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The effect of silicon (Si) on the growth and nutritional status of *Schizolobium amazonicum* seedlings subjected to zinc toxicity

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Abstract

Zinc is an essential element to plants. However, excessive zinc levels can severely damage them. *Schizolobium amazonicum* is an Amazon native species that presents desirable features to remediate environments contaminated with heavy metals. Silicon has the beneficial effect of reducing the toxicity of different contaminants. The aim of the current study is to investigate the effect of Si on the growth and nutritional status of *S. amazonicum* seedlings subjected to zinc toxicity. The study followed a completely randomized design at 4 x 2 factorial arrangement based on four zinc (1, 150, 300 and 600 µM) and two silicon (0 and 1.5 mM) concentrations with five repetitions for 30 days. Increasing Zn concentrations in the nutrient solution reduced the growth of the plant and Ca, P, Mg, Fe, Mn and Cu contents in plant tissues, increased S concentrations and led to higher toxicity in the roots than shoot of *S. amazonicum* plants. Si addition to the nutrient solution increased plant growth and the absorption of the evaluated macro and micronutrients. Si increased plant tolerance level from 42.8 to 41.3% at 600 µM Zn, which suggested that this element mitigated the phytotoxic effects of the excess of zinc. Based on the tolerance index, the species presented medium and high tolerance to the evaluated zinc doses. Bioconcentration and translocation factors have indicated the low Zn-phytoextraction capacity of *S. amazonicum* and suggested that the species may be promising for Zn phytostabilization purposes.

Keywords: bioconcentration factor, heavy metal, *paricá*, phytoremediation, translocation factor.

Abbreviations: NL_ Number of leaflets; LA_ Leaf area; SH_ Shoot height; CD_Collar diameter; RDM_Root dry matter; SDM_Shoot dry matter; TDM_Total dry matter; H/D_Shoot height/collar diameter; RDM/SDM_R/S; DM_ Dry matter; BCF_Bioconcentration factor; TF _Translocation factor; TI_Tolerance index; SD_ Standard deviations

Introduction

Soil contamination with heavy metals has been causing severe damage to different ecosystems and to human health (Huang et al., 2016, Shahid et al., 2017). The improper handling of mining, metallurgical, pesticide, fertilizer and urban wastes leads to environmental pollution with heavy metals, which depends on their concentration and can lead to toxicity of plants and animals (Niazi et al., 2015).

The phytoremediation technique based on plant cultivation is a way to decontaminate soils contaminated with heavy metals (Burges et al., 2018). Phytoextraction is a good phytoremediation strategy that consists in plant root absorption of heavy metals found in the soil or water and in their translocation to the shoot (Sharma et al., 2016). Forest species that tolerate high metal levels, produce large amounts of biomass and present a well-developed root system. They are recommended to immobilize these contaminants for long periods (Nikolić et al., 2017).

Zinc, which is the second most abundant transition metal, is essential to plant metabolism (Mateos-Naranjo et al., 2018). However, high Zn levels can damage plants (Kaya et al., 2018). The excess of Zn can lead to abnormal plant growth, leaf chlorosis and reduced absorption and translocation of essential elements in plants (Andrejić et al., 2018). Studies about the use of Amazon-native woody species for Zn phytoremediation purposes remain scarce.

Schizolobium amazonicum Huber ex Ducke is an Amazon-native plant species known as *paricá* (Gomes et al., 2019). The species is widely used in reforestation processes and its cultivation area covers 90,000 hectares throughout Brazil (Pereira et al., 2017). The interest in this plant comes from its rapid growth (yield = 38 m^3 ha^{-1} $year^{-1}$), ability to adapt to different edaphoclimatic conditions, as well as from its use as raw material for laminate and plywood production purposes (Ohashi et al., 2010). According to Nogueira et al. (2019), *S. amazonicum* can also be used for phyto-remediation purposes, since it presents cadmium-phytoextraction features.

Silicon is the second most abundant element in the soil (Sahebi et al., 2015). Plant roots can absorb it in silicic acid [$Si(OH)_4$] form and translocate it to the shoot through the xylem (Yang and Zhang, 2018). Although Si is not seen as an essential element, its beneficial effects on biotic and abiotic stress, such as the one caused by heavy metal toxicity, were found in different species (Haddad et al., 2018, Pereira et al., 2018). This beneficial nature can result from metal immobilization in the soil, antioxidant enzyme activity stimulation, and from the co-precipitation, complexation and compartmentalization of silicon/metal ions in plant tissues (Liang et al., 2015, Garg and Singh, 2018). The mechanism adopted by *S. amazonicum* to avoid Zn toxicity remains unknown.

The aim of the current study was to investigate the effect of Si on the growth and nutritional status of *S. amazonicum* seedlings subjected to zinc toxicity.

Results and discussion

Biometric characteristics

The growth of *S. amazonicum* plants was affected by different zinc and silicon concentrations in the nutrient solution (Tables 1 and 2). Increasing Zn concentrations in the culture medium decreased seedling growth. The Zn concentration of 600 µM led to decreased NL (46%), LA (66%), SH (48%), CD (61%), RDM (62%), SDM (55%), TDM (57%) and R/S (17%) in Si-free plants, compared to the control. The Si-treated seedlings recorded sharp decrease in the above metioned parameters: 35%, 51%, 36%, 47%, 53%, 46%, 48% and 11%, respectively. The decreased R/S ratio resulting from the increased zinc content in the solution shows that this metal causes more damage to the root system than to the stem and leaves.

The H/D ratio was increased as the Zn concentrations increased, which indicates higher growth in SH than in CD. The highest H/D ratio (2.21 to 2.94) was recorded for seedlings grown without silicon. The ratio between these two parameters is often used to evaluate the quality of seedlings, since seedling development is expected to be balanced. The lowest H/D ratio observed in seedlings treated with Si implied more resistant plants (Araújo Neto et al., 2000).

Reduced plant growth has been increasingly observed among Zn toxicity symptoms (Anwaar et al., 2015, Mehrabanjoubani et al., 2015, Mateos-Naranjo et al., 2018, Šimková et al., 2018). Si has shown beneficial effect on the vegetative growth of *E. urophylla* (Pinto et al., 2009), *Z. mays* (Paula et al., 2015), *G. hirsutum* (Anwaar et al., 2015) and *O. sativa* (Mehrabanjoubani et al., 2015) grown in nutrient solution enriched with zinc. The aforementioned studies recorded increased plant growth after Si addition to contaminated substrates, a fact that corroborated silicon ability to mitigate zinc toxicity to plant growth.

Zinc and silicon contents in tissues

Zn and Si contents in the roots and shoot of *S. amazonicum* plants were increased as Zn concentrations in the nutrient solution increased. On the other hand, Si addition to the substrate favored its accumulation in the roots and shoots and decreased the Zn concentration only in the shoot (Table 3).

The highest Zn contents, 3,707.9 mg kg^{-1} (treatment with Si) and 3,192.6 mg kg^{-1} (treatment without Si), were found in the roots of plants grown in the solution with 600 µM of zinc, which corresponded to Zn contents 236.2 and 203.4 times higher than the ones found in the control treatment, respectively. The shoot of plants subjected to the aforementioned Zn dose recorded metal contents of 109.4 mg kg^{-1} and 92.5 mg kg^{-1}, which corresponded to metal concentration increase by 10.5 (0.0 µM Si) and 8.9 (1.5 µM Si) times in comparison to the control. This outcome showed that the role played by *S. amazonicum* roots in Zn removal from contaminated soils is more significant than the one played by the shoot, since roots are capable of accumulating higher concentrations of this metal in their tissues. Similar results were found by Feng et al. (2018) in *Sesuvium portulacastrum*.

The highest Si content (1,547.5 mg kg^{-1}) was found when this element was available in the solution with 600 µM Zn. This silicon concentration was 3.5 times higher than the one observed in the shoot of plants subjected to the same toxicity conditions and 6.2 times higher than the one found in roots grown at Zn and Si concentrations of 1 µM and 1.5 mM, respectively.

According to Marques and Nascimento (2014) and Neumann and Zur Nieden (2001), *Cardaminopsis halleri* and *R. communis* presented silicon-zinc co-precipitation in zinc silicates, which accumulated in the vacuole of root cells and led to reduced metabolic availability and Zn translocation to the shoot. Consequently, such process increased plant tolerance to the toxic effect of this metal.

Si can also reduce the effects of Zn concentration on plant tissues and, consequently, reduce its toxicity. This element is associated with the synthesis of phenolic compounds responsible for complexing the contamination agent, as well as Zn dilution resulting from increased DM production (Kidd et al., 2001). This action was also described in other studies that evaluated the association of these elements in plants (Anwaar et al., 2015, Mehrabanjoubani et al., 2015, Paula et al., 2015).

Effect of silicon and zinc on the concentration of macro and micronutrients

Zinc concentration in the nutrient solution was inversely proportional to the nutrient contents evaluated in plant tissues, except for sulfur (Table 4). The shoot of plants treated with 0.0 mM of Si and 600 µM of Zn showed decreased Ca, P, Mg, Fe, Mn and Cu contents (19%, 26%, 26%, 31%, 36% and 47%, respectively), whereas the root system recorded 22%, 35%, 36%, 30%, 31% and 51% decrease in these very same parameters, respectively, in comparison to the control. The addition of 1.5 mM of Si to the nutrient solution mitigated the macro and micronutrient content decrease in *S. amazonicum* plants; the reduction rate in Ca, P, Mg, Fe, Mn and Cu concentrations decreased to 14%, 20%, 21%, 18%, 34% and 35% in the shoot and to 9%, 33%, 23%, 25%, 26% and 42% in the roots, respectively.

The low nutrient accumulation caused by the excess of Zn is a toxicity feature of metals that can delay the vegetative growth of plants (Pereira et al., 2018, Santos et al., 2018, Rizwan et al., 2019). Marques and Nascimento (2014) observed decreased K, Mg, P, N, Ca, Cu, B, Fe, Mo and Mn accumulation in *R. communis* plants subjected to toxic Zn levels. Mateos-Naranjo et al. (2018) observed decreased K, Mg, Ca, P and Mn levels in *Juncus acutus* grown in medium contaminated with Zn. According to the aforementioned authors, reduced nutrient contents can also affect metabolic processes such as photosynthesis.

The decreased P content resulting from the excess of Zn may be associated with the antagonism between these two elements and with the formation of P-Zn complexes, in the form of Zn phosphates or phytates, which are immobilized on the root surface and inhibit the translocation of the metal to the leaves to protect plants from toxicity (Prasad et al.,

Table 1. Number of leaflets (NL), leaf area (LA), shoot height (SH), collar diameter (CD) and Shoot height/collar diameter (H/D) ratio of *S. amazonicum* plants treated with Si and exposed to Zn toxicity.

Si (mM)	Zn (µM)	NL	LA (dm^2)	SH (cm)	CD (mm)	H/D
0	1	66.4 ± 1.1 Ba	21.2 ± 0.5 Ba	27.7 ± 0.8 Ba	12.5 ± 0.5 Ba	2.21 ± 0.02 Ad
	150	45.4 ± 0.9 Bb	13.2 ± 0.3 Bb	18.4 ± 0.7 Bb	7.1 ± 0.2 Bb	2.60 ± 0.04 Ac
	300	39.8 ± 0.8 Bc	10.0 ± 0.6 Bc	16.8 ± 0.6 Bc	6.2 ± 0.2 Bc	2.69 ± 0.02 Ab
	600	35.8 ± 0.8 Bd	7.2 ± 0.6 Bd	14.4 ± 0.5 Bd	4.9 ± 0.2 Bd	2.94 ± 0.05 Aa
1.5	1	77.4 ± 1.5 Aa	27.7 ± 1.2 Aa	30.3 ± 0.7 Aa	14.9 ± 0.4 Aa	2.03 ± 0.03 Bd
	150	48.4 ± 0.9 Ab	15.9 ± 0.7 Ab	21.4 ± 0.5 Ab	9.0 ± 0.2 Ab	2.39 ± 0.01 Bc
	300	44.4 ± 0.5 Ac	12.1 ± 0.7 Ac	18.6 ± 0.5 Ac	7.5 ± 0.2 Ac	2.49 ± 0.02 Bb
	600	43.1 ± 0.6 Ad	10.2 ± 0.6 Ad	17.7 ± 0.5 Ad	6.5 ± 0.2 Ad	2.71 ± 0.04 Ba

Columns presenting different uppercase letters between Si treatments (0 and 1.5 mM of Si at the same Zn concentration) and lowercase letters between Zn treatments (1, 150, 300 and 600 µM of Zn at the same Si concentration) indicate significant differences in the Scott-Knott test ($P < 0.05$). The herein described values represent the means and SD of five repetitions.

Table 2. Dry matter of *S. amazonicum* plants treated with Si and exposed to Zn toxicity.

Si (mM)	Zn (µM)	RDM (g)	SDM (g)	TDM (g)	R/S
0	1	10.2 ± 0.1 Ba	23.4 ± 0.2 Ba	33.6 ± 0.3 Ba	0.437 ± 0.004 Ba
	150	6.3 ± 0.1 Bb	16.0 ± 0.1 Bb	22.3 ± 0.0 Bb	0.395 ± 0.006 Bb
	300	4.6 ± 0.1 Bc	12.3 ± 0.3 Bc	16.9 ± 0.5 Bc	0.377 ± 0.002 Bc
	600	3.8 ± 0.1 Bd	10.5 ± 0.3 Bd	14.4 ± 0.4 Bd	0.365 ± 0.003 Bd
1.5	1	11.1 ± 0.2 Aa	23.8 ± 0.2 Aa	34.9 ± 0.4 Aa	0.464 ± 0.005 Aa
	150	7.3 ± 0.1 Ab	17.6 ± 0.2 Ab	24.9 ± 0.2 Ab	0.416 ± 0.003 Ab
	300	6.0 ± 0.2 Ac	15.0 ± 0.3 Ac	21.0 ± 0.5 Ac	0.397 ± 0.003 Ac
	600	4.8 ± 0.1 Ad	12.4 ± 0.2 Ad	17.2 ± 0.3 Ad	0.386 ± 0.004 Ad

RDM = root dry matter, SDM = shoot dry matter, TDM = total dry matter, R/S = root/shoot dry matter ratio. Columns presenting different uppercase letters between Si treatments (0 and 1.5 mM of Si at the same Zn concentration) and lowercase letters between Zn treatments (1, 150, 300 and 600 µM of Zn at the same Si concentration) indicate significant differences in the Scott-Knott test ($P < 0.05$). The herein described values represent the means and SD of five repetitions.

Table 3. Zn and Si contents in *S. amazonicum* plants treated with Si and exposed to Zn toxicity.

Si	Zn	Zn (mg kg^{-1} DM)		Si (mg kg^{-1} DM)	
(mM)	(µM)	Shoot	Root	Shoot	Root
0	1	10.4 ± 0.4 Ad	15.7 ± 0.6 Ad	-	-
	150	64.0 ± 2.6 Ac	1174.4 ± 9.2 Bc	-	-
	300	92.6 ± 1.2 Ab	2110.3 ± 81.0 Bb	-	-
	600	109.4 ± 3.2 Aa	3192.6 ± 131.0 Ba	-	-
1.5	1	9.1 ± 0.3 Ad	15.7 ± 0.9 Ad	205.6 ± 2.6 Ad	250.1 ± 4.5 Ad
	150	46.8 ± 1.4 Bc	1385.6 ± 14.8 Ac	356.0 ± 3.2 Ac	1114.8 ± 4.0 Ac
	300	70.3 ± 2.4 Bb	2643.4 ± 111.0 Ab	405.1 ± 3.3 Ab	1382.5 ± 19.3 Ab
	600	92.5 ± 3.4 Ba	3707.9 ± 172.3 Aa	445.7 ± 5.4 Aa	1547.5 ± 24.3 Aa

Columns presenting different uppercase letters between Si treatments (0 and 1.5 mM of Si at the same Zn concentration) and lowercase letters between Zn treatments (1, 150, 300 and 600 µM of Zn at the same Si concentration) indicate significant differences in the Scott-Knott test ($P < 0.05$). The herein described values represent the means and SD of five repetitions.

Table 4. Nutrient contents in *S. amazonicum* plants treated with Si and exposed to Zn toxicity.

Si	Zn	Ca	P	S	Mg	Fe	Mn	Cu
(mM)	(µM)	--------------------------(g kg^{-1} DM)--------------------------				--------------------(mg kg^{-1} DM)--------------------		
		Shoot						
0	1	14.8 ± 0.3 Ba	3.78 ± 0.04 Ba	2.17 ± 0.05 Bc	2.43 ± 0.03 Ba	43.8 ± 1.0 Aa	34.5 ± 0.5 Ba	1.73 ± 0.05 Ba
	150	12.6 ± 0.2 Bd	3.18 ± 0.03 Bb	2.45 ± 0.02 Bb	2.06 ± 0.04 Bb	39.8 ± 0.8 Bb	27.2 ± 0.9 Bb	1.17 ± 0.03 Bb
	300	12.2 ± 0.1 Bc	2.89 ± 0.05 Bc	2.54 ± 0.04 Ba	2.01 ± 0.02 Bb	36.3 ± 0.2 Bc	24.2 ± 0.9 Bc	1.08 ± 0.03 Bc
	600	12.0 ± 0.2 Bc	2.78 ± 0.07 Bc	2.58 ± 0.02 Ba	1.81 ± 0.03 Bc	30.4 ± 0.5 Bd	22.0 ± 0.5 Bd	0.91 ± 0.03 Bd
1.5	1	16.6 ± 0.2 Aa	3.98 ± 0.02 Aa	2.27 ± 0.04 Ac	2.67 ± 0.04 Aa	44.8 ± 0.3 Aa	37.0 ± 0.8 Aa	1.83 ± 0.01 Aa
	150	13.8 ± 0.2 Ab	3.37 ± 0.07 Ab	2.58 ± 0.01 Ab	2.29 ± 0.02 Ab	41.0 ± 0.4 Ab	29.6 ± 1.0 Ab	1.29 ± 0.04 Ab
	300	13.5 ± 0.2 Ab	3.34 ± 0.07 Ab	2.66 ± 0.02 Aa	2.09 ± 0.03 Ac	38.4 ± 0.4 Ac	26.6 ± 0.6 Ac	1.19 ± 0.02 Ac
	600	12.8 ± 0.2 Ac	3.01 ± 0.06 Ac	2.69 ± 0.02 Aa	1.93 ± 0.02 Ad	35.9 ± 0.4 Ad	22.8 ± 0.3 Ad	1.12 ± 0.02 Ad
		Root						
0	1	17.1 ± 0.5 Ba	5.78 ± 0.11 Ba	3.70 ± 0.05 Bc	8.69 ± 0.06 Ba	1027.5 ± 14.0 Aa	129.4 ± 0.9 Ba	7.03 ± 0.19 Ba
	150	15.0 ± 0.2 Bb	4.40 ± 0.09 Bb	5.77 ± 0.04 Bb	7.33 ± 0.10 Bb	843.8 ± 8.6 Bb	96.9 ± 2.7 Bb	5.31 ± 0.14 Bb
	300	14.7 ± 0.1 Bc	4.31 ± 0.14 Bb	5.85 ± 0.10 Bb	6.67 ± 0.05 Bc	772.9 ± 9.8 Bc	94.3 ± 2.1 Bb	4.76 ± 0.06 Ac
	600	13.4 ± 0.3 Bd	3.75 ± 0.10 Ac	6.64 ± 0.14 Ba	5.54 ± 0.22 Bd	724.2 ± 16.5 Bd	88.8 ± 2.1 Bc	3.43 ± 0.33 Bd
1.5	1	18.8 ± 0.6 Aa	6.66 ± 0.20 Aa	4.05 ± 0.28 Ac	8.90 ± 0.25 Aa	1035.5 ± 21.3 Aa	138.8 ± 0.8 Aa	8.27 ± 0.36 Aa
	150	16.6 ± 0.2 Ab	4.87 ± 0.08 Ab	6.57 ± 0.26 Ab	7.77 ± 0.14 Ab	895.8 ± 11.0 Ab	113.8 ± 1.3 Ab	5.94 ± 0.22 Ab
	300	15.6 ± 0.3 Ac	4.71 ± 0.10 Ab	6.66 ± 0.07 Ab	7.68 ± 0.08 Ab	876.4 ± 16.5 Ab	110.3 ± 1.6 Ab	4.98 ± 0.38 Ac
	600	15.5 ± 0.4 Ac	3.89 ± 0.16 Ac	7.64 ± 0.29 Aa	6.70 ± 0.16 Ac	774.0 ± 17.3 Ac	95.4 ± 2.3 Ac	4.10 ± 0.11 Ad

Columns presenting different uppercase letters between Si treatments (0 and 1.5 mM of Si at the same Zn concentration) and lowercase letters between Zn treatments (1, 150, 300 and 600 µM of Zn at the same Si concentration) indicate significant differences in the Scott-Knott test ($P < 0.05$). The herein described values represent the means and SD of five repetitions.

Table 5. Bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI) of *S. amazonicum* plants treated with Si and exposed to Zn toxicity.

Si (mM)	Zn (µM)	BCF	TF	TI (%)
0	1	184.0 ± 6.0 Aa	0.665 ± 0.029 Aa	-
	150	38.6 ± 0.5 Bb	0.055 ± 0.002 Ab	66.2 ± 0.5 Ba
	300	32.9 ± 1.2 Bc	0.044 ± 0.002 Ac	50.3 ± 1.5 Bb
	600	23.8 ± 1.0 Bd	0.034 ± 0.002 Ad	42.8 ± 1.6 Bc
1.5	1	174.3 ± 7.1 Aa	0.584 ± 0.017 Aa	-
	150	44.9 ± 0.5 Ab	0.034 ± 0.001 Bb	74.1 ± 0.8 Aa
	300	40.8 ± 1.5 Ac	0.027 ± 0.002 Bb	62.5 ± 1.3 Ab
	600	28.0 ± 1.2 Ad	0.025 ± 0.001 Bb	51.3 ± 1.2 Ac

Columns presenting different uppercase letters between Si treatments (0 and 1.5 mM of Si at the same Zn concentration) and lowercase letters between Zn treatments (1, 150, 300 and 600 µM of Zn at the same Si concentration) indicate significant differences in the Scott-Knott test (P < 0.05). The herein described values represent the means and SD of five repetitions.

antagonistic relation between these ions. The P supply in the form of phosphoric acid, or a combination between this acid and monocalcium phosphate, decreased Zn contents from 355 mg kg^{-1} to 208 mg kg^{-1} in the roots and from 195 mg kg^{-1} to 128 mg kg^{-1} in the shoot of *Stenotaphrum secundatum* plants.

The roots of *S. amazonicum* seedlings subjected to toxic zinc levels presented dark color and low biomass at the highest Zn concentrations (150, 300 and 600 µM). According to Pinto et al. (2009), this feature may have emerged due to calcium absorption inhibition caused by Zn. This process triggered symptoms of Ca deficiency, since this macronutrient acts on cell elongation and division and is a component of Ca pectates in the middle lamella of cell walls. Marques and Nascimento (2014) also reported lower Ca levels in *R. communis* tissues due to fertilization with Zn.

Decreased iron contents may have been caused by its competition with Zn, since the assimilation of these metals is carried out by the same transporters (ZIP transporters) (Masuda et al., 2012). The current study found chlorosis in the youngest leaves of *S. amazonicum* plants, which is a symptom of iron deficiency (Wang et al., 2009). These changes were also observed in *E. urophylla* (Šimková et al., 2018) and *O. sativa* (Mehrabanjoubani et al., 2015).

According to Monnet et al. (2001), decreased Mg, Mn, Cu, Ca and Fe contents indicate the inhibition of these elements by Zn. Because they are bivalent cations, these elements compete for the same bioactive sites and can lead to metabolic imbalance (Nocito et al., 2011). For example, it happens in Mg substitution for Zn in chlorophyll molecules subjected to zinc toxicity conditions, which lead to changes in the photosynthetic ability of plants (Küpper et al., 1998).

Seedlings subjected to Zn concentration of 600 µM presented increase S content by 89% and 19% (Si-free plants) and by 64% and 14% (Si-treated plants) in the roots and shoot, respectively, in comparison to the control. The increased incidence of sulfur in vegetative organs was caused by the excess of zinc and it can be explained by the decreased cell membrane selectivity that led to the passive influx of this mineral in the assessed plants (Šimková et al., 2018). The increased S content can also be associated with toxicity-tolerance mechanisms adopted by *S. amazonicum* plants, which can be induced to produce intercellular organic compounds formed by sulfur, such as thiol, which is found in cell wall components (Mehes-Smith et al., 2013), glutathione (Hasanuzzaman et al., 2017), metallothioneins and phytochelatins (Hernández et al., 2015), which act as chelators, participate in metal complexation processes and transport these metals to the vacuoles to prevent them from binding to proteins that act in physiological processes (Parrotta, 2015).

Silicon supply to *S. amazonicum* seedlings decreased the harmful effects of Zn by attenuating the decrease in nutrient concentrations evaluated in plant tissues. It also enabled increased availability and assimilation of these minerals, as well as increased plant growth. The action of Si as mitigator of nutrient content decrease in plants subjected to metal toxicity was also observed in *O. sativa* (Mehrabanjoubani et al., 2015, Wang et al., 2015), *E. urophylla* (Pinto et al., 2009) and *V. unguiculata* (Pereira et al., 2018).

Bioconcentration factor, translocation factor and tolerance index

The excess of zinc decreased BCF, TF and TI in *S. amazonicum* plants. However, Si application attenuated these reductions in BCF and TI (Table 5). The highest BCF values found in plants grown in solution with 1 µM of Zn were 184.0 (0.0 mM of Si) and 181.3 (1.5 mM of Si). Zn concentration of 600 µM decreased BCF by 94% and Si reduced such decrease to 91%, compared to the control. This outcome indicates that the excess of Zn in the solution reduced *S. amazonicum* ability to extract the metal from the substrate. The silicon also attenuated this effect. These results corroborate the ones recorded for *G. hirsutum* (Anwaar et al., 2015) and *O. sativa* (Mehrabanjoubani et al., 2015). According to Zayed, Gowthaman and Terry (2010), BCF is a good indicator of the metal accumulation ability of plants, since it takes into account the concentration of contaminating agents in the substrate. The aforementioned authors have reported that plants with good metal accumulation ability must have BCF > 1000. Thus, *S. amazonicum* is not a good metal accumulator.

TF values ranged from 0.465 (in the control plants) to 0.025 (in plants treated with 1.5 mM Si in association with 600 µM Zn), corresponded to 96% decrease in this parameter. This factor evaluates the phytoextraction of metals because it takes into account plant's ability to absorb contaminants from the soil or water and to transfer them to the stem and leaves of plants (Galal and Shehata, 2015).

According to Zvobgo et al. (2018), TF is classified as low (TF ≤ 0.01), moderately low (0.01 ≤ TF < 0.1), high (0.1 ≤ TF < 0.5) and very high (TF > 0.5). Low and moderately low TF indicate the incidence of mechanisms restricting the contaminant in the roots to reduce their toxic levels in the shoot (Branzini et al., 2012). According to Saraswat and Rai (2011), plants presenting these features are more tolerant to metal toxicity because they reduce the harmful effects of high Zn concentrations on leaves. On the other hand, high TF characterizes the action of physiological mechanisms that lead to high heavy metal levels in the shoot of plants (Lin and Aarts, 2012). Thus, *S. amazonicum* presented very high zinc phytoextraction ability only in substrate presenting 1 µM of Zn, as well as moderately low zinc phytoextraction ability in solution subjected to the other Zn concentrations. This outcome indicates that the excess of Zn and the presence of Si enabled increased Zn accumulation in the roots and reduced Zn translocation to the shoot of plants.

TI values ranged from 85.4% to 42.8% in Si-free plants, as well as from 92.4% to 51.3% in plants treated with 1.5 mM of Si. This index is also used to classify plant tolerance to contaminant toxicity, based on TDM production (Zvobgo et al., 2018). Si-treated seedlings presented the highest TI values. This outcome suggests that this element increased *S. amazonicum* adaptation to the zinc-contaminated environment and reduced its toxic effect. TI values found in the current study were similar to the ones recorded for species such as *Enterolobium contortisiliquum* (Silva et al., 2018) and *S. portulacastrum* (Feng et al., 2018). Lux et al. (2004) classified TI as: low ($TI < 35$), moderate ($35 \leq TI < 60$) and high ($TI > 60$). Thus, *S. amazonicum* subjected to 150 µM of Zn in the Si-free treatment, and up to 300 µM of Zn in the treatment based on Zn association with 1.5 mM of Si, presented high tolerance index in the current study. Plants subjected to the other Zn concentrations presented moderate tolerance to zinc.

According to Masarovičová, Kráľová and Kummerová (2010), BCF, TF and TI values recorded for *S. amazonicum* plants subjected to experimental conditions similar to the ones adopted in the current study showed that this species is a Zn phytostabilizer. This species is capable of tolerating the harmful effect of this metal and its accumulation it in the roots and substrate to limit its translocation to the shoot to enable plant phytostabilization (Hosman et al., 2017).

Materials and methods

Experimental site

The experiment was conducted in a greenhouse at the Agricultural Sciences Institute of Federal Rural University of Amazonia (UFRA), Belém City, Pará State, Brazil (1°27′17.3″ S; 48°26′18.0″ W), from May 8 to July 17, 2018. According to Köppen's classification, the climate in the region is Af (equatorial) (Lopes et al., 2018). Mean air temperature and relative humidity were 26.7 ± 2.9°C and 79.9 ± 13.3% (mean ± standard deviation), respectively, during the experimental period.

Plant material and growth conditions

S. amazonicum seeds were provided by Pará State Association of Timber-Exporting Industries (AIMEX), in Benevides County, Pará State, Brazil (1°20′04.9″ S; 48°14′24.5″ W). They were scarified and soaked in distilled water for 24 hours. Next, they were placed in 5-L Leonard pots filled with sterilized washed sand to germinate. Later, they were subjected to semi-hydroponic cultivation.

Four seeds were planted in each pot. Thinning was performed 7 days after seedling emergence in order to keep one seedling per pot. All plants were given distilled water for seven days. After this period, they received the nutrient solution by Hoagland and Arnon (1950), which was prepared with pure reagents for analysis purposes. The solution comprised 5 mM KNO_3, 5 mM $Ca(NO_3)_2.4H_2O$, 1 mM KH_2PO_4, 2 mM $Mg(SO_4)_2.7H_2O$, 46.26 µM H_3BO_3, 9.15 µM $MnCl_2.4H_2O$, 1 µM $ZnSO_4.7H_2O$, 0.50 µM $CuSO_4.5H_2O$, 0.09 µM $H_2MoO_4.H_2O$, 89.00 µM $FeSO_4.7H_2O$, 89.57 µM Na_2EDTA, at total volume of 800 ml per pot. The pH was adjusted to 5.8 ± 0.1 using HCl or NaOH. The ionic strength started at 25% and increased to 50% and 100%, at regular 7-day intervals. Seedlings received nutrient solution with total ionic strength, from the 22nd to the 40th experimental day.

Experimental design and treatment evaluation

The experiment followed a completely randomized design, with a 4 x 2 factorial arrangement, four Zn concentrations, with and without Si, and five repetitions, which totaled 40 experimental units with one plant per unit. The adopted Zn concentrations were: 1 (control), 150, 300 and 600 µM in the form of $ZnSO_4.7H_2O$, whereas Si concentrations were: 0 and 1.5 mM in the form of $Na_2SiO_3 \cdot 5H_2O$. Zinc levels used in the current study were based on Zn concentrations that caused toxicity symptoms in *Psidium guajava guajava* (Natale et al., 2005), *Eucalyptus maculata* and *Eucalyptus urophylla* (Pinto et al., 2009) and *Thlaspi caerulescens* (Brown et al., 1995). The herein adopted silicon concentration was based on the ones that reduced the toxicity caused by metals in *Vigna unguiculata* (Pereira et al., 2018), *E. urophylla* (Pinto et al., 2009) and *Zea mays* (Šimková et al., 2018).

Seedlings were subjected to the treatments for 30 days (from the 41st to the 70th experimental day). Morphological parameters were measured in all plants on the 70th experimental day (after Zn toxicity symptoms were visually observed) and the roots and shoot (stem + leaves) were collected for nutritional analysis.

Measurements of Morphological Parameters

The following features were evaluated: NL, LA was determined through LI-COR Mod. LI-3100; SH, CD, RDM, SDM, and TDM were quantified after oven drying with air circulation at 65ºC. H/D and R/S ratios were calculated based on these data.

Nutrient content determination

The DM samples of the plant tissues were ground and subjected to digestion in H_3NO_3 and H_2O_2. Nutrient contents were measured through inductively coupled plasma mass spectrometry (ICP-MS 7900, Agilent, USA), based on Paniz et al. (2018).

Silicon extraction and determination

The DM samples of the plant tissues were ground and subjected to digestion in H_2O_2 and NaOH. Silicon concentration was measured through spectrophotometry at wavelength 410 nm, based on Korndörfer et al. (2004).

Bioconcentration factor, translocation factor and tolerance index

The following variables were calculated based on equations 1, 2 and 3 described by Wang et al. (2018) to evaluate *S. amazonicum* phytoextraction ability and tolerance to excess of zinc: bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI).

$$BCF = Zn_p\ (mg\ kg^{-1}) / Zn_{ns}\ (mg\ l^{-1}) \qquad (1)$$

Where, Zn_p is the Zn concentration in the plant and Zn_{ns} is the Zn concentration in the nutrient solution.

$$TF = Zn_s\ (mg\ kg^{-1}) / Zn_r (mg\ kg^{-1}), \qquad (2)$$

wherein Zn_s is the Zn concentration in the shoot and Zn_r is the Zn concentration in the roots.

$$TI = MS_{Znp}\ (g) \times 100 / MS_{cp}\ (g) \qquad (3)$$

Where, MS_{Znp} is the TDM of plants grown in solution enriched with Zn and MS_{cp} is the TDM of control plants.

Data analysis

Data were subjected to analysis of variance. Means were compared through Scott-Knott test, at 5% probability level. The SD was calculated for each treatment. Statistical analyses were performed in the SISVAR software (Ferreira, 2014).

Conclusion

The addition of increasing Zn concentrations to the nutrient solution reduced plant growth, as well as Ca, P, Mg, Fe, Mn and Cu contents. It increased S concentrations and toxicity in the root system in comparison to the shoot, in *S amazonicum* plants.

Si addition to the nutrient solution increased the growth of the plant and the absorption of the evaluated macro and micronutrients. This outcome suggests that this beneficial element mitigates the phytotoxic effects of the excess of zinc. The BCF, TF and TI values have shown the low Zn phytoextraction ability of *S. amazonicum* plants and suggested that the evaluated plant species can be promising for Zn phytostabilization processes.

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