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Original Paper

Inga uruguensis response to lead: effects on growth and nitrogenous compounds

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Abstract

Lead (Pb) is a heavy metal considered one of the major soil pollutants. Phytoremediation is a sustainable and economically viable biological method for reducing Pb content in the environment. *Inga uruguensis* is a tree legume species that has characteristics favorable to phytoremediation, such as rapid growth and high biomass production. The objective of this work was an initial evaluation of tolerance and phytoremediation potential of *I. uruguensis* to Pb. The experiment was carried out in a greenhouse. In addition to the control, soil contamination was carried out with the following Pb doses: 100, 200, 300, 400, and 500 mg.dm³, with 5 repetitions in each treatment, totaling 30 vases. We assessed growth, number and mass of nodules, chlorophyll content, ureids, amino acid, protein and soluble carbohydrates in leaves, roots and nodules, tolerance index, dry matter, and tissues Pb content of *I. uruguensis*. The data were analyzed by the Tukey test using R and SISVAR software. There was no negative effect of Pb in soil on *I. uruguensis* growth, the symbiotic relationship with rhizobia was kept, even at high Pb content and the tolerance index was not lower than 0.69. *Inga uruguensis* has initial tolerance and potential to be used as phytoremediation in soils contaminated by Pb.

Key words: heavy metal, phytostabilization, phytoremediation, tree legume.

Resumo

O chumbo (Pb) é um metal pesado caracterizado como o principal poluente do solo. A fitorremediação é uma alternativa sustentável e economicamente viável para reduzir a concentração de Pb no ambiente. *Inga uruguensis* é uma espécie leguminosa arbórea com características favoráveis à fitorremediação, como crescimento rápido e alta produção de biomassa. O objetivo desse trabalho foi avaliar o potencial inicial de tolerância e fitorremediação de *I. uruguensis* para Pb. O experimento foi realizado em casa de vegetação. Além do controle, a contaminação do solo foi realizada com as seguintes doses de Pb: 100, 200, 300, 400 e 500 mg.dm³, com 5 repetições em cada tratamento, totalizando 30 vasos. Foram avaliados crescimento, número e massa de nódulos, teor de clorofila, ureídeos, aminoácidos, proteínas e carboidratos solúveis em folhas, raízes e nódulos, índice de tolerância, matéria seca e teor de Pb nos tecidos de *I. uruguensis*. Os dados foram analisados pelo teste de Tukey, utilizando os softwares R e SISVAR. Não houve efeito negativo no crescimento de *I. uruguensis*, a relação simbiótica com a rizobia foi mantida, mesmo com alto teor de Pb e o índice de tolerância não inferior a 0,69. *Inga uruguensis* apresenta tolerância inicial e potencial para ser usada como fitorremediadora em solos contaminados por Pb.

Palavras-chave: metal pesado, fitoestabilização, fitorremediação, leguminosa arbórea.

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Introduction

Heavy metals (HM) are elements occurring in the natural environment with comparatively high molecular weight. Some of them play an important role in plant nutrition, while others have deleterious effects on various components of the biosphere (Kabata-Pendias & Pendias 2001).

The amount of HM in the soil has been increasing due to different anthropic practices, such as industrial, agricultural activities (Facchinelli *et al*. 2001), mining, sewage sludge and organic compounds from urban waste recycling (Moraes 2011). This is one of the biggest problems in impacted areas, since polluting metals are not degradable and have the ability to bioaccumulate in organisms.

There are heavy metals that are classified as essential, such as copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni) and iron (Fe). However, when they exceed the required threshold, they become toxic. Non-essential heavy metals, such as cadmium (Cd), mercury (Hg) and lead (Pb), have no known biological functions and can cause serious damage to metabolism (Valls & Lorenzo 2002; Sarwar *et al*. 2017).

Lead (Pb) is characterized as one of the main soil contaminants (Gratão *et al*. 2005) and is included in the list of the US Environmental Protection Agency as the second most threatening element, behind Arsenic (ATSDR 2017). Soil contamination by Pb is a practically irreversible cumulative process (Duarte & Pasqual 2000).

According to the report from CETESB (2016) (Companhia Ambiental do Estado de São Paulo - in Portuguese), the reference value is 17 $mg.kg^{-1}$ and the prevention value is 72 $mg.kg^{-1}$ for Pb. In agricultural, residential, and industrial areas, the values 150 $mg.kg^{-1}$, 240 $mg.kg^{-1}$, and 4,400 $mg.kg^{-1}$, respectively, are considered high and require intervention.

The main route of human contamination is the ingestion or inhalation of lead particles, the toxic effects of which are the same regardless of their route of entry (ATSDR 2019). This element is retained in the body for decades and causes negative effects throughout the body, because the mechanisms that induce toxicity are common to all types of cells (ATSDR 2019). When in the human body, Pb can cause neurological, hematological, endocrinological, renal, growth, reproduction, carcinogenic, cardiovascular, gastrointestinal and liver damage (Moreira & Moreira 2004).

When in contact with plants, lead can negatively affect plant growth and development (Souza *et al*. 2011), inhibit photosynthesis and seed germination, cause leaf necrosis and senescence, cause damage to genetic material and changes in enzyme activities (Ribeiro *et al*. 2015).

Phytoremediation is a sustainable and economically viable solution to pollution and is one of the most studied techniques of bioremediation (Coutinho & Barbosa 2007), based on the use of plants and their microbiota to remove, stabilize, or degrade soil contaminants (Siliciano & Germida 1999), making them harmless to the ecosystem.

According to Pilon-Smits (2005), phytoremediation can be classified as follows: (a) phytostabilization - the contaminant is complexed in the root tissues and thus unable to move in the soil; (b) phytostimulation - the contaminant is degraded by microorganisms that develop specifically in the rhizosphere due to the particular existing conditions; (c) phytovolatilization - the contaminant absorbed has its physical state changed to a gaseous form and thus volatilized; (d) phytodegradation - a process similar to phytostimulation; however, occurring in the shoots; (e) phytoextraction - the contaminant is absorbed and most of it is transported to the shoots.

However, not all plants have properties for phytoremediation. In general, five characteristics indicate which plants could be used in phytoremediation, namely rapid growth, high biomass production, competitiveness, vigor, and tolerance to the target pollutant (Lamego & Vidal 2007).

Fast-growing tree legumes with a well-branched and deep root system have great potential as phytoremediation agents, due to the great capacity of biomass production in the shoots, in addition to the symbiotic association with N-fixing bacteria. An example is *Inga uruguensis* Hook. & Arn. (Fabaceae), a native tree legume, which maintains its symbiotic associations even in the presence of nitrate in the medium and is a kind of watercourse and can be used to phytoremediate areas with wet or flooded soils .

Even with favorable characteristics, the list of native tropical tree species that are known to be effective as phytoremediation agents is still limited (Caires *et al*. 2011). The assessment of tolerance and allocation of lead in trees shows that studies in this area are extremely necessary (Araujo *et al*. 2020).

Therefore, this study aimed to characterize tolerance and phytoremediation potential of *Inga uruguensis* to Pb for as a phytoremediator in Pb-contaminated areas.

Material and Methods

Soil preparation and Pb contamination solutions

To carry out the experiment, soil from Cerrado, collected at FEPE - UNESP's Ilha Solteira-SP Education, Research and Extension Farm, located in Selvíria - MS, and sand in a 1:1 ratio, which were stored in 2 L pots, were used. The soil was contaminated with lead acetate solution, in the following doses: 100, 200, 300, 400 and 500 $mg.dm^{-3}$, waiting for a period of 10 days for soil stabilization. Each treatment consisted of 5 repetitions, totaling 30 vessels.

Conduct of the experiment

A completely randomised design was followed in the experiment. The experiment was carried out in a greenhouse of the Laboratory of Physiology of Plant Metabolism, at UNESP in Ilha Solteira-SP. In addition to the control, the soil was contaminated with the following doses of lead: 100, 200, 300, 400, 500 $mg.dm^{-3}$. For the 100 mg treatment, 5,493 g of lead acetate was diluted in 300 mL of distilled water; for the 200 mg treatment, 10,986 g lead acetate was diluted in 300 mL of distilled water; for treatment 300 mg, 16,479 g lead acetate was diluted in 300 mL of distilled water, for treatment 400 mg, 21,972 g of lead acetate was diluted in 300 mL of distilled water and for treatment 500 mg, 27,465 g of lead acetate was diluted in 300 mL of distilled water. 20 mL of the solution were placed in 2 L pots of the respective treatments, waiting for a period of 10 days for soil stabilization. Then, seedlings of *I. uruguensis*, provided by the Program Implementation and Maintenance Division Of the São Paulo State Energy Company (CESP), were planted directly in the contaminated soil, where they remained from April 28, 2016 until September 7, 2016. During the period in which they remained in the pot, the length of the plants was measured, being measured from the base of the stem to its apical bud. Each treatment had 5 repetitions.

Experiment harvest

The material was collected on September 7, 2016. The plants were removed from the pots, with the roots carefully washed. Subsequently, they were separated into root and shoots (stem and leaves). In the laboratory, all nodules present in the roots were removed, counted and weighed.

Then, 0.5 g of samples of leaves, roots and nodules were obtained and stored in the freezer to perform the extraction and dosage of compounds of carbon and nitrogen metabolismo (quantification of soluble proteins, amino acids, soluble carbohydrates and total ureides). The rest of the material was also frozen, to be subsequently placed in an oven, in order to obtain the total dry mass and perform the quantification of lead.

Chlorophyll quantification

The chlorophyll content was analyzed one week before collection, according to the method of Hiscox & Israelstam (1979). A young leaf fililus was removed from each plant and cut into thin strips until 50 mg was obtained. The material was placed in a test tube, with 7 mL of DMSO being added to each tube. The tubes were capped with aluminum foil and placed in a water bath at 65 ºC for 30 minutes. After the solution cooled to room temperature, the spectrophotometer was read at 645 nm and 663 nm and the concentrations were calculated according to the following equations:

$$C_a = (12{,}70 \times A_{663}) - (2{,}69 \times A_{645})$$
$$C_b = (22{,}90 \times A_{645}) - (4{,}68 \times A_{663})$$
$$C_a + C_b = (20{,}20 \times A_{645}) + (8{,}02 \times A_{663})$$

Extraction and dosage of nitrogen compounds

The nitrogen compounds were extracted using the method described by Bieleski & Turner (1966). For 0.5 g of fresh material (root, leaf and nodules), 5 mL of MCW solution (60% mL Methanol, 25% mL Chloroform, 15% mL H_2O) was added. The material was crushed and then centrifuged. After centrifugation, 1 mL Chloroform + 1.5 mL H_2O was added for each 4 mL of supernatant. It was waited for 24 hours in a refrigerator, for phase separation and the water-soluble phase was used to analyze ureids, amino acids and total soluble carbohydrates. To the resulting precipitate after the first extraction, 4 mL of 0.1N NaOH was added, homogenizing it and, after centrifugation, the supernatant was used for total protein analysis.

Extraction was carried out on root, leaf and nodule samples.

Quantification of soluble proteins

The analysis of soluble proteins was carried out according to the methodology described by Bradford (1976). Where 100 µL of sample (precipitate from the extraction of soluble compounds, step 2.5) were placed in a test tube together with 5 mL of Bradford, remaining 3 minutes at room temperature. The reading was performed on a spectrophotometer with a wavelength of 595 nm.

Soluble proteins was performed on root, leaf and nodule samples.

Quantification of amino acids

The analysis of total amino acids was performed according to the methodology described by Yemm and Cocking (1955), where, in test tubes, 100 µL of sample (water-soluble phase of the extraction of soluble compounds, step 2.5) were mixed with 900 µL of distilled water. To this mixture were added 500 µL of citrate buffer (pH 5), 200 µL of ninhydrin (5% methylglycol) and 1mL of KCN. The tubes were taken to the water bath and heated to 100 ºC for 20 minutes. They were left for 10 minutes and cooled to room temperature. Afterwards, 1 mL of 60% ethyl alcohol was added to the tubes. The reading was performed on a spectrophotometer with a wavelength of 570 nm.

Amino acid quantification was performed on root, leaf and nodule samples.

Quantification of total soluble carbohydrates

The analysis of soluble carbohydrates was carried out according to the methodology described by Umbreit *et al*. (1957), where 1 mL of sample (water-soluble phase of the extraction of soluble compounds, step 2.5) was placed in test tubes, together with 1 mL of anthrone reagent, then the tube was shaken, capped and placed in a water bath at 100 ºC for 3 minutes. After cooling at room temperature, the reading was performed on a spectrophotometer with a wavelength of 660 nm

Soluble carbohydrates quantification was performed on root and leaf samples.

Quantification of total ureides (allantoin and allantoic acid)

The analysis of total ureids (allantoin and allantoic acid) was carried out according to the methodology described by Vogels and Van Der Drift (1970), which consists of 4 steps.

Step 1: 250 µL of sample (water-soluble phase of the extraction of soluble compounds, step 2.5) were placed in test tubes together with 500 µL of distilled water, 250 µL of 0.5N NaOH and a drop of 0.33% phenylhydrazine. Afterwards, they were heated in a water bath at 100 ºC for 8 minutes and cooled to room temperature.

Step 2: 250 µL of 0.65N HCl were added to the test tubes from the previous step, then they were heated in a water bath at 100 ºC for 4 minutes and cooled to room temperature.

Step 3: 250 µL of phosphate buffer (pH 7) and 250 µL of phenylhydrazine were added to the test tubes from the previous step, remaining 5 minutes at room temperature and after being placed on ice for 5 minutes.

Step 4: The tubes from the previous step were placed inside a styrofoam with ice and 1.25 ml of 37% HCl was added. Outside the Styrofoam, 250 µL of $K_3Fe(CN)_6$ were added. The tubes were then shaken and waited for 15 minutes at room temperature. The reading was performed on a spectrophotometer with a wavelength of 535 nm.

Total ureids quantification was performed on root, leaf and nodule samples.

Obtaining the dry matter and the tolerance index

The total fresh mass was taken to a closed circulation oven for 72 hours at 60 ºC to obtain the dry mass. With the dry mass data, the tolerance index for each treatment was obtained, based on the following formula: $IT = AB_T / AB_C$, where AB_T is the accumulated biomass in each treatment and AB_C is the accumulated biomass in the control treatment (Yang *et al* 2015).

Quantification of lead

After the dry material was crushed in a ball mill, the quantification of lead was performed by inductively coupled plasma optical emission spectrometry (ICP-OES). This analysis was outsourced, carried out at the Instituto Agronômico de Campinas.

Data analysis

All data were analyzed using the statistical software R and SISVAR, using the Tukey test with 5% significance.

Results

Looking at the data of the average monthly growth in height of *Inga uruguensis* in soil contaminated with Pb, it is observed that there is no statistical difference between treatments (Tab. 1). Growth of these plants was continuous, signaling optimal growth capacity even with high Pb contents in the developmental phase analyzed.

Regarding the number and mass of the nodules (Tab. 2), there were no significant differences in the number of nodules, while treatment 400 mg.dm^{-3} showed the highest mass.

The contents of photosynthesis pigments were affected by exposure to Pb (Tab. 3), with a decrease in chlorophyll A, B, and total in treatment 100 mg.dm^{-3} and subsequent recovery with a greater content in treatments 300 and 500 mg.dm^{-3}.

The highest dry mass values (Tab. 4) occurred in treatment 400 mg.dm^{-3}, both for roots and shoots. The data obtained with the tolerance index (TI) analysis (Tab. 4) showed that plants of *I. uruguensis* are tolerant to Pb, mainly in treatments 200 mg.dm^{-3} and 400 mg.dm^{-3}. Lead content (Tab. 4) was much higher in roots than in shoots and treatment 400 mg.dm^{-3} had the highest values in roots, while treatment 500 mg.dm^{-3} had the highest values in shoots.

The protein content in the leaves was affected, showing an increase in its concentration in doses with higher concentrations of lead. In nodules, the concentration of proteins was higher in the control, whereas in the roots, the highest concentration occurred in the treatment 300 mg.dm^{-3} (Tab. 5). For amino acids, there was only a significant difference in the roots showing an increase in protein content until the treatment 400 mg.dm^{-3}, where the concentration of proteins showed greater value (Tab. 5). As for the soluble carbohydrates of the roots, there was a clear drop in their concentration in all treatments that were exposed to lead (Tab. 5).

Regarding contents of total ureids in the leaves of I. uruguensis (Tab. 5), variation is low between the different treatments, except in treatment 500 mg.dm^{-3}, which had greater accumulation of total ureids. However, the pattern of ureid accumulation in the leaves was different, with the highest allantoic acid content in treatment 400 mg.dm^{-3}, while the highest allantoin content was observed in treatment 500 mg.dm^{-3}. In roots, the highest allantoin content occurred in treatment 500 mg.dm^{-3}. In nodules, the highest contents of total ureids and allantoin were observed in treatment 100 mg.dm^{-3}, while the allantoic acid contents reduced in all treatments exposed to Pb (Tab. 6).

Discussion

Lead can negatively affect plant growth, Souza *et al.* (2012) in a study analyzing the tolerance and phytoremediation potential of three species of tree legumes in soil contaminated with lead, found that the species *M. caesalpiniaefolia* Benth. and *E. speciosa* Andrews had no negative

Table 1 – Monthly growth in height (cm) and total evolution of *Inga uruguensis* in lead-contaminated soils.

VS	April	May	June	July	August	September
Treatments						
0 mg.dm^{-3}	60.68 a	61.50 a	62.84 a	64.46 a	64.60 a	64.70 a
100 mg.dm^{-3}	66.80 a	67.06 a	67.30 a	69.52 a	69.88 a	70.30 a
200 mg.dm^{-3}	61.40 a	61.90 a	65.10 a	67.02 a	67.62 a	68.20 a
300 mg.dm^{-3}	62.68 a	63.60 a	65.66 a	66.64 a	67.50 a	68.30 a
400 mg.dm^{-3}	66.28 a	66.82 a	69.56 a	71.92 a	72.28 a	72.60 a
500 mg.dm^{-3}	62.38 a	62.90 a	64.78 a	67.04 a	67.78 a	68.44 a
Pr > Fc	0.0789^{NS}	0.1061^{NS}	0.5394^{NS}	0.5455^{NS}	0.5713^{NS}	0.5898^{NS}
Overall average	63.37	63.96	65.87	67.77	68.28	68.76
SE	±1.70	±1.69	±2.53	±2.86	±2.92	±3.01

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error.

Table 2 – Number and mass (g) of nodules of *Inga uruguensis* in lead-contaminated soils.

VS	Number of nodules	Mass of nodules (g)
Treataments	**Averages**	
0 mg.dm^{-3}	27.40 a	0.18 b
100 mg.dm^{-3}	21.50 a	0.17 b
200 mg.dm^{-3}	23.00 a	0.22 b
300 mg.dm^{-3}	20.50 a	0.18 b
400 mg.dm^{-3}	28.00 a	0.57 a
500 mg.dm^{-3}	12.80 a	0.13 b
Pr > Fc	0.2768^{NS}	0.0001*
Overall average	22.20	0.24
SE	±4.76	±0.05

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error.

effect on growth, whereas the specie *S. parahyba* (Vell.) Blake presented decrease in growth as the doses of Pb increased. In the present study, we verified that the growth of *I. uruguensis* was continuous even at high doses of lead.

Legume-rhizobia symbiosis is a beneficial association between plant and bacteria, which promotes N fixation (Leite 2015). Rhizobia help plant growth (Glick 1995) via adsorption and tolerance to metals (Hao *et al*. 2013), having an important role in phytoremediation plants. Thus, the fact that rhizobia symbiosis in *I. uruguensis* species is not compromised by the presence of Pb may have positively influenced its growth.

There was no direct damage to nodulation, since the number and mass of the nodules were kept in all treatments, which may indicate tolerance of the symbiotic system to contaminated soil.

Nodules can act as a buffer area for heavy metals, reducing the risk of direct exposure of the plant to metals (Hao *et al*. 2013), a crucial symbiotic relationship for phytoremediation plants.

Table 3 – Rate of Chlorophyll A (µg g^{-1} FM), Chlorophyll B (µg g^{-1} FM), and total chlorophyll (µg g^{-1} FM) present in leaves of *Inga uruguensis* in lead-contaminated soils.

VS	Chlorophyll A (µg g^{-1} FM)	Chlorophyll B (µg g^{-1} FM)	Total Chlorophyll (µg g^{-1} FM)
Treataments		**Averages**	
0 mg.dm^{-3}	733.84 ab	234.44 ab	968.05 a
100 mg.dm^{-3}	385.34 b	97.24 b	482.48 b
200 mg.dm^{-3}	769.30 ab	189.54 ab	953.65 a
300 mg.dm^{-3}	932.71 a	281.39 ab	1213.82 a
400 mg.dm^{-3}	821.16 a	371.57 a	1192.43 a
500 mg.dm^{-3}	975.45 a	288.95 ab	1264.11 a
Pr > Fc	0.0039*	0.0130*	0.0003*
Overall average	769.63	244.35	1012.42
SE	±96.98	±49.09	±107.49

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error.

Table 4 – Average dry mass (g) and lead quantification (mg.kg^{-1} DM) of the aerial part and root and tolerance index of *Inga uruguensis* in lead-contaminated soils.

VS	Dry mass (g)		Lead quantification (mg.kg^{-1} DM)		Tolerance index
Treatments	**Shoots**	**Root**	**Aerial part**	**Root**	
0 mg.dm^{-3}	6.14 ab	2.94 ab	< 3.0** b	< 3.0** d	-
100 mg.dm^{-3}	5.71 bc	2.11 b	6.10 b	43.17 d	0.86
200 mg.dm^{-3}	7.03 ab	3.18 ab	8.87 b	187.00 c	1.12
300 mg.dm^{-3}	3.95 d	2.31 b	5.50 b	175.50 c	0.69
400 mg.dm^{-3}	7.22 a	3.75 a	11.77 b	657.50 a	1.21
500 mg.dm^{-3}	4.45 cd	2.32 b	29.4 a	301.50 b	0.75
Pr > Fc	0.0000*	0.0005*	0.0000*	0.0000*	-
Overall average	5.75	2.77	10.76	227,93	-
SE	±0.31	±0.25	±1.89	±11.33	-

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error; ** = smaller than the device's quantification limit.

The fact that the nodulation is kept in *I. uruguensis* may be an important indicator of tolerance of this plant species to Pb as well as of its capacity of N fixation.

According to Bekiaroglou & Karataglis (2002), a reduction in chlorophyll synthesis was observed when plants of species *Mentha spicata* L. were subjected to stress due to excess of Pb and Zn. In plants of species *Phaseolus mungo* L. and *Lens culinaris* Medik., Pb toxicity inhibited chlorophyll synthesis due to metabolic and enzymatic changes caused by this heavy metal (Haider *et al*. 2006). Manius *et al*. (2003), studying the effect of heavy metals nickel (Ni), copper (Cu) and zinc (Zn) in plants of species *Typha latifolia* L., observed no difference in the average chlorophyll values between treatments. In our study, there was an increase in chlorophyll synthesis in treatments with high Pb doses. This fact may have a mechanism that plants use to minimize the effects of Pb, as the increase of the chlorophyll content also increases the photosynthetic rate, which may increase plant mass, diluting the contaminant in the plant.

Kosobrukhov *et al*. (2004) reported that Pb reduced dry mass in *Plantago major* L. This effect was also seen in plants of the mesquite, jureminha and vetiver species (Alves *et al*. 2008). Iannacone & Alvariño (2005) found that the reduction of root growth was one of the main negative effects of Pb on growth of *Allium cepa* L., *Beta vulgaris* L., *Oriza sativa* L. and *Raphanus sativus* L. As Pb is preferentially retained in roots, it generally affects shoots less (Patra *et al*. 2004). The presence of Pb in plant tissues can cause disturbance in mineral nutrition, water imbalance, changes in membrane permeability, and changes in hormonal status, impairing plant growth (Sharma & Dubey 2005). In our study, this negative effect of lead was not observed.

Plants with TI above 0.8 are considered highly tolerant; plants with TI between 0.5 to 0.8 are considered moderately tolerant; and plants with TI below 0.5 are considered sensitive (Jia *et al*. 2017). The TI was not below 0.69 in any of the treatments, a strong indication of the tolerance capacity and potential for use of this species in contaminated soils. Even under conditions of higher Pb content in the soil (400 and 500 mg.dm^{-3}), the TI was greater than 0.75. Although treatment 300 mg.dm^{-3} shows a reduction in TI, it is still above 0.69, indicating that, for this developmental stage, tolerance of the species to Pb is moderate.

In *Talinum patens* (L.) Willd, the root is the main organ for Pb allocation (Souza 2017). The same effect was observed in *Eclipta prostrata* (L.) L., *Scoparia dulcis* L. and *Phyllanthus niruri* L. (Chandrasekhar & Ray 2019). It also acts as a retention barrier, hindering Pb transport to

Table 5 – Average Concentration of amino acid (mg.g^{-1} FM), protein (mg.g^{-1} FM) and soluble carbohydrates (mg.g^{-1} FM) in *Inga uruguensis* tissues in lead-contaminated soils .

VS	Amino acid (mg.g^{-1} FM)			Protein (mg.g^{-1} FM)			Soluble carbohydrates (mg.g^{-1} FM)	
Treatments	**Leaf**	**Root**	**Nodule**	**Leaf**	**Root**	**Nodule**	**Leaf**	**Root**
0 mg.dm^{-3}	12.33 a	5.14 b	3.59 a	2.60 c	0.32 ab	1.09 a	1.24 a	13.50 a
100 mg.dm^{-3}	12.50 a	5.31 b	5.10 a	4.56 ab	0.39 ab	0.63 c	1.41 a	7.41 ab
200 mg.dm^{-3}	11.23 a	6.21 ab	3.71 a	3.26 bc	0.10 b	0.73 bc	1.35 a	2.04 b
300 mg.dm^{-3}	9.81 a	6.50 ab	3.81 a	4.30 abc	0.47 a	0.79 bc	1.20 a	5.65 b
400 mg.dm^{-3}	10.18 a	8.19 a	4.15 a	5.83 a	0.16 ab	0.91 ab	1.12 a	2.88 b
500 mg.dm^{-3}	13.47 a	4.85 b	4.63 a	5.30 a	0.27 ab	0.67 bc	1.20 a	2.67 b
Pr > Fc	0.579^{NS}	0.0003*	0.4876^{NS}	0.0002*	0.0406*	0.0001*	0.5587^{NS}	0.0002*
Overall average	11.59	6.03	4.16	4.31	0.28	0.80	1.25	5.69
SE	±1.62	±0.46	±0.62	±0.44	±0.08	±0.06	±0.12	±1.59

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error.

shoots, contributing to the tolerance of *Mimosa caesalpiniaefolia*, *Erythrina speciosa*, and *Schizolobium parahyba* to Pb (Souza 2010). In addition to roots, other regions can accumulate Pb, which reaches shoots via xylem, accumulates in tissues, or is redistributed via phloem (Souza 2017). Despite a small Pb translocation to shoots, *I. uruguensis* accumulated most Pb in its roots, displaying a phytostabilizing profile.

According to Raskin *et al*. (1994), a plant can be considered in phytoremediation of Pb when it accumulates at least 1 mg.kg^{-1} of Pb in its tissues. In all treatments, *I. uruguensis* accumulated more than 1 mg.kg^{-1}, both in shoots and roots, and accumulation reached 657.50 mg.kg^{-1}, presenting as a suitable plant for phytoremediation of Pb.

According to Sharma & Dietz (2006), stress caused by heavy metals increases the content of free amino acids in different plant tissues. Proline and lead-chelating proteins (metallothioneins and phytoquelatins) are two examples of amino acids that have their synthesis increased to prevent the toxic effects of heavy metals (Pourrut *et al*. 2011). This increase in amino acids was not observed in our study, except in roots, where treatment 400 mg.dm^{-3} showed a significant increase. The amount of protein in leaves was higher in treatments with a higher Pb dose, 400 mg.dm^{-3} and 500 mg.dm^{-3}. In roots, the highest concentration was in treatment 300 mg.dm^{-3} and in nodules, the highest content was in the control treatment. The increase in the level of proteins was also observed by Sidhu *et al.* (2017) when studying the effects of lead stress in *Coronopus didymus* (L.) Sm. This is believed to be due to the induction of low molecular stress proteins under Pb stress (Sidhu *et al.* 2017). About the nodules, the symbiotic relationship with rhizobia may have reduced Pb; therefore, the stress did not increase protein synthesis.

The decrease in the content of total soluble carbohydrates was also observed by Mohan & Hosetti (1997), when studying the effects of *Lemna minor* L. exposure to lead. This decrease in the content of total soluble carbohydrates can be attributed to a reduction in photosynthetic activity or it can be correlated with the activities of starch hydrolytic enzymes (Razak 1985; Sidhu *et al.* 2017). In the current study, although there was no reduction in photosynthesis, the content of soluble carbohydrates decreased significantly in the roots. This shows that, to compensate for the possible damage caused by the accumulation of Pb, the strategy of *I. uruguensis* was to increase the rates of photosynthesis.

Ureids are the direct product of N fixation of tropical plants. Plants that have symbiotic relationships that help in N fixation have more intense synthesis of ureids. The fact that *I. uruguensis* is a tree legume that keeps its nodulation even under high Pb contents contributed to the synthesis of ureids. There was

Table 6 – Average concentration of total ureids (mg.g^{-1} FM), allantoic acid (mg.g^{-1} FM) and allantoin (mg.g^{-1} FM) in *Inga uruguensis* tissues in lead-contaminated soils.

VS	Total ureids (mg.g^{-1} FM)			Allantoic acid (mg.g^{-1} FM)			Allantoin (mg.g^{-1} FM)		
Treatments	**Leaf**	**Root**	**Nodule**	**Leaf**	**Root**	**Nodule**	**Leaf**	**Root**	**Nodule**
0 mg.dm^{-3}	6.15 ab	4.06 a	4.39 b	0.42 b	0.02 a	0.06 a	5.86 ab	4.03 ab	4.33 b
100 mg.dm^{-3}	4.65 b	4.97 a	6.68 a	0.40 b	0.02 a	0.02 b	4.13 b	4.95 ab	6.65 a
200 mg.dm^{-3}	5.41 b	3.76 a	4.76 ab	0.64 b	0.02 a	0.02 b	4.76 ab	3.74 b	4.74 ab
300 mg.dm^{-3}	5.60 b	3.80 a	3.89 b	0.63 b	0.03 a	0.02 b	4.98 ab	3.77 b	2.91 b
400 mg.dm^{-3}	5.09 b	4.88 a	3.70 b	1.66 a	0.02 a	0.02 b	4.56 b	4.86 ab	3.68 b
500 mg.dm^{-3}	8.45 a	6.34 a	2.88 b	0.78 b	0.02 a	0.02 b	7.43 a	7.69 a	2.86 b
Pr > Fc	0.0021*	0.0814^{NS}	0.0003*	0.000*	0.538^{NS}	0.000*	0.015*	0.031*	0.0000*
Overall average	5.89	4.63	4.38	0.75	0.22	0.27	5.29	4.84	4.19
SE	±0.49	±0.66	±0.48	±0.08	±0.003	±0.003	±0.56	±0.86	±0.46

Ns = not significant to the Tukey test with 5% significance; * = significant to the Tukey test with 5% significance; VS = variation source; SE = standard error.

also greater synthesis of ureids in the form of allantoin, which may indicate low activity of the enzyme allantoinase in these plants.

For N transport, the two most common forms of ureids used are allantoin and allantoic acid (Todd *et al*. 2006). The fact that allantoin and allantoic acid are rich in N, with an N:C ratio 1:1, confer a great advantage as a transport molecule to these organic compounds, reducing carbon expenditure due to N assimilation (Quiles *et al*. 2009).

Pb is a toxic element and many plants die when exposed to it, which can lead to soil exposure and future erosion. Thus, understanding the potential of tree species in phytoremediation, such as *I. uruguensis*, is essential for the treatment and rehabilitation of contaminated soils.

Inga uruguensis survived the initial exposure to Pb and was tolerant to this heavy metal even under the highest concentrations. Therefore, it has the potential to be used as a phytoremediation agent. As *I. uruguensis* accumulated most Pb in its roots, this plant has the profile of a phytostabilizer.

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