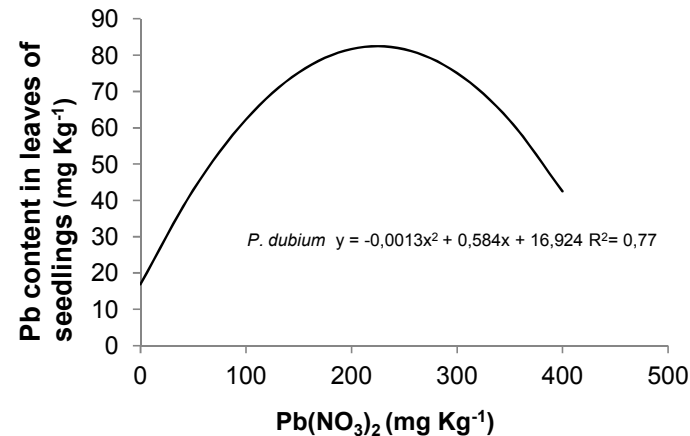


Fig. 5. Values of Pb content in leaves of seedlings of *P. dubium* cultivated in a germination chamber ($25^\circ\text{C} \pm 2$) as a function of doses of $\text{Pb}(\text{NO}_3)_2$

Similar results to those obtained for *L. leucocephala* were observed by [18] evaluating the action of Pb in *Canavalia ensiformes*, where he found that the increase in the concentration of Pb applied does not interfere with the concentration of metal present in the aerial part of the plant, unlike the root system, which showed higher concentrations of Pb as the increase of the doses, as observed in *P. dubium*.

To analyze the contents of chlorophyll in plants, the species *L. leucocephala* presented higher values of chlorophyll a (Figure 6a), chlorophyll b (Figure 6b) and total chlorophyll (Figure 6c), except in respect of chlorophyll a/b, where *P. dubium* obtained higher result (Figure 6d).

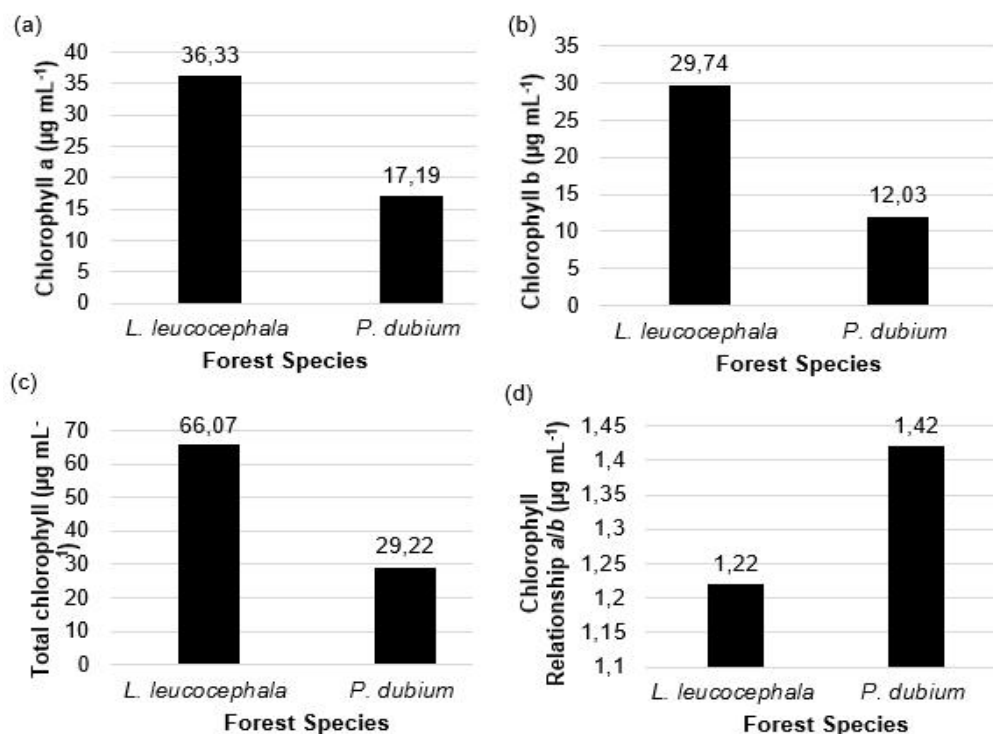


Fig. 6. Mean values of chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and the ratio of chlorophyll a/b (d) seedlings of *L. leucocephala* and *P. dubium* cultivated in a germination chamber (25 °C ± 2) as a function of doses of Pb(NO₃)₂

In a study with plants of girrasol found that the increment of Pb was able to reduce the levels of photosynthetic pigments present. This result was attributed to the possible interference of Pb in the absorption of iron and magnesium, thus having a direct effect on the synthesis of chlorophyll by the plant [20]. A similar result was obtained in a study with *Brachiaria decumbens*, where there was a reduction in the synthesis of chlorophyll content as a function of the increase of Pb concentration [21].

4. CONCLUSION

The increasing doses of Pb(NO₃)₂ influenced the germination rates and initial development of *L. leucocephala* and *P. dubium* the end of 14 days after sowing. The morphometric variables shoot length, root and stem diameter showed variations in the presence of doses, presenting significant difference for all variables, except for the stem diameter of *P. dubium*.

In the evaluations of Pb in roots and leaves, *P. dubium* presented higher concentrations, while *L. leucocephala* showed better values of chlorophyll a, chlorophyll b and total chlorophyll.

P. dubium was the species that showed greater sensitivity in the presence of doses of Pb(NO₃)₂, pointing to increased resistance of *L. leucocephala*, stating your employment in recovery of contaminated areas.

REFERENCES

1. Epstein E, Bloom AJ. Mineral nutrition of plants: principles and perspectives. 2nd ed. Londrina: Editora Planta; 2006.
2. Fadigas FS, Amaral-Nephew NMB, Mazur N, Angels LHC, Ash AA. Natural concentrations of heavy metals in some classes of Brazilian soils. *Bragantia*. 2002;61:2. Accessed 25 August 2015.

- Available: <http://dx.doi.org/10.1590/S0006-87052002000200008>
3. Andrade SAL, Abreu CA, Abreu MF, Silveira, APD. Interaction of lead, soil base saturation and arbuscular mycorrhiza in soybean growth and mineral nutrition. Brazilian Journal of Soil Science. 2003;27:5. Accessed 25 August 2015.
Available: <http://dx.doi.org/10.1590/S0100-06832003000500019>
4. Pereira MP, Pereira FJ, Rodrigues LCA, Barbosa S, Castro EM. Phytotoxicity of lead in germination and initial growth of lettuce in function of root anatomy and cell cycle. Revista Agroambiente On-line. 2013;7:1. Accessed 25 August 2015.
Available: <https://revista.ufr.br/agroambiente/article/view/895/1019>
5. Soares CRFS, Accioly AMA, Marques TCLLSM, Siqueira JOS, Moreira FMS. Accumulation and distribution of heavy metals in roots, stem and leaves of tree seedlings in soil contaminated by zinc industry tailings. Brazilian Journal of Plant Physiology. 2001;13:3. Accessed 25 August 2015.
Available: <http://www.scielo.br/pdf/%0D/rbfv/v13n3/9261.pdf>
6. Oliveira AB. Germination of leucine seeds (*Leucaena leucocephala* (Lam.) De Wit.), Var. K-72. Journal of Biology and Earth Sciences. 2008;8:2. Accessed 25 August 2015.
Available: <http://joaootavio.com.br/bioterra/workspace/uploads/artigos/18leucena-518170b397ba8.pdf>
7. Lorenzi H, Souza HM, Torres MAV, Bacher LB. Exotic trees of Brazil: woods, ornamental and aromatic. New Odessa: Instituto Plantarum; 2003.
8. Lorenzi H. Brazilian trees: manual of identification and cultivation of native plants of Brazil. 2nd ed. New Odessa: Instituto Plantarum; 2002.
9. Arnon DI. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 1949;24:1. Accessed 25 August 2015.
Available: <http://www.plantphysiol.org/content/24/1/1>
10. AOAC. Association of Official Analytical Chemists. 18th ed. 2005.
11. Welz B, Sperling M. Atomic Absorption Spectrometry, 3rd ed. 1999.
12. Ferreira DF. SISVAR: A program for analysis and teaching of statistics. Scientific Journal Symposium. 2008;6:2. Accessed 25 August 2015.
Available: <http://www.dex.ufla.br/~danielff/meusarquivospdf/art63.pdf>
13. Costa JNMNC, Durigan G. *Leucaena leucocephala* (Lam.) De Wit (Fabaceae): invasive or rude? Tree Review. 2010;34:5. Accessed 25 August 2015.
Available: <http://dx.doi.org/10.1590/S0100-67622010000500008>
14. Silva PCC, Jesus FN, Alves AC, Jesus CA, Santos AR. Growth of sunflower plants grown in environment contaminated by lead. Biocience Journal. 2013;29:5. Accessed 25 August 2015.
Available: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/15091>
15. Marques TCLLSM, Moreira FMS, Siqueira OJ. Growth and metal content of tree seedlings cultivated in soil contaminated with heavy metals. Pesquisa Agropecuária Brasileira. 2000;35:1. Accessed 25 August 2015.
Available: <http://dx.doi.org/10.1590/S0100-204X2000000100015>
16. Augusto AS, Bertoli AC, Cannata MG, Carvalho R, Bastos ARR. Bioaccumulation of heavy metals in *Brassica juncea*: relationship of toxicity with essential elements. Journal of Chemical Engineering. 2014;6:5. Accessed 25 August 2015.
Available: <http://rvq.sbg.org.br/imagebank/pdf/v6n5a07.pdf>
17. Taiz L, Zeiger E. Plant physiology. Porto Alegre: Artmed; 2013.

- 222 18. Romeiro S, Lagôa AMMA, Furlani PR, Abreu CA, Pereira BFF. Absorption of lead and
223 phytoremediation potential of *Canavalia ensiformes* L. *Bragantia*. 2007;66:2. Accessed 25 August
224 2015.
225 Available: <http://www.scielo.br/pdf/brag/v66n2/17.pdf>
226
- 227 19. Abreu CB, Mota KNAB, Sacramento BL, Neto ADA. Pigment content in sunflower *Helianthus*
228 *annuus* (Compositae) under doses of lead in nutrient solution. In: National Congress of Botany, 64.,
229 2013, Belo Horizonte, MG. *Annals...* 2013.
230
- 231 20. Serra LB, Santos NT, Rodrigues ACD. Effect of soil contamination by lead growth and the
232 photosynthetic rate of *Brachiaria decumbens*. In: TECHNICAL-SCIENTIFIC COLLECTION OF
233 UNIFOA, 7. 2013, Volta Redonda, RJ. *Anais ... Volta Redonda: UNIFOA - Centro Universitário de*
234 *Volta Redonda*, 2013. Accessed 25 August 2015.
235 Available: [http://web.unifoa.edu.br/editorafoa/wp-content/uploads/2014/06/vii-coloquio-unifoa-](http://web.unifoa.edu.br/editorafoa/wp-content/uploads/2014/06/vii-coloquio-unifoa-20131.pdf)
236 [20131.pdf](http://web.unifoa.edu.br/editorafoa/wp-content/uploads/2014/06/vii-coloquio-unifoa-20131.pdf)
237
238