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Potential of Forage Grasses in Phytoremediation of Lead through Production of Phytoliths in Contaminated Soils

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Abstract: Phytoremediation has become a promising technique for cleaning Pb-contaminated soils. Grasses have a phytoextractor potential for extracting metal from soil by transporting it and accumulating it in high concentrations in their shoots, and they have the ability to immobilize and inactivate it via phytoliths. The objective of this work was to evaluate the phytoremediation potential of forage grasses through the production of phytoliths and the occlusion of Pb in the phytoliths cultivated in Pb-contaminated soils. Three greenhouse experiments were conducted in a completely randomized design, separated by soil type (Typical Hapludox, Xanthic Hapludox and Rhodic Hapludox), in a 3 × 4 factorial scheme consisting of three forage grasses (*Megathyrsus maximus*, *Urochloa brizantha* and *Urochloa decumbens*) and four Pb rates (0, 45, 90 and 270 mg kg^{-1}) with four repetitions. The forage grasses were influenced by increases in the Pb concentrations in the soils. The higher Pb availability in Typic Quartzipsamment promoted Pb toxicity, as indicated by the reduced dry weights of the shoots, increased phytolith production in the shoots, increased Pb in the shoots and Pb occlusion in the phytoliths of the forage grasses. The production and Pb capture in the phytoliths in the grasses in the Pb-contaminated soils were related to the genetic and physiological differences in the forage grasses and the Pb availability in the soils. *Urochloa brizantha* was the most tolerant forage to the excess Pb, with a higher production of phytoliths and higher Pb occlusion in the phytoliths, making it a forage grass that can be used in the future for the phytoremediation of Pb-contaminated soils.

Keywords: *Urochloa*; *Megathyrsus maximus*; lead; biomineralization; Entisol; Oxisol

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1. Introduction

Plants are targets for a wide range of pollutants that vary in concentration, speciation and toxicity. Lead (Pb) is among the most toxic pollutants that affect plants [1–6], being found in all environmental compartments, such as soil, water, the atmosphere and living organisms [5,7]. The capacity for environmental contamination by Pb results from its persistence in soil, which it can achieve via its low mobility and non-degradation in soil [6,8–12].

Lead tends to accumulate on the soil surface, and its concentration is reduced deeper in the soil [5,6,8–12]. Lead, which plants easily uptake and accumulate [1,13,14], is considered a subtle and slow-acting general protoplasmic poison, and it is highly toxic, even in small concentrations [6,8–12]. Lead toxicity causes changes in enzymatic, nutritional, hormonal and water balances and changes in photosynthesis, respiration and membrane structure and permeability, resulting in a reduction in growth, chlorosis and the darkening of the root system of plants [1,6,15]. High concentrations of Pb in a plant may eventually promote cell death, resulting in reduced crop yields becoming a serious problem for agriculture [1,6].

The remediation of Pb-contaminated soils is a major challenge for many industries and government agencies. Lead-contaminated sites have been remedied through a relatively narrow range of engineering-based technologies [1]. Phytoremediation uses plants to remediate areas contaminated with heavy metals, and it is a promising technique with great potential for cleaning Pb-contaminated soils [1,5]. Phytoremediation may involve several processes, including phytoextraction, which is based on easy cultivation and the use of fast-growing plants capable of extracting heavy metal from soil by transporting it and accumulating it in high concentrations in their shoots [1]. Grasses have a potential phytoextractor of heavy metals due to tolerance and accumulation of metals in tissues, besides presenting high growth rate, biomass production and abundant root system [5,16] combined with their ability to produce phytoliths [17–19]. Plant tolerance to Pb depends on genotype and physiological characteristics [1,5,20], with the existence of different defense strategies that provide protection against its harmful effects [1,6,21]. Phytolith production is one of these defense strategies that enable plant survival under such extreme conditions [17–19,22,23].

Phytoliths are amorphous silica particles between 1 and 250 μm in size, resulting from the uptake of silicic acid from the soil solution by plant roots [24,25]. Structures form via the polymerization process of silicic acid, which causes amorphous silica to precipitate along with metals in the cells of some plants [17–19,26]. Silica bodies can occlude harmful metal ions in some parts of plants, reducing the stress caused by these metals in many terrestrial plants, especially in species of the Cyperaceae and Poaceae families [17–19,22,24]. They also reduce soil-soluble metals, mainly in contaminated areas and, most importantly, without the risk of contamination of the food chain due to its stability [12,22,23,25,27–29].

Studies have reported that phytoliths produced in a plant can contribute to the immobilization and subsequent inactivation of the plant's tolerance to toxic metals [22,25,27]. The production and concentration of plant phytoliths depend on phylogenetic characteristics, such as species and genus, and they vary with plant phenology, as well as soil type [17–19,22,23]. Some grass species exhibit characteristics of a high growth rate and biomass yield capacity, and they can tolerate and accumulate toxic metal [28] with a high phytolith yield capacity [17,19,22]. The grasses evaluated in this study are *Megathyrsus maximus* and *Urochloa* genera, with high biomass production and rapid growth [5], classified according to their management and soil fertility requirements [5,16]. *Megathyrsus maximus* is known worldwide for having high productivity and for being able to adapt to different soil and climatic conditions [16], and *Urochloa* grasses stand out for being more rustic and having a good ability to adapt to diverse environments [16], but the phytolith yield capacities of these grasses are unknown. Thus, the present study aimed to evaluate the phytoremediation potential of forage grasses through the production of phytoliths and the occlusion of Pb in the phytoliths cultivated in Pb-contaminated soils.

2. Material and Methods

2.1. Soil Characterization and Experimental Design

Three greenhouse experiments were conducted in Diamantina (18°15′ S, 43°36′ W, 1250 m a.s.l.), Minas Gerais, Brazil. The soils were a Typic Quartzipsamment (TQ), a Xantic Hapludox (XH) and a Rhodic Hapludox (RH), classified according to Soil Taxonomy [30], with different chemical and textural characteristics. The soils were collected in a condition of native "Cerrado" to ensure the absence of metal contamination of the surface soil layer (0–0.2 m depth). A subsample was air-dried and sieved (2.0 mm) for chemical analyses and soil texture determination [31] (Table 1). The available Pb concentrations in the soils were determined by using the USEPA 3052 method [32] (Table 1).

Table 1. Soil attributes before application of basic fertilizer and Pb rates.

Attribute	Unit	Soil [(6)]		
		TQ	XH	RH
pH [(1)] water	-	5.1	5.4	5.5
P [(2)]	mg kg^{-1}	0.2	0.1	0.2
K [(2)]	$mmol_c$ kg^{-1}	0.4	0.1	0.2
Ca [(3)]	$mmol_c$ kg^{-1}	6.7	4.50	8.1
Mg [(3)]	$mmol_c$ kg^{-1}	3.5	1.8	3.9
Al [(3)]	$mmol_c$ kg^{-1}	7.8	4.2	1.6
Cation-exchange capacity	$mmol_c$ kg^{-1}	40.6	71.4	49.2
Organic carbon	g kg^{-1}	3.5	5.8	5.2
Pb [(4)]	mg kg^{-1}	0.0	0.0	0.0
Maximum P adsorption	mg kg^{-1}	100	200	250
Sand [(5)]	g kg^{-1}	830.0	580.0	310.0
Loam [(5)]	g kg^{-1}	110.0	70.0	180.0
Clay [(5)]	g kg^{-1}	60.0	350.0	510.0

[(1)] Soil:water 1:2.5. [(2)] Mehlich-1 extractor. [(3)] KCl 1 mol L^{-1} extractor. [(4)] USEPA 3052. [(5)] Pipette method. [(6)] TQ: Typic Quartzipsamment. XH: Xantic Hapludox. RH: Rhodic Hapludox.

The liming of soils was carried out with dolomitic limestone to increase the base saturation to 45%. The lime requirement (LR) was calculated as LR (Mg ha^{-1}) = (($V_2 - V_1$) × CEC)/100, where V_2 is the base saturation recommended for the grasses (45%), V_1 is the base saturation in the soil analysis, and CEC is the cation-exchange capacity (Table 1). The soils were incubated for 30 days under field capacity conditions, controlled by daily weighing and maintained throughout the experiment.

The basal fertilization rates were 180 mg N (Urea, $NH_4H_2PO_4$, $(NH_4)_2SO_4$, $Pb(NO_3)_2$), 150 mg K (KCl), 50 mg S ($(NH_4)_2SO_4$), 1 mg B (H_3BO_3), 1.5 mg Cu ($CuCl_2$), 5.0 mg Fe ($FeSO_4.7H_2O$-EDTA), 4.0 mg Mn ($MnCl_2.H_2O$) and 4 mg Zn ($ZnCl_2$) per kg of soil. The phosphate fertilization was based on the maximum P adsorption capacity of each soil (Table 1), estimated from the data of the Langmuir isotherm second region [33]. The applied phosphorus rate was 200 mg for the TQ, 350 mg for the XH and 450 mg for the RH per kg of soil with source NaH_2PO_4. Nutrients were applied as pure reagents for analyses, and they were mixed completely and incubated for 15 days in each soil. The Pb rates were applied after liming, and a basic fertilizer was applied as a pure lead nitrate reagent for planting and soil incubation for 15 days.

Three experiments were conducted in a completely randomized design, with a 3 × 4 factorial scheme and three replications. The factors were three forage grasses (*Urochloa decumbens* (Stapf) R.D. Webster cv. Basilisk; *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu e *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Mombaça) and four Pb rates (0, 45, 90 and 270 mg per kg of soil) examined in three soils. The choice of forage grasses was due to their rusticity, ability to adapt to different environments, easy handling and good market acceptance [16]. The Pb rates were based on the investigation values of the soils [34].

The forage grasses were seeded in pots with 3 kg of soil. Then, 7 days after seedling emergence, thinning was performed, leaving only one plant per pot. The four top-dressing fertilizations of 30 mg N (urea) per kg soil were carried out every 15 days, after the thinning of the grasses. The pots were irrigated daily with distilled water to maintain soil moisture at field capacity, which was checked daily by weighing the plots.

2.2. Measurements and Analytical Determinations

The plant shoots were harvested 120 days after the thinning of the forage grasses. The shoot samples were dried in a forced-air oven at 65 °C until constant weight, and they were ground and weighed to determine their dry weights. The Pb was extracted in a microwave oven (CEM Mars^{TM} 6) with nitric acid (65% *v/v* - Merck), and its concentration was determined using atomic absorption spectrometry in a graphite oven (AAnalyst 800, Perkin-Elmer, Waltham, MA, USA). The quality of the Pb analysis of the plant tissue was assured by using certified reference material (NIST SRM 1573a tomato leaf) and reagent blanks.

The phytoliths in the shoots of the forage grasses were prepared and separated using the process detailed in [35]. The separated phytoliths were opened by using the USEPA 3052 method [32]. The Pb concentrations in the filtered solutions were determined using atomic absorption spectrometry and a graphite oven (AAnalyst 800, Perkin-Elmer, Waltham, MA, USA).

2.3. Statistics

The data were subjected to an analysis of joccccint variance, which consisted of a study of the Pb rates and the forage grasses within each soil type. The means of the forage grasses and soil types were compared by using Tukey's test at a 5% significance level. The regression equations were adjusted for the variables in the function of Pb rates.

3. Results

The shoot dry weights of the forage grasses decreased with an increase in the Pb rates applied to the soils ($p < 0.01$), and the forages that were grown in the TQ presented lower reductions in their shoot dry weights than those that were grown in the other soils (Figure 1). Based on the regression coefficients (Figure 1), *Urochloa brizantha* had the highest tolerance to Pb than the other forages evaluated and the lowest reduction in dry matter yield when grown in all evaluated soils. The higher tolerance of the forages in the TQ and the lower sensitivity to Pb presented by the forage *Urochloa brizantha* may be related to the characteristics of the species.

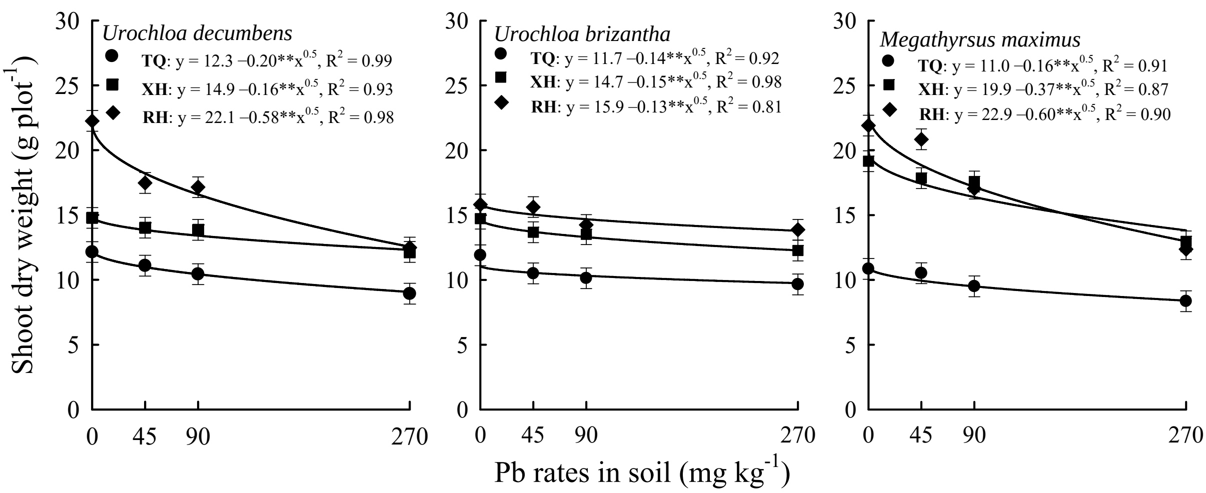

Figure 1. Shoot dry weights of forage grasses with increases in Pb rates within 120 days of thinning in three soils (TQ: Typic Quartzipsamment. XH: Xantic Hapludox. RH: Rhodic Hapludox) (significant at ** $p = 0.01$).

Phytolith production in the forage shoots increased with an increase in the Pb rates applied to the soils ($p < 0.01$). The three forages presented phytolith production, independent of soil and species, with *Urochloa brizantha* presenting the highest phytolith production (Figure 2). This may also explain the higher phytolith production observed in the forages that were grown in the TQ than in the forages that were grown in the other evaluated soils (Figure 2). Pb toxicity caused a greater increase in the phytoliths in *Urochloa brizantha* than in the other forages evaluated, with the higher yield reflecting the genetic and physiological differences between the forage grasses in producing phytoliths.

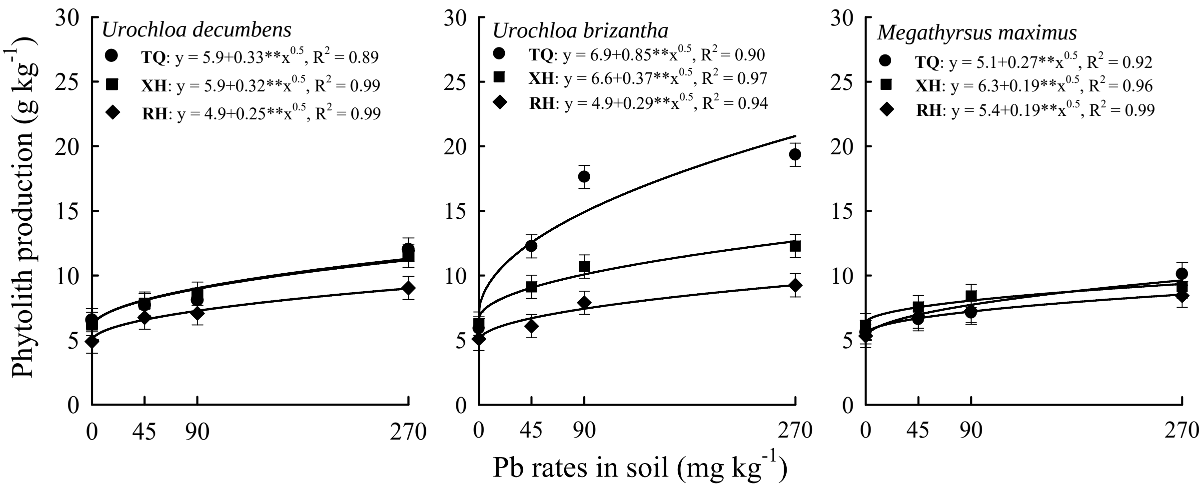

Figure 2. Phytolith production in forage grasses with increases in Pb rates within 120 days of thinning in three soils (TQ: Typic Quartzipsamment. XH: Xantic Hapludox. RH: Rhodic Hapludox) (significant at ** p = 0.01).

The lead concentrations in the shoots and phytoliths were evaluated to verify the Pb uptake and Pb occlusion by the grasses, respectively. The Pb addition to the soils linearly increased the Pb concentrations in the shoots (Figure 3) and phytoliths (Figure 4). The forages grasses only differed in Pb uptake (Figure 3) and Pb occlusion in the phytoliths (Figure 4) when cultivated in the TQ.

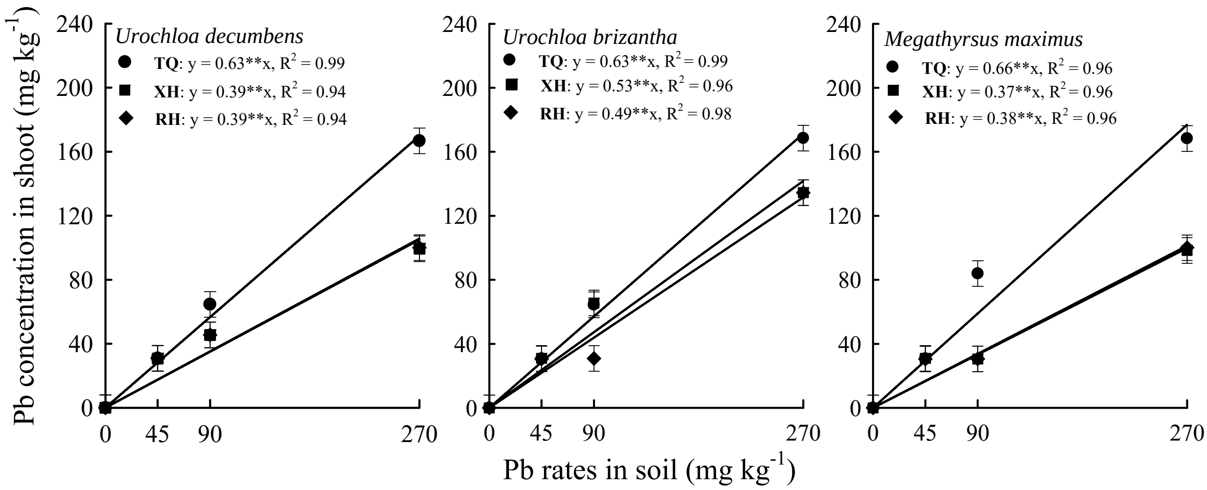

Figure 3. Pb concentrations in shoots of forage grasses with increases in Pb rates within 120 days of thinning in three soils (TQ: Typic Quartzipsamment. XH: Xantic Hapludox. RH: Rhodic Hapludox) (significant at ** p = 0.01).

Higher Pb concentrations in the shoots were found in the grasses that were cultivated in the TQ than in the grasses that were cultivated in the other soils (Figure 3); this was due to the higher Pb availability in the TQ, being a soil with a sandy texture (Table 1), reflecting the toxic effect of Pb on the dry matter yield of grasses (Figure 1). On average, the Pb concentration in the shoot of *Urochloa brizantha* was 17% higher than that in the other two forage grasses when grown in the soils at the highest applied rate (270 mg kg^{-1}).

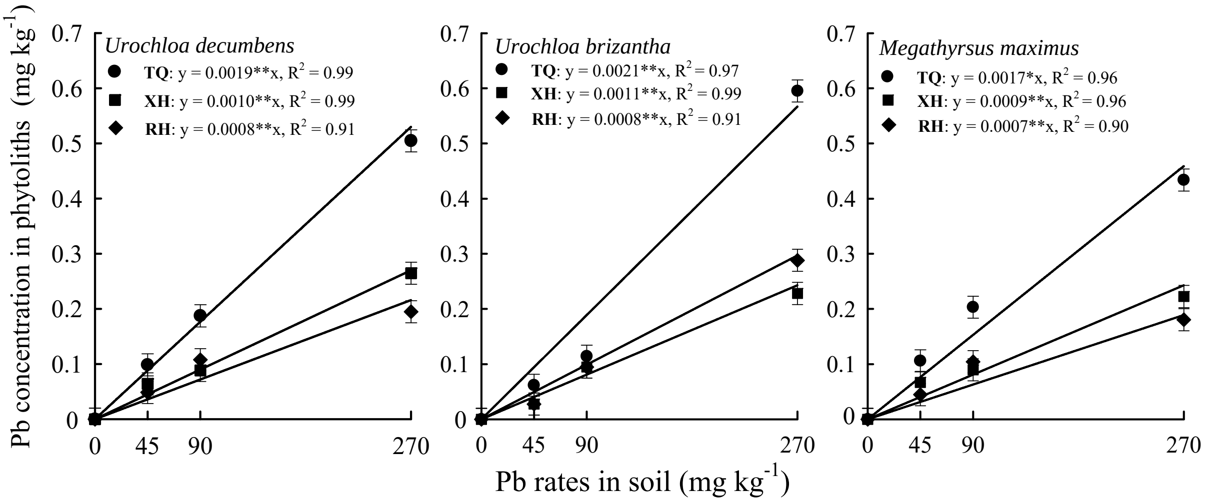

Figure 4. Pb concentrations in phytoliths of forage grasses with increases in Pb rates within 120 days of thinning in three soils (TQ: Typic Quartzipsamment. XH: Xantic Hapludox. RH: Rhodic Hapludox) (significant at ** $p = 0.01$).

The Pb occlusion in the phytoliths was higher in the grasses that were grown in the TQ than in the grasses that were grown in the other soils (Figure 4); this occurred in response to the greater toxic effect of Pb due to the higher availability of Ni in the TQ, which is related to this soil having a sandier texture than the other evaluated soils (Table 1). *Urochloa brizantha* had a higher Pb occlusion in the phytoliths than the other evaluated forage grasses, which may be related to the tolerance mechanism of this grass in response to Pb toxicity.

The lead concentrations in the shoots increased as a function of the Pb rates applied to the three soils ($p < 0.01$) for all the forages evaluated (Figure 3). The lead concentrations in the phytoliths increased with an increase in the Pb concentrations in the three soils ($p < 0.01$). The phytoliths produced in the forages in the different soils (Figure 2) were able to capture Pb metal in the three soils (Figure 4). A higher Pb concentration was observed in the phytoliths in the forages that were grown in the TQ, with *Urochloa brizantha*, in general, being the forage that presented the highest Pb occlusion in the phytoliths, based on the regression coefficients (Figure 4), with a 17% greater P occlusion compared to the other two forage grasses at the highest Pb rate in the soil (270 mg kg^{-1}).

The increase in the Pb concentrations in the soils, in general, provided the greatest reduction in the production of shoot dry matter (Figure 1), the greatest production of phytoliths (Figure 2) and the highest Pb concentrations in the shoots of the forage grasses (Figure 3) and in the phytoliths (Figure 4). *Urochloa brizantha*, in general, was the forage that showed the lowest reduction in biomass production in the shoot (Figure 1), the highest production of phytoliths (Figure 2) and the highest concentration of Pb in the phytoliths (Figure 4). The forages cultivated in the TQ showed the lowest dry matter production in the shoots (Figure 1), the highest production of phytoliths (Figure 2), the highest Pb concentrations in the shoots (Figure 3) and the greatest Pb occlusion in the phytoliths (Figure 4) at the maximum dose of Pb applied (270 mg kg^{-1}).

4. Discussion

The low Pb supply rate reduced the shoot dry weights of the forage grasses in the evaluated soils (Figure 1). The results prove the toxic effect of Pb, since the growth and development of plants grown in Pb environments are affected by several negative effects that occur after Pb absorption by plants [1–6]. The nutritional imbalance caused by Pb may have occurred in the forage grasses evaluated in the present study, contributing to the reductions in the dry weights of the shoots (Figure 1). Nutritional imbalance in different plant species [1], such as reductions in the absorption, distribution and accumulation of macro- and micro-nutrients in various bean crop organs [3], affects their biomass production and development [2,3,5,6].

Lead has a higher availability in sandy soils [5], but the forages cultivated in the TQ showed the lowest reductions in the dry weights of the shoots (Figure 1). The results confirm that the phytotoxicity of Pb [2,3,5,6] depends not only on its availability and concentration [6,8–12] but also on the period of exposure to the metal, the species and its physiological characteristics, the affected organ or tissue and the tolerance mechanisms [1,6,7,21]. Studies have shown that Pb affects plants differently, with the characteristics of each species resulting in different sensitivities to the adverse effects of Pb [1,13,14].

The phytolith production in the forages proves that they are potential producers of silica bodies (Figure 2) in soils with different textures (Table 1), compositions and Pb concentrations. Studies have claimed that Poaceas are major phytolith producers [17–19,22,24]. The forages exhibited increased phytolith production when higher Pb rates were applied to the soils (Figure 2), confirming that a higher concentration and availability of Pb in soil may influence phytolith production in plant organs [22,26]. That is, the higher phytolith production in the forages was related to the higher Pb concentrations and availability [6,8–12] in the soils in the present study. Soils that are more sandy with a low clay concentration, a cation-exchange capacity and organic matter are known to have a higher bioavailability of Pb [6,8–12], which indicates the importance of these factors in the adsorption and desorption of Pb to soil and, consequently, in the production of phytoliths in plants [22].

Urochloa brizantha produced more phytoliths with increases in the Pb rates applied to the three soils (Figure 2). Plant phytolith production depends on phylogenetic characteristics, such as genus and species, but species phenology, soil characteristics and environmental conditions are also factors that influence the production of silica bodies [12,22,26]. Under experimental conditions, the results indicate that the higher tolerance of *Urochloa brizantha*, demonstrated by the lower reduction in the dry weights of the shoots, may reflect higher phytolith production (Figure 2). Silica bodies called phytoliths provide mechanical strength and help protect against physical, chemical and biological stresses on the plant [17–19,22,23].

The forages evaluated could extract Pb from the soil, and their absorption was not limited by increases in the Pb rates (Figure 3), noting their phytoextracting capacity; it is possible that they were tolerant because they have effective physiological and biochemical mechanisms in place to reduce Pb toxicity in tissues [1,13,14].

At first, the forages evaluated may not be considered plants of interest for Pb phytoremediation due to their reduced growth when the Pb concentrations increased in the soils (Figure 1). In addition, Pb-hyperaccumulating plants can extract, tolerate and accumulate high Pb concentrations in tissues when grown in soils contaminated with Pb. Lead tolerance may be at concentrations exceeding 1.000 mg kg^{-1} of Pb in dry matter [5], and these plants may be able to extract and accumulate it in tissues, reaching Pb concentrations of up to 1% of the dry matter produced [11], although this was not observed in the present study.

In general, almost all hyperaccumulating plants reported in the literature have high concentrations of heavy metals in their dry mass and produce a low biomass, which results in a low metal uptake per area [20]. In this sense, the high dry mass production of the forages [5,16] and their Pb accumulation capacity demonstrate their potential use in Pb phytoremediation programs. In addition, the phenotype of metal overaccumulation in shoots is an extreme plant response to soils with high metallic concentrations and is acquired throughout a plant's evolution [15]. Therefore, the evaluated forages are bioindicator plants, since this classification is given to plants that absorb toxic metals and have internal metal concentrations that reflect external concentrations [20], and, therefore, they may be plants with the potential for Pb phytoextraction in contaminated soils.

The most well-known toxic metal tolerance mechanisms are summarized as mechanisms that act to expel absorbed metal or prevent root entry and detoxification by sequestering the metal into plant-specific organelles, particularly vacuoles [1]. However, other potential mechanisms, such as the intra- and extra-cellular binding of toxic metals and their isolation in a non-vital compartment, are the subject of speculation, discussion and study [12,27]. The potential mechanisms include phytolith production and metal occlusion [17–19,22,23]. In the present study, the Pb applied to soils provided increases in the Pb concentrations in shoots and Pb occlusion in phytoliths (Figure 4), with a higher phytolith production (Figure 2) in the forages. It is possible that the capture and accumulation of Pb by phytoliths are due to a forage defense mechanism, which may have helped the evaluated forages to reduce Pb toxicity.

Among the functions attributed to plant phytolith production [17–19] is the relief of physiological stresses on plant growth due to heavy metal toxicity from the capture and immobilization of these metals by the silico phytoliths [12,22,23,25,27–30]. A higher production of phytoliths (Figure 2) and Pb occlusion in the phytoliths were observed when the forages were grown in the TQ (Figure 4), possibly due to the higher availability and/or concentrations of Pb and Si in this soil, because its low clay concentration, cation-exchange capacity and organic matter (Table 1) are characteristics that increase soil Pb availability [5]. In contrast, in the clay soils with higher Fe oxide concentrations (XH and RH) (Table 1), the forages presented lower Pb toxicity with lower reductions in the dry weights of the shoots (Figure 1) when compared to the sandy soil (TQ). The lower effect of Pb phytotoxicity can be attributed to the lower Pb availability in clay soils, whose components have a strong adsorptive capacity, and to the higher Pb binding energy in soils with higher clay mineral concentrations, which reduces the availability of Pb to plants and, consequently, ensures a toxic potential that is lower than that of sandy soils [5,6,8–12].

The available Pb concentration is adsorbed in an exchangeable form in soil, indicating high Pb mobility and immediate bioavailability [6,8–12], while concentrations resulting from intense chemical bonds are present in fractions of organic matter and oxides of amorphous and crystalline Mn and Fe, and they indicate that the metal is immobilized, poorly mobile in the environment and has a low availability to plants, presenting a lower risk of environmental contamination [6,8–12]. In addition to the availability of Pb in the soil, the production and chemical composition of phytoliths can be influenced by metal uptake, climatic conditions, the silicon concentration in the soil, plant species, location, disease resistance and fertilizer requirements [22,25,26].

5. Conclusions

The production of phytoliths by forage grasses in Pb-contaminated soils can increase the tolerance of forages to Pb through the detoxification, immobilization and inactivation of Pb due to the stable nature of these siliceous bodies. *Urochloa brizantha* can be a future forage grass used for the phytoremediation of Pb-contaminated soils. However, there is a need for further studies to evaluate the role of phytolith formation in Pb sequestration in forage grasses.

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