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Effects on growth, membrane integrity, and phytoremediation potential of *Arachis pintoi* in soil with excess boron

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Abstract

Boron (B) is a micronutrient that plays a structural role in the cell wall and the correct functioning of the plasma membrane; however, excess B in soil is an emerging environmental problem that can hinder plant development. In this context, phytoremediation, which uses plants to contain, destroy, or extract pollutants from the environment, presents a sustainable solution. Therefore, this study aimed to evaluate the growth, membrane integrity, and phytoremediation potential of forage peanuts (*Arachis pintoi* Krapov. & W. C. Greg.) grown in soil with high concentrations of B. The experiment was carried out in a greenhouse, under a completely randomized design, using 6 concentrations of B (0.5, 30, 60, 90, 120, and 150 mg B dm^{-3} soil) in the form of boric acid and 6 replicates for each treatment. After 60 days of cultivation, increasing B doses resulted in a decline in the number of leaves, root dry mass, fresh mass, and number of nodules. *Arachis pintoi* was tolerant to up to 60 mg B dm^{-3} of soil and a high rate of B translocation was observed, so that B accumulation was predominant in the shoots. Furthermore, damage to cell membranes intensifies from 90 mg B dm^{-3} soil. In conclusion, *A. pintoi* is tolerant to excess B up to a 60 mg B dm^{-3} soil dose and can be classified as a phytoextractor when accumulating B in the shoots. However, boron toxicity causes oxidative damage and impairs growth, limiting its establishment in more severely contaminated environments.

Keywords Micronutrient toxicity, Oxidative damage, Soil health, Tolerance, Toxicity

1 Introduction

Boron (B) is a mineral micronutrient, so its concentration must remain between 0.5 and 2 mg kg^{-1} in the soil for normal plant development, as higher concentrations can cause toxicity [1]. Among the best-known functions of this chemical element, its structural role in the cell wall, involvement in the integrity and correct functioning of the plasma membrane, role in phenolic metabolism, nitrogen assimilation, and carbohydrate transport stand out [2, 3]. Excess B, however, can result in a series of problems, such as reduced plant growth, compromised fruit production and quality, spotted chlorosis

followed by necrosis of leaf and stem tissues, among other harmful effects reported in agriculture and ecosystems [4].

B contamination is documented primarily in arid and semiarid regions worldwide, occurring in the form H_3BO_3 and $B(HO)_4^-$ [5] in soil; B occurs at concentrations ranging from 10 to 300 mg/kg of soil [5] and the element's toxicity is already observed in Australia, Jordan, Malaysia, India, Israel, Mediterranean areas (Türkiye, Morocco), and the United States [6]. However, contamination also occurs in tropical soils, as evidenced in Argentina [7], Chile [8], and Peru [9].

The increase of B in the soil surface layer and in water appears to be an emerging problem, so that its contamination can occur through different anthropogenic means, such as inadequate fertilization, irrigation using water rich in B, use of organic fertilizers, and industrial activities (production of glass, ceramics, and detergents) [6, 10]. These industrial activities are responsible for depositing B residues that reach water bodies, which consequently lead to contamination of the soil through use in agriculture [6]. Thus, research aimed at solving the problem has become necessary and has considerably increased in recent decades [11].

Therefore, one of the strategies used to mitigate the problem is phytoremediation, a technique that uses the plants themselves to contain, destroy, or extract contaminants present in the environment [12, 13]. It is an attractive alternative because, in addition to being efficient, it is an easy-to-implement, economical, environmentally friendly mechanism, with broad public acceptance, which has received great global attention in recent years due to its series of advantages, when compared to conventional physical–chemical methods [14–16]. Leaching, for example, has limitations due to its high water requirements; adding lime is a short-term solution, and highly saline soils cannot be treated this way; surface soil can be replaced with uncontaminated soils, but this is a costly strategy, and boron can migrate [6]. Furthermore, due to the ineffectiveness or impossibility of using these practices, phytoremediation is an important technological alternative that can manage this issue, since it increases microbial activity, reduces soil erosion, decreases dust formation and sequesters carbon [6].

Furthermore, phytoremediation is classified according to the strategy used by the plant, including phytoextraction, when the plant absorbs the contaminant through the roots and translocates it to the shoots; phytovolatilization, when the contaminant is transformed into a less toxic form for later emission into the atmosphere; rhizofiltration, when plants absorb pollutants present in bodies of water; phytostabilization, when contaminants are stabilized in the root; and rhizodegradation, when microorganisms in the rhizosphere degrade organic pollutants in the soil [17]. Therefore, it is a mechanism strongly capable of treating a wide range of contaminants in the most varied environments.

Forage peanut (*Arachis pintoi* Krapov. & W. C. Greg.) is an herbaceous and perennial species, endemic to Brazil, which presents creeping and stoloniferous growth, as well as adaptation to acidic soils with low fertility [18]. Used as a permanent green cover, it is characterized by high dry matter production, high nitrogen fixation capacity, good tolerance to shading and aluminum, good yield and vigor, and resistance to pests and diseases [18]. These characteristics are particularly beneficial considering that contaminated soils often present adverse conditions unfavorable for plant growth [19]. Therefore, the plants used in these phytoremediation processes must be tolerant and resilient enough to adapt

to and establish themselves in such environments [19]. Perennial species are also specifically advantageous for phytoremediation, as they typically exhibit high biomass production, allowing them to immobilize contaminants for extended periods [20].

Forage plants help control erosion and retain nutrients, reducing the risk of them being transported into water bodies [21]. *Arachis pintoi* is a legume capable of competing with grasses of the genera *Brachiaria* and *Cynodon*, thus helping to control weeds [22]. Forage species, specifically legumes, also have high economic value due to their ability to provide soil cover and enrich the environment with nitrogen [22, 23]. This contributes to soil conservation by providing a better environment for subsequent crops, improving their growth and productivity [22, 23]. Therefore, legumes represent an elite group for soil restoration, contributing to ecosystem improvement and promoting positive impact on agriculture [23]. For these reasons, *Arachis pintoi* appears to be a promising species for studies involving soil decontamination.

Different plant species require and tolerate different amounts of B [5]; therefore, studies aimed at identifying species for use in soil decontamination help to fill a knowledge gap by assessing the tolerance limit of such species to this micronutrient, since the threshold for B deficiency and toxicity is very narrow [24].

Thus, the purpose of the present study is to evaluate the growth, membrane integrity, and phytoremediation potential of forage peanut (*Arachis pintoi* Krapov. & W. C. Greg.) grown in soil with high concentrations of B. We hypothesize that *A. pintoi* is tolerant to excess boron and that its phytoremediation potential is influenced by membrane integrity and nutrient partitioning between roots and shoots.

2 Materials and methods

2.1 Experiment implementation and soil preparation

The experiment was conducted in a greenhouse at Ilha Solteira, SP (20° 25′ 58″ S, 51° 20′ 33″ W). A completely randomized design was used, with six replicates per treatment. Irrigation was applied six times daily for 2 min each. Six concentrations of boron in the soil were tested, with boric acid (H_3BO_3) as the source, in order to obtain treatments of 0.5 (control—minimum to eliminate deficiency symptoms [25]), 30, 60, 90, 120 and 150 mg B dm^{-3} of soil. This work seeks to understand the contamination levels that forage peanuts can tolerate, including possible future scenarios, so that the highest concentration chosen demonstrates extreme environmental conditions.

The sandy soil classified as Oxisol [26] was collected from the experimental area of the teaching, research and extension farm (FEPE), plant production sector, Selvíria, MS, Brazil. The soil granulometric analysis [27] found the following proportions: 166, 809, and 25 g kg^{-1} of clay, sand, and silt, respectively. The soil chemical properties presented the following values [28]: pH = 5.5 (determined with 0.01 M $CaCl_2$); organic matter = 17.0 g dm^{-3}; P = 7.0 mg dm^{-3} (resin); K = 1.6, Ca = 14.0, Mg = 15.0 $mmol_c$ dm^{-3} (resin); B = 0.11 mg dm^{-3} (barium chloride); Cu = 0.8, Fe = 15.0, Mn = 12.9, Zn = 0.5 mg dm^{-3} (DTPA); potential acidity = 13.0 $mmol_c$ dm^{-3} (SMP buffer); Al = 0.0 $mmol_c$ dm^{-3}; sum of bases = 30.6 $mmol_c$ dm^{-3}; cation exchange capacity = 43.6 $mmol_c$ dm^{-3}, and base saturation = 70%.

To add the B treatments, the soil was sieved and homogenized, and then, for every 5 L of soil, a solution of boric acid in 500 mL of distilled H_2O was added for each treatment. To stabilize the contaminant, the soil remained incubated for 30 days in plastic bags [29].

The soil was then transferred to pots sealed with paper towels to prevent runoff, with a capacity of 5 L of soil. *Arachis pintoi* seeds were purchased commercially, and four seeds were planted per pot. Thinning was performed 20 days after sowing, with one plant per pot.

2.2 Growth assessments

To monitor the vegetative development of the species 30 days after sowing, a ruler was used to assess plant length up to the apical meristem, length up to the tallest leaf, and number of leaves. The forage peanuts were harvested after 60 days of cultivation. The plants were washed in running water, dried on paper towels, and separated into shoots, roots, and nodules. The assessments performed over the 30-day period were repeated at the end of the experiment, and root length was measured, as well as the number of flowerings throughout the cultivation period.

Fresh mass of the shoots, roots, and nodules was quantified. The number of nodules was measured, and to evaluate functional and active nodules, three nodules from each treatment were cut with a razor and then photographed under a stereomicroscope. The collected material was then placed in forced circulation ovens at 60 °C for 72 h to obtain the dry mass of the shoots and roots.

2.3 Nutritional analysis

The dry material was grinded, and a composite sample obtained from the 6 replicates of each treatment was used to perform a nutritional analysis to observe the concentration of B and other nutrients in the plant tissues. Nitrogen (N) concentration was obtained through sulfuric digestion: 400 mL of digestion mixture (catalyst salts) + 3 mL of concentrated sulfuric acid was added to 100 mg of the dry sample. In a digestion block, the sample was heated until it reached 350 °C. After cooling, 10 mL of deionized water was added, and the extract was subsequently transferred to the nitrogen distiller. Nitroperchloric digestion was performed to determine the concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). To 250 mg of the sample, 3 mL of nitric acid + perchloric acid solution were added. In the digestion block, the temperature was gradually increased from 80, 160 to 210 °C. The remaining extract was adjusted to 25 mL of deionized water. Readings were performed by atomic absorption spectrophotometer for Ca, Mg, K, Cu, Fe, Mn and Zn. For P and S, a UV–VIS spectrophotometer was used. The B analysis was performed through incineration digestion, whereby 200 mg of the sample was placed in an electric oven at 550 °C for 3 h. Subsequently, 10 mL of 0.1N HCl was added, and to 2 mL of the supernatant, 2 mL of buffer solution + 2 mL of 0.45% azomethine H were added. The reading was performed on a UV–VIS spectrophotometer [30]. An average was calculated to determine the B concentration in the roots (RBC) and shoots (SBC) (mg kg^{-1} DW).

2.4 Phytoremediation assessments and soil analysis

The accumulation of B in the roots (RAC), shoots (SAC), and in the total biomass (TAC) was calculated from the nutritional analysis [31] and the dry mass (g) of the roots (RDM), shoots (SDM), and total biomass (TDM), as shown in the following equations:

$$0026; RAC = (RDM * RBC)$$
$$0026; SAC = (SDM * SBC)$$
$$0026; TAC = (RAC + SAC)/TDM$$

Furthermore, a pre- (BC1) and post-cultivation (BC2) soil analysis was performed to verify the concentration of B (mg dm^{-3} of soil) available in the soil. The soil sample for each treatment was composed of subsamples taken from each of the replicates. To determine B levels, 10 g soil samples were sieved and subjected to microwave-assisted digestion with 20 mL of barium chloride extractant solution and, subsequently, inductively coupled plasma optical emission spectrometry (ICP-OES) [32].

Thus, it was possible to determine the phytoremediation potential of *A. pintoi*, by estimating the tolerance index (TI), translocation index for the shoot (IT%) and root (RC%) [33], and transfer factor (TF) [34], determined by the following equations:

$$0026; TI = TDMtreatment/TDMcontrol\ treatment$$
$$0026; \mathrm{IT}\% = (SAC/RAC + SAC) * 100$$
$$0026; RC\% = (RAC/RAC + SAC) * 100$$
$$0026; TF = RAC + SAC \in milligrams/BC1 \in mg/kg$$

2.5 Membrane stability: electrolyte leakage and evans blue dye test

The electrolyte leakage test (EL%) [35] was performed using 0.2 g of fresh leaves, cut into strips, and placed in containers containing 20 mL of distilled H_20 at 25 °C. After 30 min, the initial conductivity (E1) was measured using a conductivity meter. Subsequently, the samples were placed in a water bath at 90 °C for 30 min. The final conductivity (E2) was determined after 30 min at room temperature, and the results were expressed as a percentage following the equation:

$$EL\% = (E1/E2) * 100$$

To identify the loss of plasma membrane integrity, three *A. pintoi* leaflets from each treatment were collected and placed in Petri dishes containing Evans Blue reagent [36]. The reagent remained in contact with the leaflets for 20 min, after which they were washed three times with a $CaCl_2$ solution (pH 5.6). The abaxial and adaxial surfaces were observed and photographed using a stereomicroscope.

2.6 Statistical analysis

A factorial model was used to compare growth between 30 and 60 days of cultivation, so that the data were subjected to regression and a mean test (Tukey's test ($p < 0.05$)). Data collected only after material collection, on the other hand, were subjected to regression analysis, as well as the data from the electrolyte leakage test.

To evaluate the phytoremediation data, the data were subjected to a mean test (Tukey's test ($p < 0.05$)) and a factorial model was used to verify the accumulation of B in different organs (RAC, SAC, TAC) and treatments, verifying the comparison of means between treatments and between organs. The R 4.3.0 software (Package ExpDes.pt) [37] was used in all statistical analyses and the graphs were made using SigmaPlot 12.5 software.

Furthermore, the data were also subjected to multivariate analysis using Pearson correlation ($p < 0.05$) and Principal Component Analysis (PCA) and the graphs were made using R 4.3.0 software (Packages: corrplot, FactoMineR and factoextra).

3 Results

3.1 Forage peanut cultivation

At the end of 60 days of cultivation, there was a decrease in the number of *A. pintoi* leaves as the B doses increased (Fig. 1a). When comparing the plant development in relation to the first cultivation month, a greater number of leaves was observed in all treatments at the end of the second month (Fig. 1b). Regarding the length of the leaves

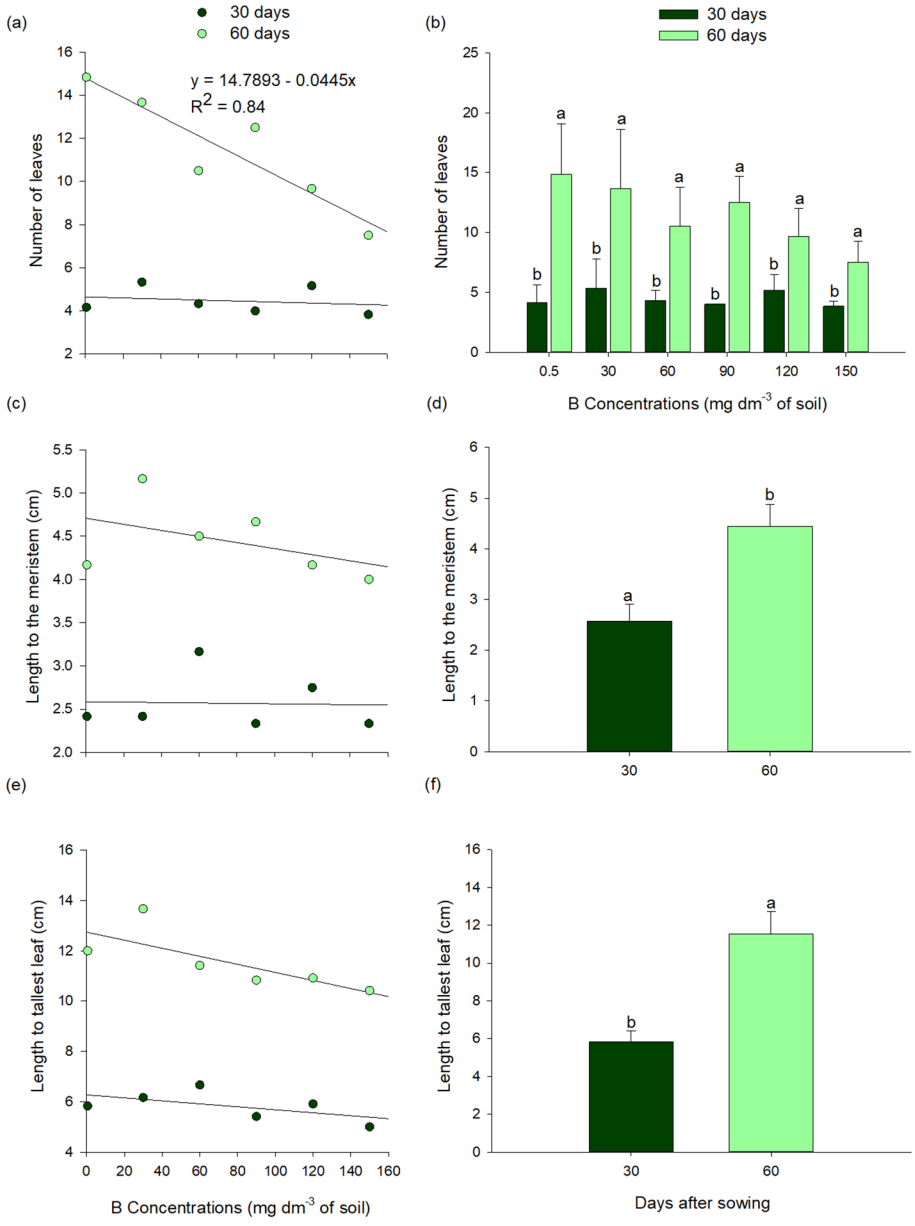

Fig. 1 **a** Evaluation of the number of leaves, **c** length to the apical meristem and **e** length to the tallest leaf throughout the B treatments. Comparison between cultivation time (30 and 60 days) for the **b** number of leaves, **d** length to the meristem and **f** length to the tallest leaf

up to the meristem and length up to the tallest leaf, there was no significant interaction between treatments and cultivation time (Supplementary material—Table 1), so that significant results were found only for cultivation time; therefore, regardless of the treatments, higher values were obtained at the end of forage peanut cultivation (Fig. 1df).

The highest doses of B caused a significant decrease in root dry mass (Fig. 2b), as well as in the number and fresh mass of nodules (Fig. 2cd). The control treatment showed the highest number of plants with (Fig. 2f), followed by the treatment with 30, 120, 90, and 150, and 60 mg B dm^{-3} soil. Physical changes in peanuts are visually noticeable, such as reduced root branching (Fig. 3b) and reduced lateral growth of the plant (Fig. 3a), due to the smaller number of leaves. Furthermore, the nodules present in the roots of forage peanut showed a red hue in all treatments (Fig. 4), indicating that they were active and functional.

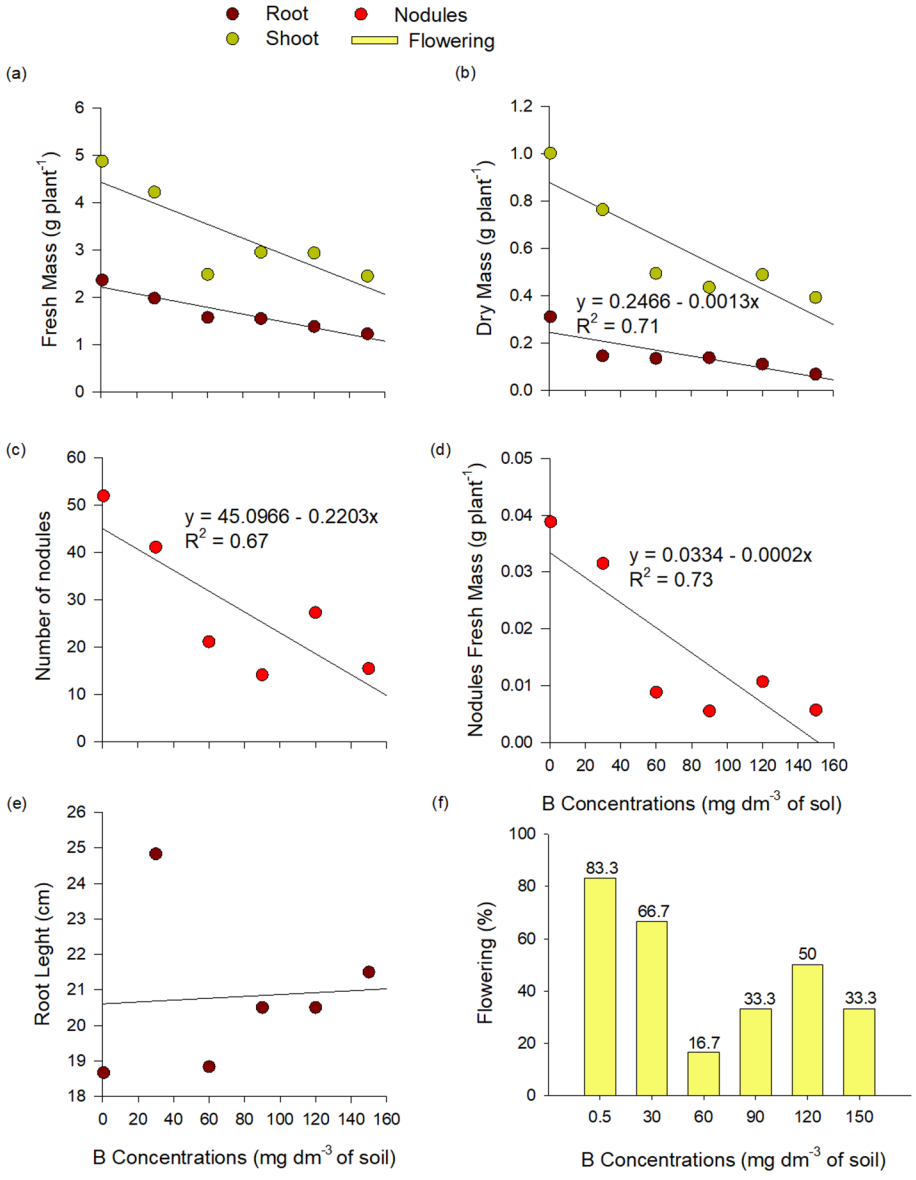

Fig. 2 **a** Fresh mass and **b** dry mass of root and shoot, **c** number of nodules, **d** fresh mass of nodules, **e** root length and **f** inflorescences of *A. pintoi* subjected to different treatments of B

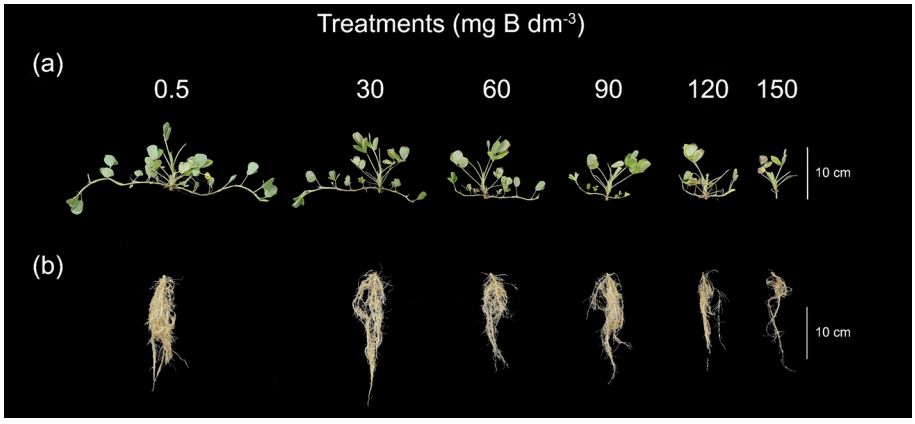

Fig. 3 **a** Shoot and **b** root of *A. pintoi* after 60 days of cultivation in soil contaminated by B

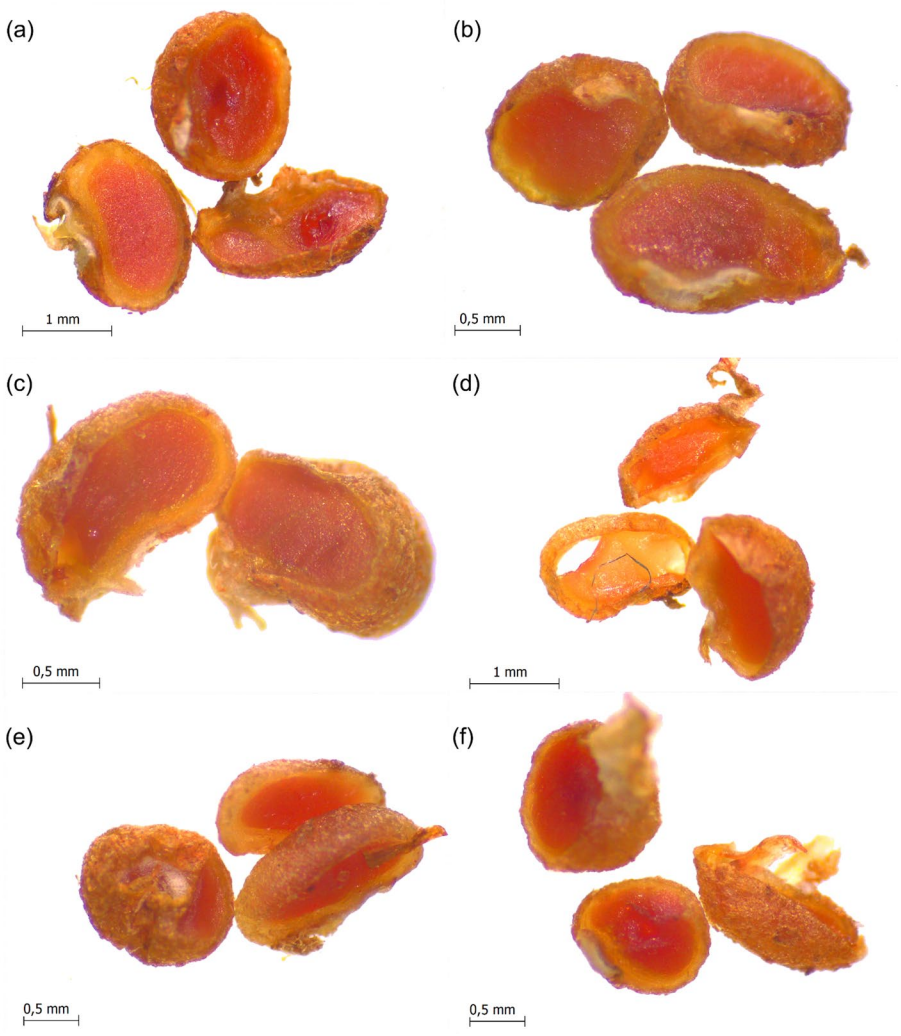

Fig. 4 Nodules of *A. pintoi* plants subjected to concentrations of **a** 0.5, **b** 30, **c** 60, **d** 90, **e** 120 and **f** 150 mg B dm^{-3} of soil

3.2 Determination of phytoremediation potential

Soil B availability after forage peanut cultivation decreased in all treatments (Fig. 5a). The treatment with 120 mg B dm^{-3} soil showed a higher value of pre- (11.79 mg B dm^{-3} soil) and post-cultivation (3.91 mg B dm^{-3} soil), demonstrating that there was a significant drop in the micronutrient availability.

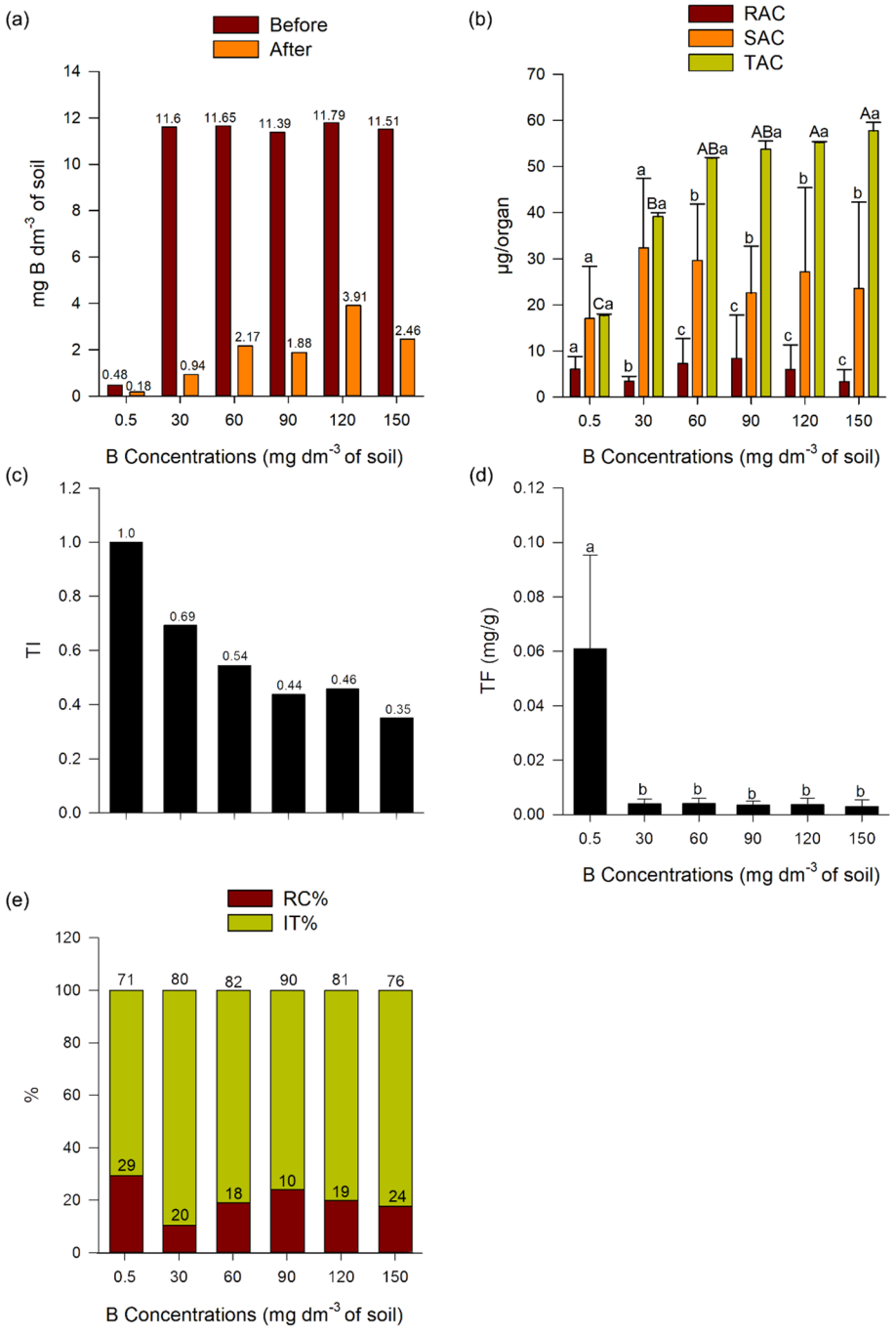

Fig. 5 **a** Soil B availability before and after *A. pintoi* cultivation; **b** B accumulation in roots—RAC, shoots—SAC, and total biomass—TAC; **c** B tolerance index, **d** transfer factor, and **e** translocation factor for shoots—IT% and roots—RC%. Capital letters compare total biomass across B treatments, and lowercase letters compare accumulation in different plant parts within the same treatment. RAC and SAC did not present significant results when compared between treatments

Treatments of 30, 60, 90, 120, and 150 mg B dm^{-3} soil showed greater accumulation of B throughout the plant—TAC (Fig. 5b), with the 30 mg B dm^{-3} soil dose only differing from the two highest treatments. Thus, in TAC, the control showed lower accumulation of B.

When comparing the B accumulation in the organs (root—RAC and shoot—SAC) in each treatment, the accumulation did not vary between the shoot, root, and total biomass for the control treatment (Fig. 5b). At 30 mg B dm^{-3} soil, TAC and SAC obtained the highest means, with no significant difference between them. In the following treatments, higher means were observed in TAC, followed by SAC, both differing significantly. Thus, except for the control, RAC obtained the lowest accumulation means in all treatments, indicating that B is accumulated in the plant shoot.

Arachis pintoi was tolerant up to a 60 mg B dm^{-3} soil concentration, with a tolerance index of 0.54 (Fig. 5c). The B transfer rate from the soil to the plant was higher in the control treatment and decreased in the other treatments (Fig. 5d), while the translocation rate of the micronutrient to the shoot was high (Fig. 5e), obtaining a lower percentage in the control treatment (71%) and a higher percentage with 90 mg B dm^{-3} soil (90%).

After the forage peanut cultivation, the values obtained by nutritional analysis demonstrate that the control treatment provided the lowest concentration of B for the shoot and root (Table 1).

A gradual increase in B concentration was observed in the roots of the plants up to the 90 mg B dm^{-3} treatment. The same B concentrations were found in the 60 and 120 mg B dm^3 treatments, while the higher dose resulted in B levels higher only than the control and the 30 mg B dm^{-3} treatment. In the shoots, B concentration was also increased, reaching its highest value at the 150 mg B dm^{-3} treatment.

The K concentration in the shoot (Table 4—Supplementary material) decreased as the number of treatments increased. There was a decline in the P concentration observed in the 60 mg B dm^{-3} soil treatment. The 90 and 120 mg B dm^{-3} soil treatments obtained the same P concentration (0.17 g kg^{-1} DW), compared to that, there was a slight increase in the highest dose (0.20 g kg^{-1} DW), however, all treatments maintained values below the control. The 60 mg B dm^{-3} soil treatment had a slightly lower concentration of Ca than the control, however, a decline is prominently observed in the two higher treatments, resulting in values of 2.50 and 2.83 g kg^{-1} DW, respectively.

3.3 Membrane stability: electrolyte leakage and Evans blue

The Evans Blue test indicated rupture of membranes on the abaxial surface of *A. pintoi* plants in the 60 and 90 mg B dm^{-3} soil treatments (Fig. 6fh), as well as on both leaf

Table 1 Nutritional analysis containing the concentration of B (mg kg^{-1} DM) in shoot and root of *A. pintoi*

Treatments mg B dm^{-3} of soil	Root	Shoot
0.5	19.45	17.02
30	23.74	42.34
60	54.01	51.32
90	60.78	51.83
120	54.01	55.50
150	48.95	59.99

B Boron, *DM* dry mass

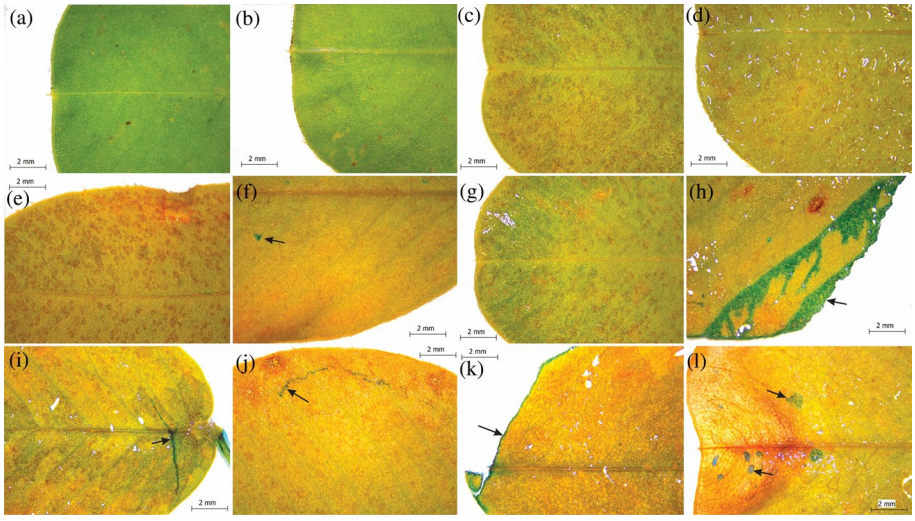

Fig. 6 **a**, **c**, **e**, **g**, **i** and **k** Adaxial surface of treatments with 0.5, 30, 60, 90, 120, and 150 mg B dm^{-3} of soil, respectively; **b**, **d**, **f**, **h**, **j** and **l** abaxial surface of treatments with 0.5, 30, 60, 90, 120, and 150 mg B dm^{-3} of soil, respectively. Arrows indicate positive results for the Evans Blue test

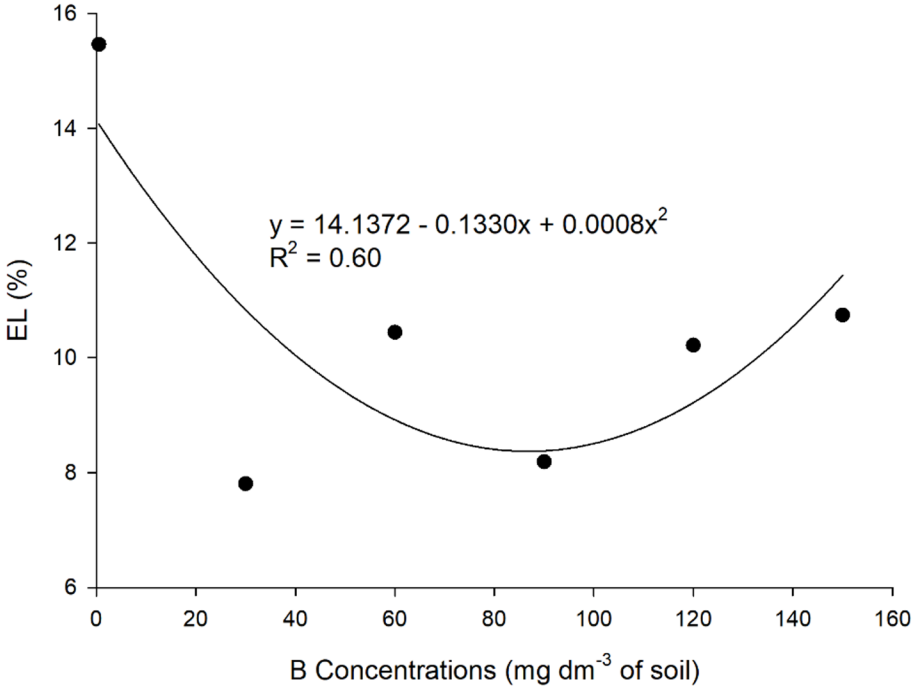

Fig. 7 Electrolyte leakage from *A. pintoi* plants as a function of soil B concentrations

surfaces of the 120 and 150 mg B dm^{-3} soil treatments (Fig. 6ijkl), as indicated by the arrows in Fig. 6.

The increase in B concentrations resulted in a decrease in the percentage of electrolyte leakage until reaching a minimum point at 83.13 mg B dm^{-3} soil (Fig. 7). After this concentration, the percentage starts to increase.

3.4 Pearson correlation and PCA

The B accumulation for the total plant biomass—TAC was the variable with the highest number of negative correlations (Fig. 8), relating to fresh root mass, electrolyte leakage, transfer factor, fresh root mass, number of leaves, fresh shoot mass, dry shoot mass and total dry mass, tolerance index, number of nodules, and fresh nodule mass. Furthermore, the strong negative correlation between the translocation index to the shoot and the translocation of B to the roots is highlighted.

In contrast, the accumulation of B in the shoot—SAC was one of the variables with the highest number of positive correlations (Fig. 8), relating to the tolerance index, length to the meristem, translocation index to the shoot, fresh root mass, root length, fresh nodule mass, number of nodules, total dry mass, dry mass of the shoot, fresh mass of the shoot, number of leaves, and length to the tallest leaf.

The two principal components (Fig. 9) explain 88.2% of the data variation. The control treatment predominantly influenced root dry mass and transfer factor, while the 30 mg B dm^{-3} soil treatment influenced the length to the meristem and tallest leaf. The other treatments influenced the accumulation of B in the total biomass—TAC.

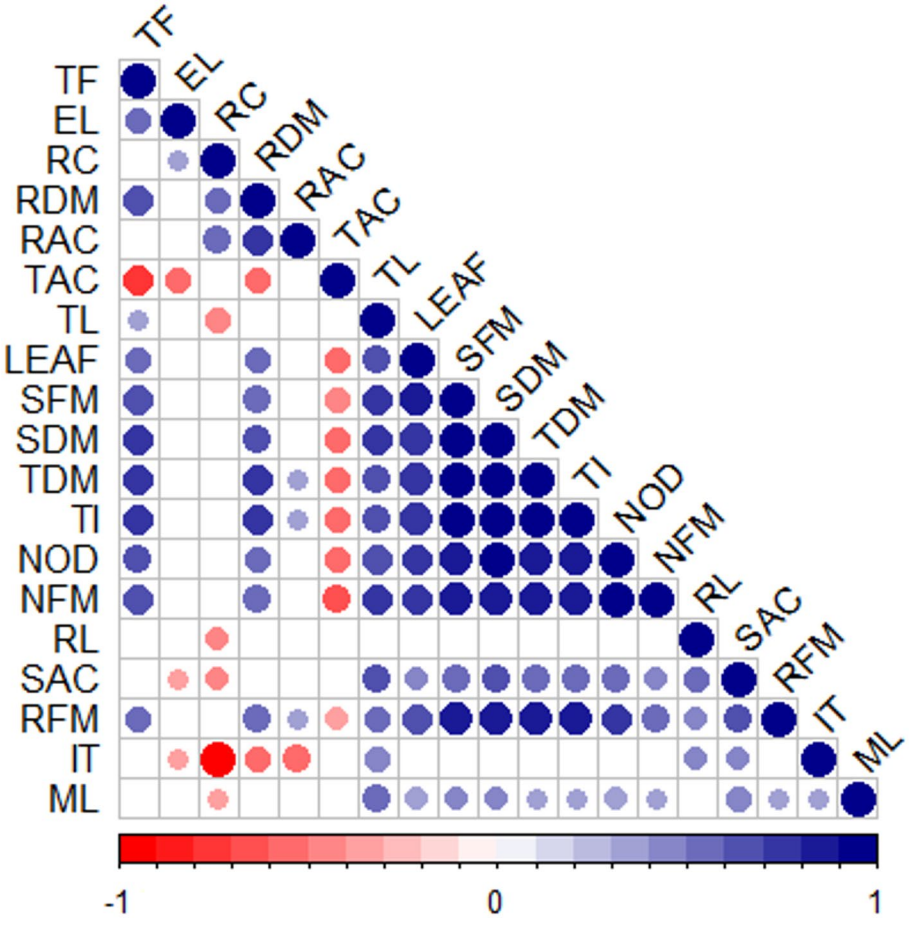

Fig. 8 Correlation matrix, blank spaces indicate non-significant correlations for Pearson's correlation ($p < 0.05$). *TF* transfer factor, *EL* electrolyte leakage, *RC* root translocation index, *RDM* root dry mass, *RAC* root B accumulation, *TAC* B accumulation in the whole biomass, *TL* length to the tallest leaf, *LEAF* number of leaves, *SFM* shoot fresh mass, *SDM* shoot dry mass, *TDM* total dry mass, *TI* tolerance index, *NOD* number of nodules, *NFM* nodule fresh mass, *RL* root length, *SAC* shoot B accumulation, *RFM* root fresh mass, *IT* shoot translocation index, *ML* length to the meristem

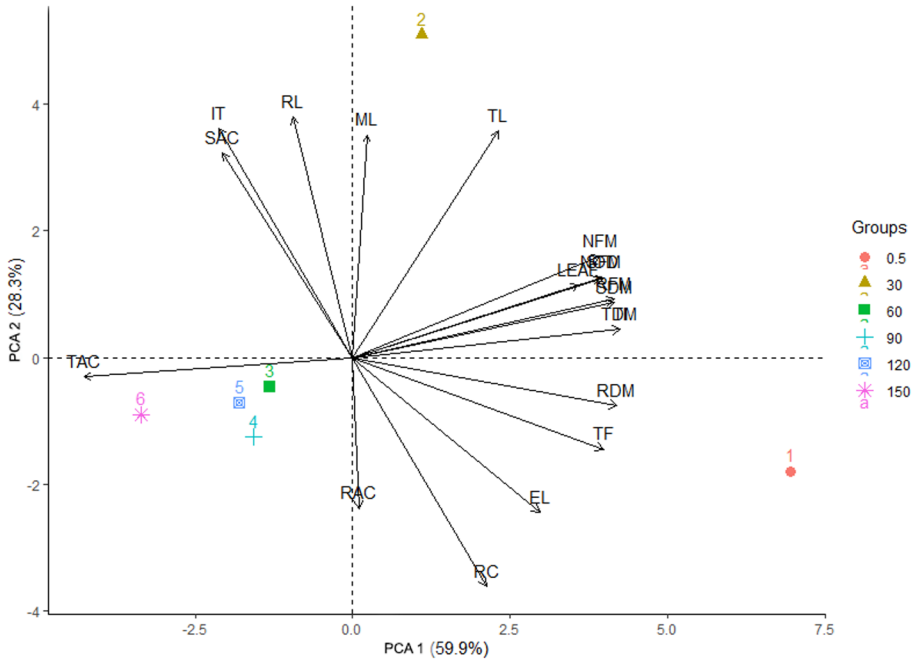

Fig. 9 Principal component analysis

4 Discussion

The toxicity generated by excess B negatively influences different aspects of plant development, so that decreased cell expansion, chlorosis and leaf necrosis (leading to a decrease in the number of leaves), delayed flowering and decreased dry mass are some of the effects known in the literature [38], and reflect the element's ability to interrupt the plant physiological processes [11], resulting in the observed consequences.

Furthermore, it is noteworthy that the root is greatly affected by the nutritional imbalance of B, so that, in toxicity situations, general weakness of the organ and a decrease in lateral roots can be observed [24], which may explain the significant decrease in fresh mass and number of nodules present due to the above-mentioned increase in B concentration. However, despite such consequences caused by the subjected growth conditions, it is important to highlight that compared to the first month of cultivation, *A. pintoi* was able to increase its length and number of leaves, demonstrating its efficiency in developing in a stressful environment.

The data on B accumulation in plant organs, associated with a high translocation index (TI%) and a drastic decrease in B availability in the soil after cultivation, demonstrate the ability of forage peanuts to absorb and translocate the chemical element in their plant body, accumulating it in the shoot. Such characteristics are common in phytoextracting plant species [17]; therefore, forage peanut presents three essentially important characteristics to obtain such classification: Tolerance to the element present in toxic levels (60 mg B dm^{-3} soil); capacity for high biomass production; and accumulation of the element in parts that are easy to collect [39, 40].

The transfer factor demonstrated a decrease in the transfer of micronutrients from the soil to the plant in relation to the control treatment. However, it is noteworthy that this factor represents the ratio between the contaminant accumulation in the plant tissues and the amount of B available in the soil [34]. Thus, the control treatment naturally

presents a lower quantity of the nutrient in the soil, and this ratio will be consequently higher in relation to the other treatments. Therefore, even with the B increase in the soil, the plant was able to carry out a good transfer of the nutrient to the plant, remaining the same in all treatments.

The plant tolerance mechanism can encompass a series of processes, such as the synthesis of organic compounds that chelate B, increase in the oxidizing apparatus, compartment of B in different organelles and locations, and even the efflux of the element through the roots [4]. This may explain the ability to establish active nodules in all treatments, because even if excess B impairs nodulation, such strategies associated with high translocation and accumulation of the element in the shoot may allow the biological nitrogen fixation process to remain functional. Furthermore, it is known that adequate B levels are necessary for the correct establishment of the rhizobia infection channel, in addition to helping maintain the cell wall structure, stabilizing the root nodule tissue membrane and strengthening the symbiotic interaction [41, 42].

Nodule size can also be used to assess N_2 fixation efficiency, as smaller or with reduced mass nodules are generally related to lower nitrogen-fixing activity [43, 44]. In the *Arachis* genus, during early growth stages of nodules, the rhizobium-infected area becomes dark red as the nodule diameter increases [43]. This coloration indicates the presence of the enzyme leghemoglobin, which is associated with high N_2 fixation activity [43].

Excess B can also disrupt electron transport, leading to overaccumulation of reactive oxygen species (ROS), thus causing oxidative damage that can lead to membrane disruption and even cell death [11, 45]. Thus, small visual damage to cell membranes can be observed after the treatment of 60 mg B dm^{-3} soil, in addition to the prominent increase in electrolyte leakage after a 83.13 mg B dm^{-3} of soil concentration, which agrees with the tolerance data of this study, indicating that the three treatments with higher concentrations of B in the soil lead to strong oxidative damage, making it impossible for the plant to establish itself in this medium.

Electrolyte leakage is a method used to indicate damage in cell membrane permeability as a response to stress [46, 47]. B maintains membrane structure through cis-diol complexation with glycoproteins (structural constituents of the membrane), and its deficiency is known to alter the plasma membrane potential and reduce the proton-pumping activity of ATPase [48]. Changes in B concentration can also alter the permeability of K^+ and sugars [48], consequently resulting in membrane damage.

In dicotyledonous plants, B status regulates the ability to absorb ions, since the element participates in membrane-associated processes [49]. When we address the nutritional status of forage peanuts, a decrease in the P and K concentration at B high concentrations is highlighted. The antagonistic relationship between B and P occurs due to competition for the same absorption and transport system. Furthermore, B also favors the accumulation of K in plant tissues. However, opposite effects can occur in extreme situations, since the balance of the elements is disrupted, influencing all vital processes of the plant [42]. Although a large accumulation of B is evident in the shoot, the nutritional analysis demonstrates high concentrations of B in both organs observed, thus, the element toxicity can cause other nutritional problems that limit the plant establishment in contaminated areas.

Ca, along with B, are elements that play a role in maintaining the cell wall structure and functionality [42]. However, high doses of B increase its concentration and decrease

the concentration of Ca in the cell walls of leaves [50, 51], which explains the decrease in the Ca concentration in plants subjected to higher treatments. As Ca is capable of attenuating oxidative damage caused by excess B by improving the antioxidant activity of enzymes [51, 52], such an imbalance in plants may reflect greater difficulty for *A. pintoi* to withstand more acute doses.

Furthermore, correlation analysis demonstrates that the B accumulation in the *A. pintoi* tissues appears to be the key to understanding the variables studied in this work. The strong positive correlations between SAC and several variables linked to growth and phytoremediation potential indicate that the accumulation of the nutrient in the *A. pintoi* shoot intensifies its tolerance; however, high concentrations of B also intensify damage to cell membranes. It is known that B is highly required in meristematic regions, this requirement being fundamental for correct cell division and tissue elongation [49]. However, the accumulation of the micronutrient in the plant upper parts generates variation in cell wall components, oxidative stress, and membrane peroxidation, as observed for rice seedlings [53].

For forage peanut, stress caused by B high concentrations in plant tissues results in variations in growth factors, nutritional status, and membrane integrity, which directly influences the micronutrient tolerance capacity. To alleviate the toxic effects of B on plants, the application of nutrients, plant growth regulators, plant growth-promoting microbes, lime, water, and organic matter are strategies that can be adopted [11]. However, B toxicity is a difficult condition to study and improve, so the cultivation of tolerant species often presents itself as a solution to this problem [49]. Furthermore, the biomass produced by plants used in the phytoremediation of boron-contaminated soils can be used as timber, bioenergy, animal fodder, or organic fertilizers in boron-deficient agricultural soils [6].

5 Conclusion

Arachis pintoi is tolerant up to a 60 mg B dm^{-3} soil concentration, classifying itself as a phytoextracting plant by accumulating and translocating B in its shoot. The toxicity generated by B in forage peanut plants is closely related to the reduction of variables linked to their growth; however, accumulation in the shoot, despite causing oxidative damage, enhances their tolerance.

Compared to the first month of cultivation, *A. pintoi* was able to develop and has the ability to establish functional nodules in all treatments. However, it is noteworthy that B negatively affected peanut development, both for the shoots and for the roots, as evidenced by the decrease in the number of leaves, dry mass of the roots and fresh mass of the nodules.

Concentrations above 90 mg B dm^{-3} of soil cause damage to the plant's plasma membrane due to oxidative damage, which leads to higher electrolyte leakage. Excess B influences nutrient absorption, primarily causing an imbalance in the concentrations of K, P, and Ca in the shoots of plants, which limits the plant's ability to establish itself in these soils. As future perspectives, the use of strategies to manage oxidative damage generated by B toxicity may be beneficial to enhance phytoremediation and plant tolerance in this environment.

Supplementary Information

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Supplementary Material 1.

Author contributions

The study conception and design were carried out by LSC and RPdS. Material preparation, data collection and analysis were performed by RPdS and TS. The manuscript was written by RPdS and revised by LSC. All authors read and approved the final manuscript.

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Data availability

The data generated during the current study are partially included in this published article and the Supplementary Material. For the full version, a request to the corresponding author is required.

Declarations

Ethics approval and consent to participate

The *Arachis pintoi* seeds used in this study were commercially acquired via BrSeeds®. The plant material was properly identified and stored following all ethical guidelines of the Department of Biology and Zootechny at the São Paulo State University "Júlio de Mesquita Filho". This study did not involve human or animal participants and therefore did not require any license or approval to be conducted.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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