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ARTICLE

Boron phytoremediation: *Stizolobium aterrimum* is tolerant and can be used for phytomanagement of boron excess in soils

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ABSTRACT

Soil contamination by toxic elements causes concern and is increasing through industrial development, mining activities and the overuse of chemical fertilisers. Some toxic elements, such as boron, also play a structural role. The present study aimed to evaluate the behaviour of *S. aterrimum* plants growing in boron artificially contaminated soils. It has been found that *S. aterrimum* is tolerant to low doses of boron, moderately tolerating doses of up to 240 mg.dm⁻³. But high doses (doses above the adequate zone) cause decreased plant growth and present symptoms of toxicity, such as foliar chlorosis. The dose of 240 mg.dm⁻³ can already be considered toxic, since the tolerance index (TI) was less than 50%. The species can be used as a phytoextractor in low doses of boron because it presented a high tolerance index (TI) and demonstrated the ability to uptake and accumulate boron in root tissue.

KEYWORDS

Phytoremediation; soil pollution; environmental protection

Introduction

Soil pollution by toxic elements and other potentially toxic elements is a growing problem related to industrial and mining activities as well as the use of sewage sludge in agricultural soils [1]. Contamination by metals and non-metals is of great concern because they are not metabolised by living organisms and accumulate at different trophic levels of the food chain. Further, not all metal or non-metal elements are merely toxic. Many are micronutrients and are required so that the plant can complete its cycle, for example, copper (Cu⁺⁺) and boron (H₃BO₃).

Both Cu and B may have their concentrations increased in soil through the use of fertilisers and agrochemicals [2]. In Brazil, there is no legislation specifying safe levels of B in soils, although the toxicity of this element has been under investigation. For example, it has been found that a B concentration in soil of 3.0 mg.dm⁻³ can induce toxic effects in many plants [3].

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B was the first element to have been proven essential for plant growth in 1920 [4,5]. It belongs to the group of semiconductors, having intermediate properties between metal and non-metal. At a pH close to neutral, B is found in most biological fluids (~96%) as boric acid (H_3BO_3), and as borate $\text{B}(\text{OH})^{4-}$ anion, both of which form complexes with a wide range of (OH^-) concentrations [6]. In soil, it is generally found as boric acid and as borate anion, with H_3BO_3 being the predominant form in the most common pH values of soils. $\text{B}(\text{OH})^{4-}$ predominates only above a pH of 9.2 [7].

Several physical and chemical methods, combined with engineering knowledge, can be applied to remediate soils contaminated with toxic elements [8]. These methods are often expensive and may generate other kinds of impact on the environment.

Phytoremediation (green remediation) is the use of vegetation to decrease the risk of soil and groundwater contamination. More than a simple restoration of vegetal cover, phytoremediation requires the use of selected plants adapted to the chosen treatment [9]. This remediation technique occurs through the following mechanisms: (A) absorption and accumulation of heavy metals in plant aerial tissues (phytoextraction); (B) retention of elements in the root system or incorporation of elements in the cell wall matrix, precipitating the metals in insoluble forms and, subsequently, immobilising these contaminants (phytoadsorption); (C) release in to the soil of compounds that can immobilise toxic elements (phytostabilisation); and (D) stimulation of bioremediation by fungi or other microorganisms located in the root-soil system (rhizoremediation) [10]. Studies have evaluated four species for their ability to tolerate and concentrate B on the aerial part (*Brassica juncea*, *Lotus corniculatus*, *Festuca arundinacea* (fescue) and *Hibiscus cannabinus* (kenaf)) [11]. The species were cultivated in soils with high levels of B, ranging from 1 to 10 mg.kg^{-1} of free B. The average concentration in dry mass was 105 mg.kg^{-1} of B, in which there was no difference between the studied species. The reduction of boron content in the soil in the 0–60-cm layer was between 48 and 52% in the first year and then between 13 and 24% in the second year of cultivation. Similar results were found using as test plants, in a greenhouse experiment, *Brassica juncea* (mustard), *Raphanus sativus*, *Amaranthus cruentus* and *Hibiscus cannabinus* (kenaf), cultivated in soil contaminated with Cu, Zn, Mn, Pb, and B [12]. All species were efficient in removing boron, with emphasis on kenaf and mustard. Among the elements, boron presented the highest rates of removal, with the possibility of reducing its level by 50% in less than one year of cultivation. Despite a large number of studies on B toxicity in different crop species, such as wheat and barley, physiological mechanisms affecting differential expression of B toxicity stress between genotypes is not well understood [13].

Other plants such as sunflower, jack bean (*Canavalia ensiformis*), Velvet bean (*Stizolobium aterrimum*), castor bean (*Ricinus communis*), among others, have been extensively explored for their tolerance and phytoremediation potential of potentially toxic elements [14–18].

The species *Stizolobium aterrimum* is an herbaceous legume, which has the favourable characteristics mentioned above, and is also used as green manure in agricultural systems. It has already been characterised as tolerant to lead [19] and it is moderately tolerant to copper (unpublished data: previous studies of our research group), and so the evaluation of tolerance and potential phytoremediation of this plant for boron is of great scientific importance with respect to the characterisation of plant tolerance.

Materials and methods

The experiment was carried out in the greenhouse at UNESP university, under controlled temperature $27 \pm 2^\circ\text{C}$ conditions, with a completely randomised design containing 6 B concentrations (control, 30, 60, 120, and 240 mg.dm^{-3}). The control treatment was considered as the natural B content in soil. The plants were installed in pots with 2 dm^{-3} capacity and each treatment was composed of 4 biological repetitions containing 2 plants per pot. A red dystrophic latosol with 60% sand and B addition to the soil was prepared by adding 10 mL dm^{-3} of a boric acid solution containing the respective B amounts to obtain the soil B concentrations mentioned above. After B addition to the soil, it was allowed to stabilise over 20 days before the seeds were sown.

Seeds of *S. atterimum* were surface sterilised for 15 min in 10% hypochlorite solution (v/v), and then were immersed in distilled water for one hour before sowing. The plants were allowed to grow for 45 days and then were harvested and separated into roots and shoots. One plant of each pot had roots and shoots washed in water and dried on paper before being dried at 60°C in a forced circulation oven for 3 days for further biomass and boron analysis; the remaining plants had only their leaves harvested, frozen in liquid nitrogen and stored at -80°C for further biochemical analysis.

Boron was quantitated at the plant nutrition laboratory of DEFERS-FEIS-UNESP using atomic absorption spectrometry after nitric-perchloric digestion of plant roots and shoots. Nitrogenous compounds were extracted from leaves in a liquid-liquid extraction using methanol:chloroform:water (MCW) solution in the proportion 12:5:3 [20]. Total soluble protein was extracted from leaves using 0.1 M NaOH. Then were quantitated total soluble proteins, total soluble amino acids, total ureides, allantoic acid and allantoin [21–24].

Chlorophyll extraction was carried out in 50 mg leaf discs using dimethyl sulfoxide [25]. The extracts were read in a spectrophotometer at 645 and 663 wavelengths and calculated according to the formula: $C_a = (12,70 \times A_{663}) - (2,69 \times A_{645})$; $C_b = (22,90 \times A_{645}) - (4,68 \times A_{663})$; $C_a + C_b = (20,20 \times A_{645}) + (8,02 \times A_{663})$ [26].

The phytoremediation indices such as plant relative growth (PRG), tolerance index (TI%), and extraction potential (EP) were calculated based on data from plant mass, B concentration in soil and plant organs.

All data were subjected to analysis of variance and regression analysis in SISVAR® software. Regression model adjustment was considered significant when $p < 0.05$.

Results and discussion

Boron effects on growth and phytoremediation potential

Boron has toxic potential that is closely related to its concentration in soil and, consequently, in plant tissues. Its toxicity is mainly related to irrigated lands in arid and semiarid regions worldwide [27]. Growth is one of the most commonly used features to evaluate plant tolerance to any excess trace elements in soils. Although boron is found generally under low concentrations, it has also a toxic potential that broadly varies between plant species [5].

Several characteristics can be considered with regard to growth, such as height, leaf area and plant biomass, which are often altered when soils are contaminated. Other symptoms can also occur, for example changes in leaves and stem coloration, chlorosis and/or tissue necrosis. We observed a significant variation in the production of fresh and dry biomasses in roots. It is evident that the biomass decreases with higher concentrations of boron in the soil (Table 1). This was directly reflected in the tolerance index (TI). Concentrations of 30 to 120 mg.dm⁻³ presented TI greater than 50%. At a concentration of 30 mg.dm⁻³ the TI reached 95% TI (Table 2), indicating that this soil boron concentration is favourable for the growth and development of *S. atterimum*. When we compare these data with the concentration of total protein and amino acids, we can infer that the increase of the concentration of these metabolites in the treatment at 30 mg.dm⁻³ is in fact a positive effect for this as a nutrient. The fact that the species present a TI greater than 50% at concentrations up to 120 mg.dm⁻³ and close to 50% at 240 mg.dm⁻³, may indicate the potential use of the species for phytoremediation of soils with contamination rates of 200 mg.dm⁻³ of boron.

Boron effects on photosynthetic pigments and nitrogenous compounds

Plant performance under stressful conditions is highly dependent on a proper biochemical functioning. In this way, photosynthesis and nitrogen use are of great importance to overcome adverse situations. Photosynthetic performance may be inferred from the amount of photosynthetic functional pigments, chlorophylls, and carotenoids. We evaluated the effects of increasing B concentrations on chlorophylls a and b, the ratio of chlorophylls a and b, and total chlorophyll. In these observations we found that all of these parameters were not affected by increasing B in soil (Table 3), suggesting no interference of this element with chlorophyll biosynthesis. Excess of boron interferes in nitrate reductase activity and thus affect the synthesis of amino acids [28], in special

Table 1. Shoots and roots dry mass (g) and boron accumulation in shoots and roots (mg.kg⁻¹) of *Stizolobium atterimum* under increasing B concentrations in soil.

Boron (mg.dm ⁻³)	Dry mass		B concentration	
	Shoots	Roots	Shoots	Roots
0	0.90 ± 0.19	0.78 ± 0.11	18 ± 2.94	111 ± 15.37
30	0.99 ± 0.17	0.60 ± 0.03	55 ± 9.92	128 ± 6.89
60	0.63 ± 0.12	0.52 ± 0.08	65 ± 9.80	130 ± 20.09
120	0.62 ± 0.18	0.44 ± 0.08	70 ± 13.48	115 ± 7.51
240	0.73 ± 0.07	0.37 ± 0.02	41 ± 4.87	114 ± 7.35
Regression	ns	x **	x ² **	ns

ns – non-significant ($p > 0.05$); ** $p < 0.01$; x: linear regression adjustment; x²: quadratic regression adjustment.

Table 2. Plant relative growth (PRG %), boron translocation index (TI in %) and boron extraction (BE) per cycle (g.ha⁻¹) in *Stizolobium atterimum* under increasing B concentrations in soil.

Boron (mg.dm ⁻³)	PRG (%)	TI (%)	BE (g.ha ⁻¹)
0	1.00 ± 0.00	25.05 ± 5.37	200 ± 23.55
30	0.95 ± 0.61	34.17 ± 3.62	325 ± 74.29
60	0.68 ± 0.12	22.17 ± 2.94	150 ± 33.80
120	0.63 ± 0.16	43.25 ± 6.45	379 ± 86.59
240	0.49 ± 0.15	40.31 ± 1.89	224 ± 14.70

Table 3. Chlorophyll *a*, *b* and total chlorophyll ($\mu\text{mol g}^{-1}$) in leaves of *Stizolobium aterrimum* under increasing B concentrations in soil.

Boron (mg.dm^{-3})	Chlorophyll content			
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll ratio	Total Chlorophyll
0	8.54 ± 1.24	3.53 ± 0.42	2.40 ± 0.10	12.13 ± 1.65
30	6.47 ± 1.18	2.53 ± 0.31	2.50 ± 0.16	9.06 ± 1.49
60	6.38 ± 0.73	2.68 ± 0.19	2.40 ± 0.27	9.11 ± 0.80
120	7.33 ± 1.38	3.13 ± 0.70	2.39 ± 0.09	10.51 ± 2.08
240	8.37 ± 0.55	3.47 ± 0.16	2.41 ± 0.16	11.91 ± 0.67
Regression	ns	ns	ns	ns

ns – non-significant ($p > 0.05$)

glutamic acid that can lead to imbalance in chlorophyll production once this molecule is synthesised from alpha-aminolevulinic acid, derived from glutamic acid. So, as we did not find any effects in chlorophyll content we could assume that it had no effect on nitrogen metabolism, although we showed some opposite results for other plants in another study [29]. In that work, nitrogen sources were evaluated to overcome B toxicity in lentil and barley plants and the authors found a decrease in chlorophyll content in both species. We shall investigate this question in future work.

Boron deficiency was found to be a huge growth restriction in *Dittrchia viscosa* but it did not impair photosynthesis [30].

We can confirm that plant metabolism varies from species to species in a manner which confers tolerance. In our case, we can suggest that photosynthetic activity may be less impaired by B, as we have found no effect in chlorophyll content under increasing doses of B.

Together with photosynthetic metabolism, we must consider plant N status. N has a central role in the metabolism of all living organisms. Any disturbance in N uptake, assimilation and use will impair the development of the organism. In this way, some nitrogenous metabolites serve as indicators of stress severity and, sometimes, indicate a plant's strategy to overcome several kinds of stress.

We found that the amino acid content was kept stable in leaves while in roots their content decreased according to an increase of B concentration (Table 4). The same pattern was observed for total soluble protein which was compromised in both leaves and roots (Table 4), although a slight increase in leaf proteins occurred up to a dose of 60 ppm of B in the soil.

It was found that toxicity significantly decreased leaf biomass, leaf relative growth rate RGR(L), organic N, soluble proteins, and nitrate reductase (NR) and nitrite reductase (NiR) activities [31]. The lowest NO_3^- and NH_4^+ concentration in leaves was recorded when plants were supplied with 2.0 mM B in root medium. Total B,

Table 4. Total soluble amino acids ($\mu\text{mol g}^{-1}$) and proteins (mg g^{-1}) in leaves of *Stizolobium aterrimum* under increasing B concentrations in soil.

Boron (mg dm^{-3})	Amino acids		Proteins	
	Leaves	Roots	Leaves	Roots
0	2.84 ± 0.11	5.89 ± 0.14	1.08 ± 0.32	1.59 ± 0.16
30	3.32 ± 0.52	3.90 ± 1.03	1.47 ± 0.39	1.51 ± 0.23
60	3.31 ± 0.42	3.37 ± 0.33	1.68 ± 0.62	0.53 ± 0.11
120	2.29 ± 0.19	3.55 ± 0.19	0.32 ± 0.09	0.69 ± 0.10
240	2.39 ± 0.06	3.45 ± 0.21	0.29 ± 0.02	0.48 ± 0.07
Regression	ns	x*	x*	x***

ns – non-significant ($p > 0.05$); * $p < 0.05$; *** $p < 0.001$; x: linear regression adjustment.

Table 5. Allantoic acid, allantoin and total ureides ($\mu\text{mol g}^{-1}$) in leaves of *Stizolobium aterrimum* under increasing B concentrations in soil.

Boron (mg.dm^{-3})	Total ureides		Allantoic acid		Allantoin	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
0	4.15 ± 0.43	5.56 ± 0.67	3.65 ± 0.56	2.29 ± 0.09	1.68 ± 0.30	3.26 ± 0.66
30	4.63 ± 0.30	5.65 ± 0.97	2.71 ± 0.41	3.57 ± 0.42	1.96 ± 0.44	2.52 ± 0.86
60	4.84 ± 0.71	3.92 ± 0.39	2.85 ± 0.19	2.41 ± 0.11	2.42 ± 0.49	2.66 ± 0.29
120	4.14 ± 0.60	4.40 ± 0.64	1.97 ± 0.10	2.73 ± 0.34	2.17 ± 0.67	2.13 ± 0.47
240	3.59 ± 0.18	3.74 ± 0.60	2.18 ± 0.08	3.28 ± 0.08	1.41 ± 0.13	1.39 ± 0.20
Regression	ns	ns	x**	x**	ns	x*

ns – non-significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; x: linear regression adjustment.

amino acids, activities of glutamine synthetase (GS), glutamine oxoglutarate amino-transferase (GOGAT) and glutamate dehydrogenase (GDH) increased under B excess. Data from the present study show that B excess caused inhibition of NO_3^- reduction and increased NH_4^+ assimilation in tomato plants. Another study, considering excess B effects on *Schizolobium parahyba*, suggests that the increase in proline amino acid content is responsible for activating tolerance mechanisms [32].

Allantoin and allantoic acid are the two most common forms of ureides used to transport nitrogen, especially in species of the Phaseolae family, and they are, therefore, used as a mechanism for evaluating the efficiency of biological fixation. Many studies have indicated that these metabolites may be related to responses to abiotic stresses (hydric or nutritional) and can be used as a nitrogen reserve and, therefore, are not only related to nitrogen fixation [33]. The advantage of transport N as allantoin and allantoic acid is related to the efficiency of nitrogen transport in relation to the carbon atoms that make up the molecule, once the N:C ratio is 1:1 [34].

In the present study, there was no significant variation in the total content of ureides in both aerial parts and roots (Table 5). But, we found a significant variation in allantoic acid concentration in leaves and roots (Table 5). It might be inferred that a slight decrease in boron may impact nitrogen fixation [35] (Table 5). In relation to the roots, the treatment with the highest concentration of allantoic acid used 30 mg.dm^{-3} . The alteration in the form of ureides transported or stored in response to some abiotic stress has been reported in several studies [36–38]. This may indicate adaptation or acclimatisation to the stress situation.

Conclusion

Stizolobium aterrimum was shown to be tolerant to excess boron in the soil. Thus, it should be suitable for cultivation in arid and semiarid soils that have a high B content produced by irrigation water. It would also be interesting to consider biomass produced as a complement in B deficient soil, it could be considered as phytomanagement for improving the mineral nutrition status of cultivated plants without adding manufactured fertilisers or generation of plant material for bioenergy purposes [39].

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- [1] Chen, H.M., Zheng, C.R., Tu, C. and Shen, Z.G., 2000, Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere* **41**, 229–234. PMID: 10819205
- [2] Luo, C., Liu, C., Wang, Y., Liu, X., Li, F., Zhang, G. and Li, X., 2011, Heavy metal contamination in soils and vegetables near an e-waste processing site, South China. *Journal of Hazardous Materials* **186**, 481–490. doi:10.1016/j.jhazmat.2010.11.024 PMID: 21144651
- [3] Abreu, C.A., Raji, B., Abreu, M.F. and Gonzalez, A.P., 2005, Routine soil testing to monitor heavy metals and boron in soils. *Scientia Agricola* **62**, 1–6.
- [4] Warrington, K., 1923, The effect of boric acid and borax on the broad bean and certain other plants. *Annals of Botany* **37**, 629–672. doi:10.1093/oxfordjournals.aob.a089871
- [5] Camacho-Cristóbal, J.J., Rexach, J. and González-Fontes, A., 2008, Boron in plants: deficiency and toxicity. *Journal of Integrative Plant Biology* **50**, 1247–1255. doi:10.1111/jipb.2008.50.issue-10
- [6] Bolaños, L., Lukaszewski, K., Bonilla, I. and Blevins, D., 2004, Why boron? *Plant Physiology and Biochemistry* **42**, 907–912. doi:10.1016/j.plaphy.2004.11.002 PMID: 15694285
- [7] Lindsay, W.L., 1979, Inorganic phase equilibria of micronutrients in soil. In: J.J. Mortvedt, P.M. Giordano and W.L. Lindsay Eds. *Micronutrients in Agriculture* (Madison: Soil Science Society of America), pp.41–57.
- [8] Yao, Z., Li, J., Xie, H. and Yu, C., 2012, Review on remediation technologies of soil contaminated by heavy metals. *Procedia Environmental Sciences* **16**, 722–729. doi:10.1016/j.proenv.2012.10.099
- [9] USEPA – United States Environmental Protection Agency, 2009, *Brownfields Technology Primer: Selecting and Using Phytoremediation for Site Cleanup*, (Washington, DC), EPA/542/R-01/006, 2001
- [10] Accioly, A.M.A. and Siqueira, J.O., 2000, Contaminação química e biorremediação do solo. In: R.F. Novais, V.H. Alvarez and C.E. Schaefer Eds. *Tópicos Em Ciência Do Solo* (Viçosa: Sociedade Brasileira de Ciência do Solo) pp.299–352.
- [11] Bañuelos, G.S., Cardon, G., Mackey, B., Ben-Asher, J., Wu, L., Beuselinck, P., Akohoue, S. and Zambrzuski, S., 1993, Boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species. *Journal of Environmental Quality* **22**, 786–792. doi:10.2134/jeq1993.00472425002200040021x
- [12] Santos, G.C.G., 2005, Comportamento de B, Zn, Cu, Mn e Pb em solo contaminado sob cultivo de plantas e adição de fontes de matéria orgânica como amenizantes do efeito tóxico [thesis]. (Piracicaba: Universidade de São Paulo).
- [13] Torun, A.A., Yazici, A., Erdem, H. and Çakmak, I., 2006, Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes. *Turkish Journal of Agricultural and Forestry* **30**, 49–58.

- [14] Andrade, S.A.L., Jorge, R.A. and Silveira, A.P.D., 2005, Cadmium effect on the association of jackbean (*Canavalia ensiformis*) and arbuscular mycorrhizal fungi. *Scientia Agricola* **62**, 389–394. doi:10.1/1.828.9837
- [15] Andrade, S.A.L., Silveira, A.P.D., Jorge, R.A. and Abreu, M.F., 2008, Cadmium accumulation in sunflower plants influenced by arbuscular mycorrhiza. *International Journal of Phytoremediation* **10**, 1–13. doi:10.1080/15226510701827002 PMID: 18709928
- [16] Adhikari, T. and Kumar, A., 2012, Phytoaccumulation and tolerance of *ricinus communis* L. to nickel. *International Journal of Phytoremediation* **14**, 481–492. doi:10.1080/15226514.2011.604688
- [17] Souza, L.A., Andrade, S.A.L., Souza, S.C.R. and Schiavinato, M.A., 2013, Evaluation of mycorrhizal influence on the development and phytoremediation potential of *Canavalia gladiata* in Pb-contaminated soils. *International Journal of Phytoremediation* **15**, 465–476. doi:10.1080/15226514.2012.716099
- [18] Souza, L.A., Piotto, F.A., Dourado, M.N., Schmidt, D., Franco, M.R., Boaretto, L.F., Tezotto, T., Ferreira, R.R. and Azevedo, R.A., 2017, Physiological and biochemical responses of *dolichos lablab* L. to cadmium support its potential as a cadmium phytoremediator. *Journal of Soils and Sediments* **17**, 1413–1426. doi:10.1007/s11368-015-1322-0
- [19] De Souza, L.A., Andrade, S.A.L., Souza, S.C.R. and Schiavinato, M.A., 2011, Tolerance and phytoremediation potential of *Stizolobium aterrimum* associated to the arbuscular mycorrhizal fungi *glomus etunicatum* in lead-contaminated soil. *Revista Brasileira De Ciência Do Solo* **35**, 1441–1451. doi:10.1590/S0100-06832011000400038
- [20] Bielecki, L.R. and Turner, N.A., 1966, Separation and estimation of aminoacids in crude plant extracts by thin-layer electrophoresis and chromatography. *Analytical Biochemistry* **17**, 278–293.
- [21] Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing principle of protein-dye-binding. *Analytical Biochemistry* **72**, 248–254.
- [22] Yemm, E.M. and Cocking, E.C., 1955, Estimation of amino acids by ninhydrin. *The Analyst* **80**, 209–213. doi:10.1039/AN9558000209
- [23] Vogels, G.D. and Van der Drift, C., 1970, Differential analyses of glyoxylate derivatives. *Analytical Biochemistry* **33**, 143–157. PMID: 5413235
- [24] Ferreira, D.F., 2006, *Sisvar - Sistema De Análise De Variância*, (Unregistered free software).
- [25] Hiscox, J.D. and Israelstam, G.F., 1979, A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**, 1332–1334. doi:10.1139/b79-163
- [26] Lichtenthaler, H.K. and Wellburn, A.R., 1983, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* **11**, 591–592. doi:10.1042/bst0110591
- [27] Türker, O.C., Vymazal, J. and Türe, C., 2014, Constructed wetlands for boron removal: A review. *Ecological Engineering* **64**, 350–359. doi:10.1016/j.ecoleng.2014.01.007
- [28] Marschner, H., 2011, *Mineral Nutrition of Higher Plants*, (Oxford:Academic Press), 3rd.
- [29] Tepe, M. and Aydemir, T., 2011, Antioxidant responses of lentil and barley plants to boron toxicity under different nitrogen sources. *African Journal of Biotechnology* **10**, 10882–10891. doi:10.5897/AJB10.1076
- [30] Stavrianakou, S., Liakopoulos, G. and Karabourniotis, G., 2006, Boron deficiency effects on growth, photosynthesis and relative concentrations of phenolics of *Dittrichia viscosa* (Asteraceae). *Environmental and Experimental Botany* **56**, 293–300. doi:10.1016/j.envexpbot.2005.03.007
- [31] Cervilla, L.M., Blasco, B., Ríos, J.J., Rosales, M.A., Rubio-Wilhelmi, M.M., Sanchez-Rodriguez, E., Romero, L. and Ruiz, J.M., 2009, Response of nitrogen metabolism to boron toxicity in tomato plants. *Plant Biology* **11**, 671–677. doi:10.1111/j.1438-8677.2008.00167.x PMID: 19689774

- [32] Silva, P.F.N., Lobato, E.M.S.G., Souza, P.R., Santos, H.J.M., Braga, R.O., Moura, A.S. and Lobato, A.K.S., **2016**, Proline but not glutathione actively participates in the tolerance mechanism of young *schizolobium parahyba* var. Amazonicum plants exposed to boron toxicity. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **44**, 215–221. doi:[10.15835/nbha44110102](https://doi.org/10.15835/nbha44110102)
- [33] Todd, C.D., Tipton, P.A., Blevins, D.G., Piedras, P., Pineda, M. and Polacco, J.C., **2006**, Update on ureide degradation in legumes. *Journal of Experimental Botany* **57**, 5–12. doi:[10.1093/jxb/erj013](https://doi.org/10.1093/jxb/erj013) PMID: 16317038
- [34] Camargos, L.S., Soares, C.R.S., Justino, G.C. and Aguiar, L.F., **2013**, Alocação de compostos nitrogenados de reserva durante a germinação de sementes de *Canavalia brasiliensis*. *Biotemas* **26**, 1–10. doi:[10.5007/2175-7925.2013v26n4p1](https://doi.org/10.5007/2175-7925.2013v26n4p1)
- [35] Peoples, M.B., Sudin, M.N. and Herridge, D.F., **1987**, Translocation of nitrogenous compounds in symbiotic and nitrate-fed amide-exporting legumes. *Journal of Experimental Botany* **38**, 567–579. doi:[10.1093/jxb/38.4.567](https://doi.org/10.1093/jxb/38.4.567)
- [36] Canatto, R.A., Aguiar, L.F., Justino, G.C., Souza, L.A. and Camargos, L.S., **2014**, O nitrato influencia o metabolismo de compostos nitrogenados em calopogônio (*Calopogonium mucunoides*) ao longo do ciclo de vida. *Biotemas* **27**, 1–8. doi:[10.5007/2175-7925.2014v27n1p1](https://doi.org/10.5007/2175-7925.2014v27n1p1)
- [37] Camargos, L.S., Souza, A.C., Souza, L.A., Justino, G.C. and Aguiar, L.F., **2012**, *Calopogonium caeruleum* apresenta baixa tolerância do sistema simbiótico à presença de nitrato. *Acta Iguazu* **1**, 9–16.
- [38] Camargos, L.S., **2007**, Alterações no metabolismo de compostos nitrogenados em *Calopogonium mucunoides* em resposta a diferentes fontes de nitrogênio: efeitos na nodulação e na fixação [thesis]. (Campinas: Universidade Estadual de Campinas).
- [39] Souza, L.A., Camargos, L.S. and Carvalho, M.E.A., **2018**, Toxic metals phytoremediation using high biomass non- hyperaccumulator crops: new possibilities for bioenergy resources. In: V. Matichenkov Ed. *Phytoremediation: Methods, Management and Assessment* (New York Nova Science Publishers), pp.1–25.