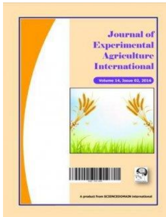

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Lead Nitrate in the Germination and Initial Development of *Leucaena leucocephala* and *Peltophorum dubium*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NKC, JAB and MELC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SSO, LSC, LLOR and DB managed the analyses of the study. Author SSO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to determine the influence of lead nitrate ($Pb(NO_3)_2$) on the germination and initial development of leucaena (*Leucaena leucocephala*) and canafístula (*Peltophorum dubium*). The experimental design was a randomized complete block design in a 2x6x4 factorial scheme [two forest species and six $Pb(NO_3)_2$ doses], with 4 replicates per treatment, totaling 48 sample units. Statistical analyzes were performed to analyse the variables: tukey test and regression at 5% probability. 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 were conditioned to germinate for 14 days in the presence of $Pb(NO_3)_2$ 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

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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 $Pb(NO_3)_2$ 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 $Pb(NO_3)_2$ 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 the restoration of degraded areas are *Leucaena leucocephala* (Lam.) de Wit. and *Peltophorum dubium* (Spreng.) Taub., belonging to the family Fabaceae, known as leucaena and canafístula, 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 behaviour and the development of seedlings of *L. leucocephala* and *P. dubium*, in order to characterise the sensitivity of species in the treatments.

2. MATERIALS AND METHODS

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 24º 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 24º 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 2x6x4 factorial scheme [two forest species and six doses of $Pb(NO_3)_2$, with 4 repetitions per treatment, totaling 48 sample units. Statistical analyzes were performed to analyse the variables: tukey test and regression at 5% probability in the statistical software Sisvar [9].

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 ± 2ºC) 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 (25ºC ± 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 (SL), root length (RL) and stem diameter (SD), 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 ºC. 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 [10]. 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 (PBL) and Pb concentration in the roots (PBR)] 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 24º 33' 22" South, longitude 54° 03' 24" W and altitude 410 m, by means of a nitro perclórica digestion [11] and by atomic absorption spectrometry, modality flame [12].

3. RESULTS AND DISCUSSION

When evaluating the results of the analysis of variance it was possible to observe a significant difference in the behavior of the interaction between forest species and concentrations of $Pb(NO_3)_2$ at the level of significance ($P<0.01$) at the end of 14 days after sowing to: RL, PBL and PBR. Still in the same table, there are significant differences for the dose factor and for the SD (Table 2).

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 SL of *L. leucocephala* showed behavior with quadratic function ($Y = -40.689x^2 + 11.519x + 5.8097$ $R^2 = 0.90$) to the maximum point at the 0.14 mg L^{-1} $Pb(NO_3)_2$, with a maximum shoot length of 6.57 cm. In this, there was first an increase of SL, and concentrations between 0.10 and 0.16 mg L^{-1} $Pb(NO_3)_2$ provided the highest values for SL, and later, a decrease, demonstrating the harmful action of increasing doses of treatments. (Fig. 1a). For the *P. dubium*, the mean values of the percentage obtained presented a behavior with quadratic function ($Y = 1.3676x^2 - 1.245x + 5.0552$ $R^2 = 0.71$) to the minimum point 4.83 cm. This result was observed for the values between 0.27 and 0.29 mg L^{-1} $Pb(NO_3)_2$ provided the lowest values for the variable in question, and a decrease in the values presented shows that the *P. dubium* presented greater sensitivity to the doses, when compared to the values presented for *L. leucocephala*, affirming the higher resistance of this specie (Fig. 1b).

The initial increase presented in the SL in *L. leucocephala* demonstrates that the species has a tolerance in the presence of doses of $Pb(NO_3)_2$, 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

Table 2. Summary of variance analysis containing mean square values for the variables SL, RL, SD, PBL and PBR of *L. leucocephala* and *P. dubium* cultivated in a germination chamber (25 ºC ± 2) as a function of doses of $Pb(NO_3)_2$

F.V.	GL	RL	SL	SD	PBL	PBR
Species (SP)	1	17,93**	82,89**	0,0516**	3836,02**	974,23**
Doses (DO)	5	1,89^{ns}	0,43^{ns}	0,0041**	3911,75**	1025,54**
(ES) * (DO)	5	2,37^{ns}	0,86**	0,0003^{ns}	833,66**	977,36**
Repetitions	3	0,67^{ns}	0,32^{ns}	0,0001^{ns}	7,05^{ns}	3,54^{ns}
Residues	37	11,19	0,23	0,0001	4,59	5,78
Total	51					
C.V.		10,01	16,47	6,17	28,89	28,66
D.M.S.		0,31	0,69	0,06	6,17	6,23

^{ns} *not significant,* ** *(P<0.01%),* * *(P<0.05%)*

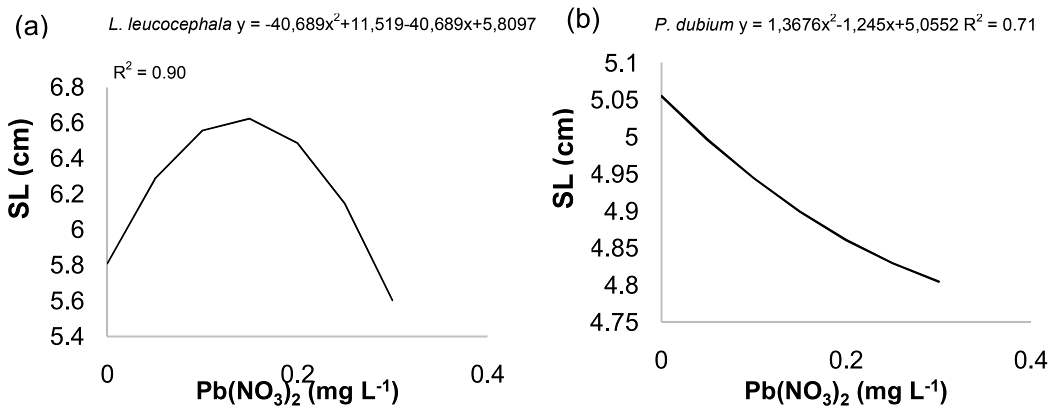

Fig. 1. SL (cm) of seedlings of *L. leucocephala* (a) and *P. dubium* (b) cultivated in a germination chamber (25 ºC ± 2) as a function of doses of $Pb(NO_3)_2$ ($P<0.05$)

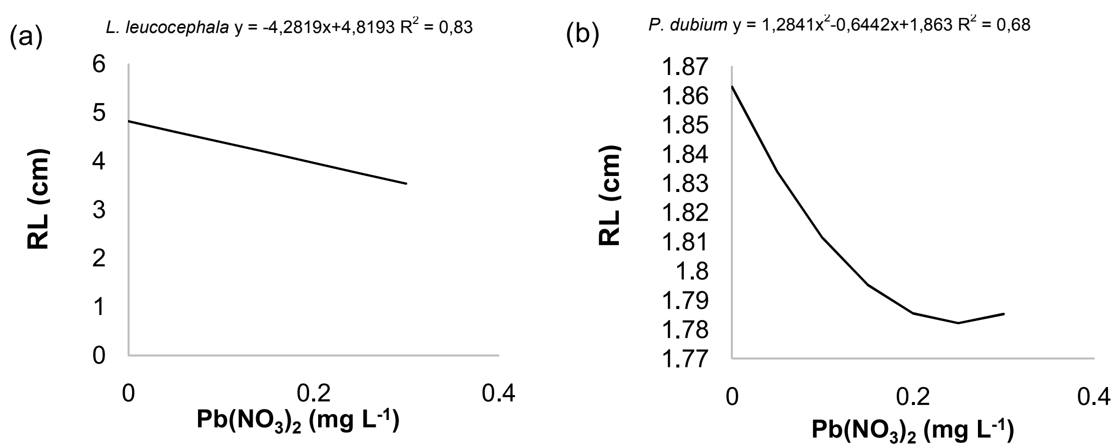

Fig. 2. RL (cm) of seedlings of *L. leucocephala* (a) and *P. dubium* (b) cultivated in a germination chamber (25 ± 2 ºC), as a function of doses of $Pb(NO_3)_2$ ($P<0.05$)

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} $Pb(NO_3)_2$, (Fig. 2a). To the extent that concentrations of $Pb(NO_3)_2$ increased, there was a decrease in the length of the root system, this being the most affected region in relation to the carrier.

The RL of *L. leucocephala*, there was a linear decrease ($Y = -4.2819x + 4.8193$ $R^2 = 0.83$), showing the lowest values between 0.27 and 0.29 mg L^{-1} $Pb(NO_3)_2$, with values of 3.83 cm of RL (Fig 2a). In relation to the RL of *P. dubium*, the mean values of the percentage obtained showed a behavior with quadratic function (Y = 1.2841x2 - 0.6442x + 1.863 R2 = 0.68) 1.78 cm of a curve to the minimum point at a concentration of 0.25 mg L^{-1} $Pb(NO_3)_2$ (Fig. 2b). The decrease was higher for the root system in relation to the carrier and in relation to the values obtained in the RL of *L. leucocephala*, demonstrating that the root of this species was more susceptible to the damaging action of Pb.

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].

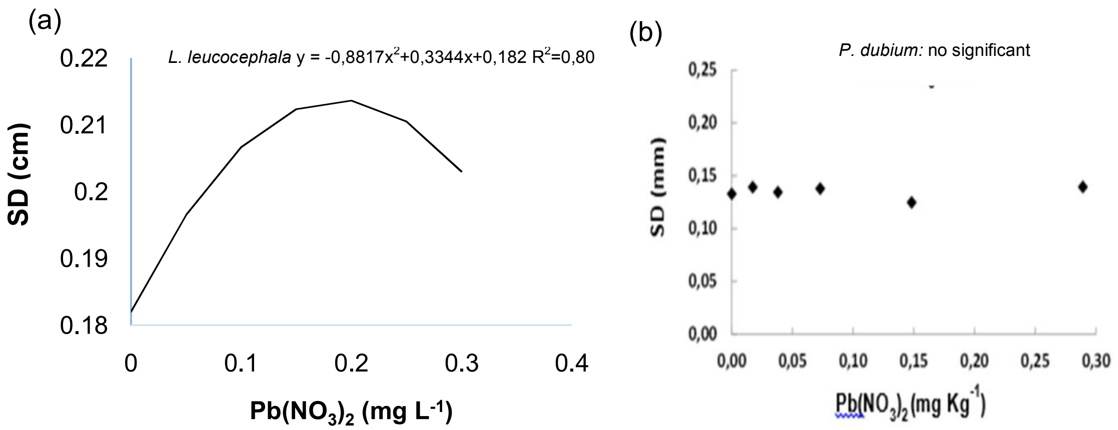

Fig. 3. SD (mm) of seedlings of *L. leucocephala* (a) and *P. dubium* (b) grown in a germination chamber (25 ± 2 ºC), as a function of doses of $Pb(NO_3)_2$ (P<0.05)

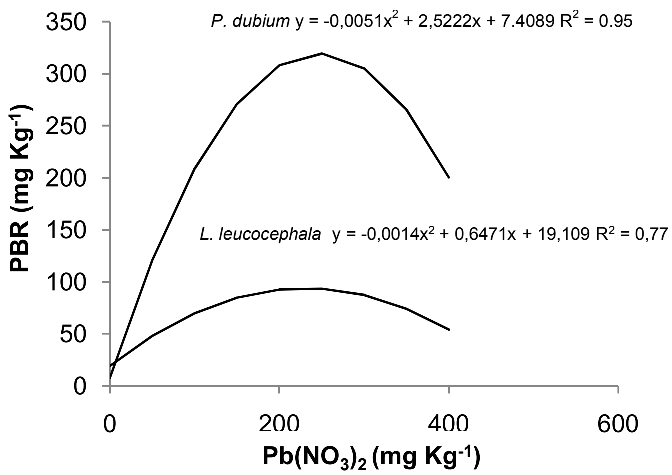

Fig. 4. Values of PBR of seedlings of *L. leucocephala* and *P. dubium* cultivated in a germination chamber (25 ºC ± 2) as a function of doses of $Pb(NO_3)_2$ (P<0.05)

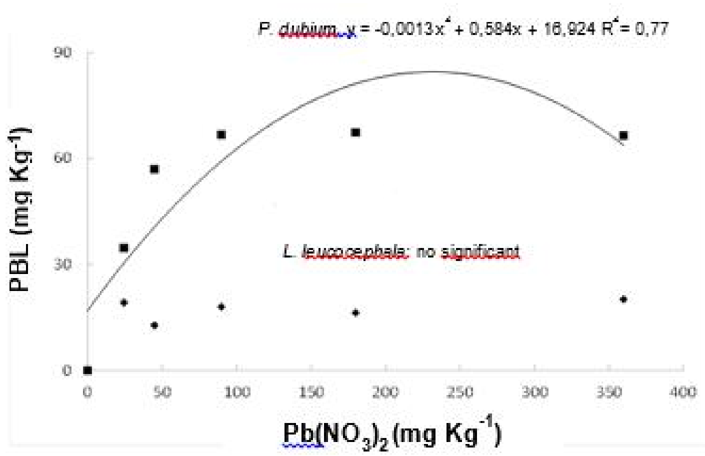

Fig. 5. Values of PBL of seedlings of *P. dubium* cultivated in a germination chamber (25ºC ± 2) as a function of doses of $Pb(NO_3)_2$ (P<0.05)

In the SD, the mean values of the percentage obtained in *L. leucocephala* showed behavior with quadratic function ($Y = -0.8817x^2 + 0.3434x + 0.182$ $R^2 = 0.80$) up to the maximum point, where the highest values for the variable 0.19 mg L^{-1} $Pb(NO_3)_2$ for SL of 0.21 mm (Fig. 3a), while the values of *P. dubium* proved indifferent to the doses, did not differ statistically (Fig. 3b).

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 PBR in *P. dubium* presented a quadratic function ($Y = -0.0014x^2 + 0.6471x + 19.109$ $R^2 = 0.77$), as well as for the *P. dubium* ($Y = -0.0051x^2 + 2.5222x + 7.4089$ $R^2 = 0.95$). *P. dubium* there was a maximum concentration of 317.94 mg kg^{-1} of Pb in roots, while *L. leucocephala* obtained less concentration in the roots, regardless of the concentration used, presenting a maximum value of 76.32 mg kg^{-1} of PBR (Fig. 4).

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.*

As in the previous results for the root system, the PBL variable for *P. dubium* presented a quadratic function ($Y = -0.0013x^2 + 0.584x + 16.924$ $R^2 = 0.77$). It was possible to determine increasing doses of Pb, whereas $Pb(NO_3)_2$ was given to the seeds in gerbox, presented a maximum point in 224.62 mg mL^{-1} $Pb(NO_3)_2$ and a value of 81.20 mg kg^{-1} Pb in the leaf tissue, but in *L. leucocephala*, the doses of Pb in the leaves did not present significant difference as the increase of the doses of applied treatments (Fig. 5).

Similar results to those obtained for *L. leucocephala* were observed by Romeiro et al. [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*, chlorophyll *b* and total chlorophyll, except in respect of chlorophyll a/b, where *P. dubium* obtained higher result (Table 3).

Table 3. 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 (25ºC ± 2) as a function of doses of $Pb(NO_3)_2$

Pigments ($\mu g\ mL^{-1}$)	*L. leucocephala*	*P. dubium*
Chlorophyll *a*	36,33	17,19
Chlorophyll *b*	29,74	12,03
Total Chlorophyll	66,07	29,22
Chlorophyll *a*/*b*	1,22	1,42

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 [19]. 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 [20].

4. CONCLUSION

The increasing doses of $Pb(NO_3)_2$ 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 $Pb(NO_3)_2$, pointing to increased resistance of *L. leucocephala*, stating their employment in recovery of contaminated areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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