



# Sulfate supply decreases barium availability, uptake, and toxicity in lettuce plants grown in a tropical Ba-contaminated soil

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## Abstract

Barium (Ba) is a non-essential element that can cause toxicity in living organisms and environmental contamination. Plants absorb barium predominantly in its divalent cationic form  $Ba^{2+}$ . Sulfur (S) can decrease the availability of  $Ba^{2+}$  in the soil by causing its precipitation as barium sulfate, a compound known for its very low solubility. The objective of this study was to evaluate the effect of soil sulfate supply in soil Ba fractions, as well as on plant growth, and Ba and S uptake by lettuce plants grown in artificially Ba-contaminated soil under greenhouse conditions. The treatments consisted of five Ba doses (0, 150, 300, 450, and 600  $mg\ kg^{-1}$  Ba, as barium chloride) combined with three S doses (0, 40, and 80  $mg\ kg^{-1}$  S, as potassium sulfate). The treatments were applied to soil samples (2.5 kg) and placed in plastic pots for plant cultivation. The Ba fractions analyzed were extractable-Ba, organic matter-Ba, oxides associated-Ba, and residual-Ba. The results indicate that the extractable-Ba fraction was the main one responsible for Ba bioavailability and phytotoxicity, probably corresponding to the exchangeable Ba in the soil. The dose of 80  $mg\ kg^{-1}$  of S reduced extractable-Ba by 30% at higher Ba doses while it increased the other fractions. Furthermore, S supply attenuated the growth inhibition in plants under Ba exposure. Thus, S supply protected the lettuce plants from Ba toxicity by reduction of Ba availability in soil and plant growth enhancement. The results suggest that sulfate supply is a suitable strategy for managing Ba-contaminated areas.

**Keywords** Barium fractionation · *Lactuca sativa* · Plant stress · Soil chemistry · Sulfur

## Introduction

Barium (Ba, CAS number 7440-39-3) is a non-essential metal element that may cause harmful effects in living organisms and ecosystems. Chronic exposure to Ba results in hypertension, cardiovascular diseases, and other related health problems in humans and animals (WHO 2022). Furthermore, high Ba concentrations can inhibit plant growth and development (Peana et al. 2021; Sleimi et al. 2021). The recommended environmental safety limit values range from 0.7 to 1.3  $mg\ L^{-1}$  Ba in water (USEPA 2004; CONAMA 2005; WHO 2022) and are around 330  $mg\ kg^{-1}$  Ba in soils

(USEPA 2005), with prevention and investigation threshold concentrations in Brazilian soils being set at 150 and 300  $mg\ kg^{-1}$  Ba, respectively (CONAMA 2009). Although Ba has high safety values compared to other contaminants, some studies have reported Ba concentrations above the recommended limits in soils and water bodies contaminated with industrial, mining, and domestic waste (Lamb et al. 2013; Ferreira et al. 2020; Viana et al. 2021). Thus, Ba contamination can be an environmental and public health problem, mainly in waste deposits with high Ba concentrations.

Although Ba has no known benefits for plants, it can be absorbed, probably as  $Ba^{2+}$ , a divalent cation similar to nutrients like  $Ca^{2+}$  and  $Mg^{2+}$  (Kabata-Pendias 2010). Indeed, results of sequential extraction experiments indicated that the exchangeable Ba fraction plays an important role in the retention of Ba in a tropical climate (Gong et al. 2020). Barium plant uptake makes phytoremediation a potential strategy for managing Ba-contaminated areas. Phytoremediation consists of a low-cost and environmentally

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friendly strategy to remove contaminants from soil and water through plant uptake and extraction (Antoniadis et al. 2017). Previous studies have demonstrated that phytoremediation is a suitable strategy for managing Ba contamination (Carvalho et al. 2019; Ferreira et al. 2019). In contrast, Ba plant uptake might increase risks to humans and animals by the possible ingestion of edible parts with high Ba content (Kravchenko et al. 2014). Thus, studies about Ba uptake are important for phytoremediation and risk assessment of contaminated areas (Myrvang et al. 2016). Among the aspects that influence contaminant uptake and extraction, plant nutritional status has a crucial role, affecting the amount of contaminant extracted through the effect on plant growth and antioxidant systems (Anicésio and Monteiro 2022; Cardoso et al. 2022).

Among the nutrients that enhance plant resistance, sulfur (S) has demonstrated positive effects on plant tolerance to several contaminants (Rabêlo et al. 2018; Gonçalves and Monteiro 2022), including Ba in nutrient solution (de Souza Cardoso and Monteiro, 2021a, b). Some studies indicate that sulfate ( $\text{SO}_4^{2-}$ ) possibly reduces  $\text{Ba}^{2+}$  availability in soils, probably by causing the precipitation of Ba as barium sulfate (Ippolito and Barbarick 2006; Melo et al. 2014; Cappuyns 2018). Thus, S supply may reduce Ba availability in soils, reducing the toxicity risk. In contrast, S can induce Ba accumulation by plant growth improvement (de Souza Cardoso and Monteiro 2021a), enhancing Ba phytoremediation, yet it can also increase Ba content in foods and, thus, toxicity risks. The study of Ba chemical fractions can elucidate the dynamics, mobility, and availability of Ba in soils (Nogueirol and Alleoni 2013); however, the influence of sulfate in Ba fractions was unknown. Here, we evaluated the effect of sulfate supply in Ba chemical soil fractions, as well as on plant growth, and Ba and S uptake by lettuce, an important vegetable and soil pollution bioindicator, cultivated in a tropical soil artificially contaminated with Ba under greenhouse conditions.

## Material and methods

### Experimental design

The experimental design consisted of a completed randomized design with fifteen treatments and four replicates. The treatments consisted of combinations between five Ba doses (0, 150, 300, 450, and 600 mg  $\text{kg}^{-1}$  Ba) and three S doses (0, 40, and 80 mg  $\text{kg}^{-1}$  S) in soil, and each experimental unit consisted of one pot with 2.5 kg soil and two plants. The Ba doses were set based on Ba prevention and investigation values established for Brazilian soils (CONAMA 2009), and S doses were determined based on previous studies about S supply in pot experiments (Huang et al. 2021; Cardoso et al. 2022). Barium and S sources were barium

chloride ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ , CAS number 10326-27-9) and potassium sulfate ( $\text{K}_2\text{SO}_4$ , CAS number 7778-80-5), respectively, with K and other nutrients being balanced between the treatments (Table S1). The treatments were applied as liquid-solution form along with basic fertilization.

## Experimental conditions

### Soil characterization

The soil sample was collected in an Oxisol (0–20-cm depth) classified as Rhodic Haplustox under natural vegetation (USDA 1999). The sample was grounded, sieved (4-mm mesh), and mixed for homogenization, and sub-samples were analyzed for chemical and physical attributes. The results are presented in Table S2. For Ba pseudo-total determination, samples (0.5 g) were digested in 10 mL of concentrated hydrochloric and nitric acid solution (HCl/ $\text{HNO}_3$  ratio 4:1) under heating in a digestion block system (60°C for 180 min, 105°C for 60 min, and 140°C for 30 min) (McGrath and Cunliffe 1985). The analysis quality—QA/QC protocol—was verified with certified soil (NIST SRM 2710a, Montana Soil). After cooling, the extracts were filtered, and the volume was adjusted using deionized water and volumetric flasks (10 mL). The extracts were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES), and Ba concentrations were calculated using a standard curve. The Ba pseudo-total concentration was 3.9 mg  $\text{kg}^{-1}$  Ba in soil. For S- $\text{SO}_4$  determination, samples (2.5 g) were reacted with 25 mL calcium phosphate (500 mg  $\text{L}^{-1}$  P) diluted in an acetic acid solution (2.0 M), and S concentrations were determined by the turbidimetric method (Ajwa and Tabatabai 1993). The S- $\text{SO}_4$  concentration was 12 mg  $\text{kg}^{-1}$  S in the soil.

### Liming and fertilization

Prior to plant cultivation, soil samples were transferred to plastic pots (2.5-L capacity) and received 2.3 g  $\text{kg}^{-1}$  of limestone powder (41% CaO and 10.5% MgO), aiming to increase base saturation to 80% for soil acidity correction. Then, the samples were incubated for 20 days. After the incubation period, the samples received the treatments and the following basic fertilization, as liquid-solution form (mg  $\text{kg}^{-1}$ ): 200 N ( $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{NH}_4\text{NO}_3$ ), 450 P ( $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{KH}_2\text{PO}_4$ ), 300 K ( $\text{KH}_2\text{PO}_4$ ), 0.8 B ( $\text{H}_3\text{BO}_3$ ), 1.3 Cu ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ), 3.6 Mn ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ), 0.15 Mo ( $\text{H}_2\text{MoO}_4$ ), and 4.0 Zn [ $(\text{ZnNO}_3)_2$ ] (Table S1). Then, the samples were incubated for 10 days. The soil moisture was maintained at 60% field capacity during the incubation period with deionized water.

## Plant cultivation and harvest

Lettuce seeds (*Lactuca sativa* L. cv. Solaris) were germinated in trays with vermiculite under Hoagland solution irrigation (10% ionic strength) under greenhouse conditions (spring season,  $25 \pm 3^\circ\text{C}$ , 14 h light/10 h dark). Ten days after germination, three seedlings were selected according to uniformity (two 3-cm-length true leaves) and transplanted to the pots containing the treatments. After 7 days, one seedling was removed, and two seedlings remained per pot until the harvest. The soil moisture was maintained at 60% field capacity at plant cultivation with deionized water. Fifteen days after the transplant, a top-dress soil fertilization with  $100 \text{ mg kg}^{-1} \text{ N}$  ( $\text{NH}_4\text{NO}_3$ ) was applied in solution form. The plants were harvested 30 days after transplant. The shoots were harvested, and the roots were washed using sieves and tap water. Then, shoots and roots were rinsed with calcium chloride solution (2 mM) and deionized water. The plant material was dried for 72 h in an oven with air circulation ( $65^\circ\text{C}$ ) and weighed using a digital scale.

## Barium and sulfur in plants

Dried plant material was ground, and samples (0.5 g) were digested in 8 mL of concentrated perchloric and nitric acid solution ( $\text{HClO}_4/\text{HNO}_3$  ratio 3:1) under heating in a digestion block system (50, 100, and  $150^\circ\text{C}$  for 60 min each, and  $200^\circ\text{C}$  until translucent extracts, around 30 min). The analysis followed a rigid QA/QC protocol with the use of a certified reference material (NIST SRM 1573a, Tomato Leaves). After cooling, the plant extract volume was adjusted using volumetric flasks and deionized water (25 mL). The extracts were analyzed by ICP-OES, and element concentrations were calculated using standard curves.

## Barium indices in plants

Barium bioconcentration (Ba-BCF) and translocation (Ba-TF) factors were calculated according to Antoniadis et al. (2017) using the following equations: 1.  $\text{Ba} - \text{BCF} = [\text{Ba in plants}]/[\text{Ba in soil}]$ , 2.  $\text{Ba} - \text{TF} = [\text{Ba in shoots}]/[\text{Ba in roots}]$ , where [Ba in plants] is the weighted average of Ba concentrations in plant tissues, [Ba in soil] is the Ba dose applied, and [Ba in shoots] and [Ba in roots] are the Ba concentrations in shoots and roots, respectively.

## Sequential extraction of Ba fractions

The sequential extraction was carried out according to Nogueirol and Alleoni (2013) with some modifications. Before seedlings transplant, soil samples (50 g) were collected, dried (room temperature), and ground, and sub-samples (0.5 g) were added to centrifuge tubes (50 mL). The

fractions were extracted in the following sequence: Ba in extractable fraction (extractable-Ba, 1); Ba bound to organic matter (organic matter-Ba, 2); Ba bound to Al, Fe, and Mn oxides (oxides associated-Ba, 3); and Ba in residual fraction (residual-Ba, 4). Extractable-Ba was obtained with 15 mL calcium chloride solution (0.1 M  $\text{CaCl}_2$ ). Samples were shaken in a horizontal shaker at 120 rpm for 2 h (room temperature), centrifuged (1400 g for 20 min), and supernatants were collected, filtered, and transferred to volumetric flasks (25 mL). Then, samples were resuspended with 5 mL of sodium chloride solution (0.1 M  $\text{NaCl}$ ) in a vortex shaker and centrifuged as described. Supernatants were collected, filtered, and transferred to the previous flasks. The volume was adjusted with deionized water. Then, samples were dried overnight in an oven ( $60^\circ\text{C}$ ) and weighed to check soil loss.

Organic matter-Ba was extracted with 5 mL sodium hypochlorite solution ( $\text{NaOCl}$  5% v/v, pH 8.5). The samples were kept in a water bath ( $90\text{--}95^\circ\text{C}$ ) for 30 min and, after cooling, were centrifuged, resuspended, and dried, and supernatants were collected as described. The oxides associated-Ba was extracted with 20 mL solution (pH 3.0) with ammonium oxalate [0.2 M ( $\text{NH}_4$ ) $_2\text{C}_2\text{O}_4$ ], oxalic acid (0.2 M  $\text{C}_2\text{H}_2\text{O}_4$ ), and ascorbic acid (0.1 M  $\text{C}_6\text{H}_8\text{O}_6$ ). The samples were kept in a water bath ( $90\text{--}95^\circ\text{C}$ ) for 30 min and, after cooling, were centrifuged, resuspended, and dried, and supernatants were collected as described. At last, the samples were transferred to glass digestion tubes for residual-Ba extraction (pseudo-total concentration). The samples were digested in 10 mL of hydrochloric ( $\text{HCl}$ , 37% v/v) and nitric acid ( $\text{HNO}_3$ , 65% v/v) solution,  $\text{HCl}/\text{HNO}_3$  ratio 4:1, under heating in a digestion block system ( $60^\circ\text{C}$  for 180 min,  $105^\circ\text{C}$  for 60 min, and  $140^\circ\text{C}$  for 30 min). After cooling, the extracts were filtered, and the volume was adjusted as described. The extracts were analyzed by ICP-OES, and Ba concentrations were calculated using a standard curve.

## Statistical analysis

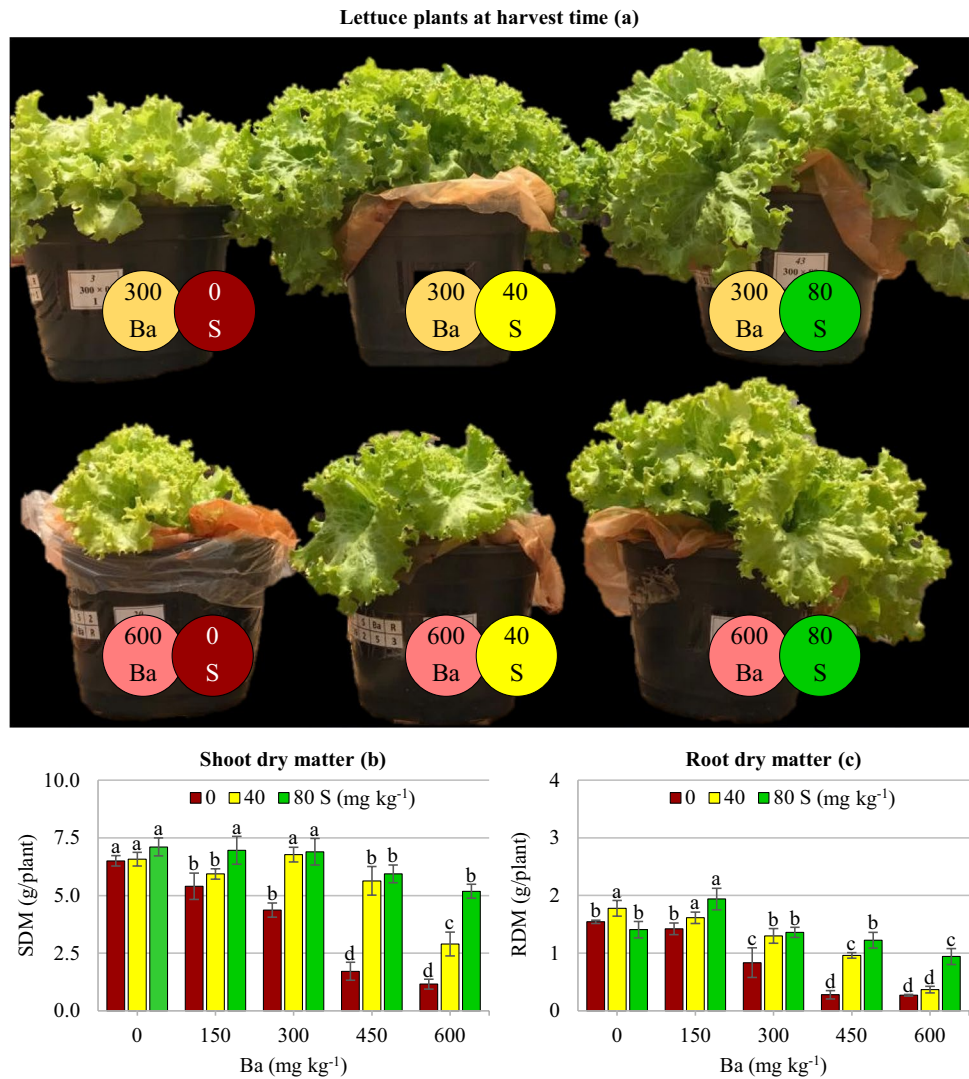
Data normality and variance analyses were performed using SAS® Studio (SAS® OnDemand for Academics), and treatments were grouped by the Scott-Knott test ( $p < 0.05$ ) using the SISVAR® software (Ferreira 2019). Pearson correlation and regression analyses were performed using SAS® Studio.

## Results

### Plant growth and toxicity symptoms

The treatments severely affected plant growth (Fig. 1). Barium exposure inhibited lettuce growth, mainly in plants cultivated without S supply (Fig. 1(b, c)). Conversely, S doses attenuated Ba toxicity, mainly in plants cultivated under

**Fig. 1** Lettuce growth under Ba and S doses in soil ( $\text{mg kg}^{-1}$ ). Plants at harvest time (a). Shoot dry matter (SDM, b) and root dry matter (RDM, c). Columns with the same letters were grouped by the Scott-Knott test ( $p < 0.05$ )



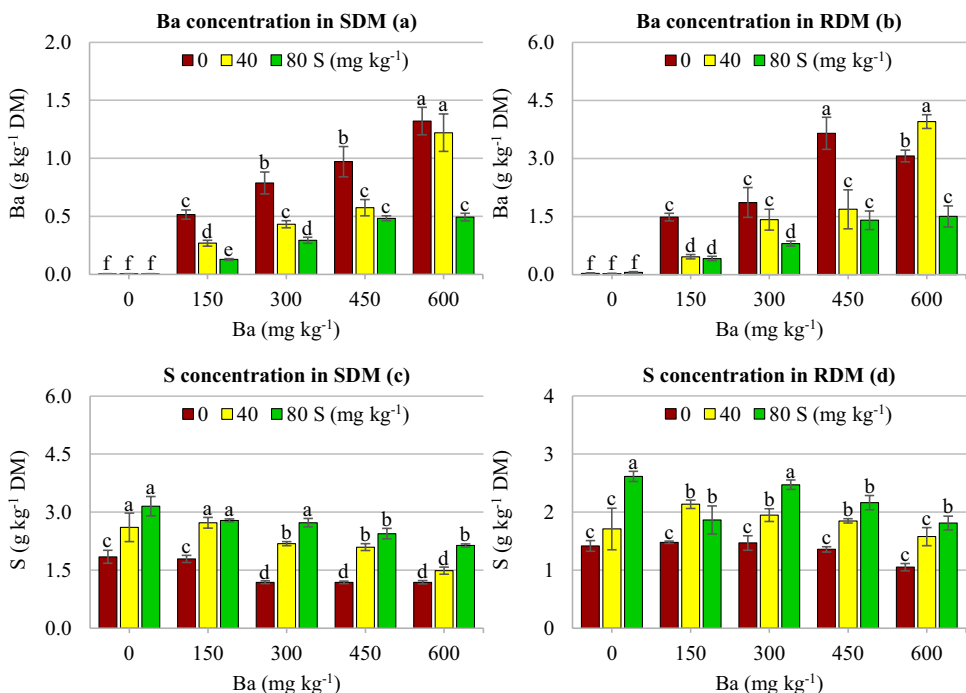
the  $80 \text{ mg kg}^{-1}$  S supply. The addition of  $600 \text{ mg kg}^{-1}$  Ba decreased the shoot and root dry matter production by 85% in plants grown without S supply, compared with the control treatment ( $40 \text{ mg kg}^{-1}$  S without Ba addition). However, the same Ba dose decreased shoot and root dry matter productions by 21 and 47%, respectively, in plants grown with  $80 \text{ mg kg}^{-1}$  S supply compared with the control treatment. Similarly, Ba doses induced leaf chlorosis, mainly in plants grown without S supply, which was less severe in plants cultivated with S supply (Fig. 1(a)). Interestingly,  $40 \text{ mg kg}^{-1}$  S dose presented equivalent results to  $80 \text{ mg kg}^{-1}$  S until  $450 \text{ mg kg}^{-1}$  Ba addition, while  $80 \text{ mg kg}^{-1}$  S dose promoted better plant tolerance at  $600 \text{ mg kg}^{-1}$  Ba dose.

### Barium and sulfur concentrations

The treatments influenced both Ba and S concentrations in lettuce tissues (Fig. 2). Barium doses increased

Ba concentrations in lettuce shoots and roots, mainly in plants grown without S addition. At the same time, this effect was less pronounced under S supply (Fig. 2(a, b)). Plants cultivated under the addition of  $600 \text{ mg kg}^{-1}$  without S supply exhibited Ba concentrations of  $1.3 \text{ g kg}^{-1}$  Ba in shoots and  $3 \text{ g kg}^{-1}$  Ba in roots. In contrast, in plants grown under the same Ba dose with  $80 \text{ mg kg}^{-1}$  S supply, the Ba concentrations were  $0.5 \text{ g kg}^{-1}$  Ba in shoots and  $1.5 \text{ g kg}^{-1}$  Ba in roots. Likewise, in plant growth results,  $40 \text{ mg kg}^{-1}$  S dose promoted Ba concentrations in lettuce tissues equivalent to  $80 \text{ mg kg}^{-1}$  S until  $450 \text{ mg kg}^{-1}$  Ba addition, while  $80 \text{ mg kg}^{-1}$  S dose promoted lower Ba concentrations at  $600 \text{ mg kg}^{-1}$  Ba. Regarding S in plants, Ba doses decreased S concentrations in shoots and roots. The addition of  $600 \text{ mg kg}^{-1}$  Ba decreased S concentrations by 30% in shoots and roots of plants grown with  $80 \text{ mg kg}^{-1}$  compared with the same S dose without Ba addition (Fig. 2(c, d)).

**Fig. 2** Ba and S concentrations in shoot (SDM) and root dry matter (RDM) of lettuce grown under Ba and S doses in soil. Ba concentrations in SDM (a) and RDM (b). S concentrations in SDM (c) and RDM (d). Columns with the same letters were grouped by the Scott-Knott test ( $p < 0.05$ )

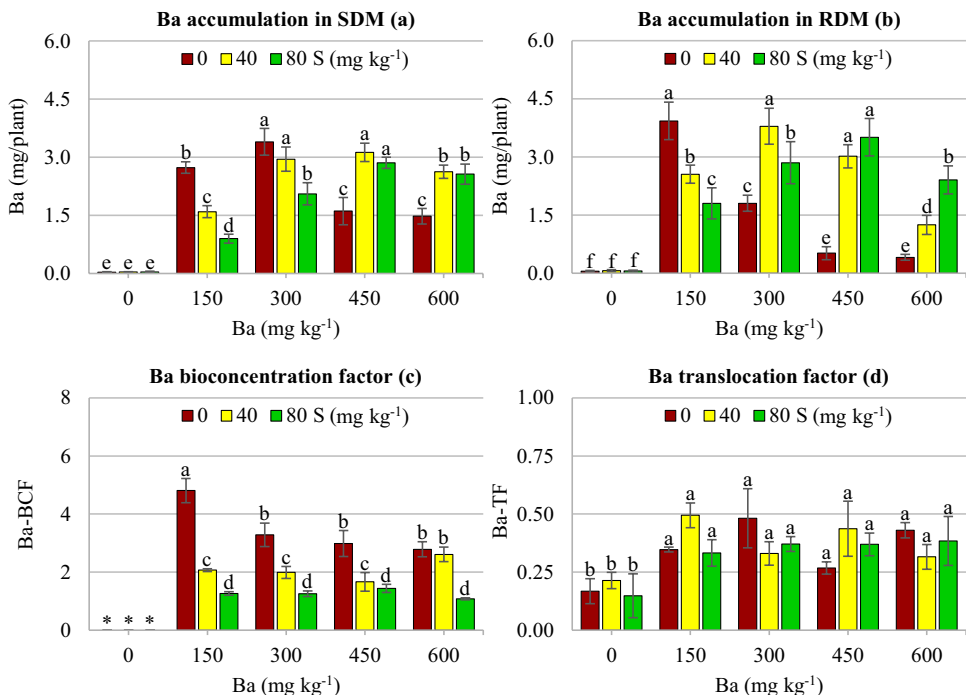


**Barium indices and accumulation**

The treatments influenced the Ba indices and accumulations in lettuce plants (Fig. 3). S supply decreased the Ba accumulation in shoots until 300 mg kg<sup>-1</sup> Ba dose, and in roots at 150 mg kg<sup>-1</sup> Ba, mainly at 80 mg kg<sup>-1</sup> S supply (Fig. 3(a, b)). This S dose decreased the Ba accumulation in shoots by

40% at 300 mg kg<sup>-1</sup> Ba and in roots by 54% at 150 mg kg<sup>-1</sup> Ba, compared with the same Ba doses without S supply. However, the S supply increased Ba accumulation in plants at higher Ba doses. The 80 mg kg<sup>-1</sup> S supply increased the Ba accumulation by 74% in shoots and 6-fold in roots at 600 mg kg<sup>-1</sup> Ba dose, compared with the same Ba dose without S supply. Regarding Ba indices, the S supply decreased

**Fig. 3** Ba indices and accumulation in lettuce grown under Ba and S doses in soil. Ba accumulations in shoot (SDM, a) and root dry matter (RDM, b). Ba bioconcentration (Ba-BCF, c) and translocation factors (Ba-TF, d). Columns with the same letters were grouped by the Scott-Knott test ( $p < 0.05$ )



Ba-BCF, mainly at 80 mg kg<sup>-1</sup> S dose (Fig. 3(c)). This S dose decreased the Ba-BCF by 74% in plants grown under 150 mg kg<sup>-1</sup> Ba compared with the same Ba dose without S supply. Conversely, the Ba addition increased the Ba-TF by 2.2-fold compared with the control treatment, regardless of Ba and S doses (Fig. 3(d)).

### Barium fractionation

The treatments affected all Ba chemical fractions evaluated (Fig. 4). The Ba doses increased all fractions regardless of S supply, with higher increments in extractable-Ba, residual-Ba, and oxides associated-Ba (Fig. 4(a, c, d)). The addition of 600 mg kg<sup>-1</sup> Ba combined with 40 mg kg<sup>-1</sup> S yielded 352 mg kg<sup>-1</sup> of extractable-Ba, 115 mg kg<sup>-1</sup> of residual-Ba, 66 mg kg<sup>-1</sup> of oxides associated-Ba, and 14 mg kg<sup>-1</sup> of organic matter-Ba. In contrast, the S supply decreased extractable-Ba at Ba doses higher than 150 mg kg<sup>-1</sup>, mainly at 80 mg kg<sup>-1</sup> S, while it increased the other fractions. Both 40 and 80 mg kg<sup>-1</sup> S doses decreased extractable-Ba by around 25% at 300 and 450 mg kg<sup>-1</sup> Ba, compared with the same Ba doses without S supply (Fig. 4(a)). However, just the higher S dose decreased extractable-Ba at 600 mg kg<sup>-1</sup> Ba, decreasing it by 30% compared with the same Ba dose without S supply. Conversely, S doses increased by around 2-fold the residual-Ba, oxides associated-Ba, and organic matter-Ba at 450 and 600 mg kg<sup>-1</sup> Ba, compared with the same Ba doses without S supply. Analyzing the relative distribution of Ba fractions, we observed that, at Ba doses higher than 150 mg kg<sup>-1</sup>, the higher S dose decreased extractable-Ba from 77

to 55%, while increased residual-Ba from 15 to 29%, and oxides associated-Ba from 6 to 14% (Fig. 5).

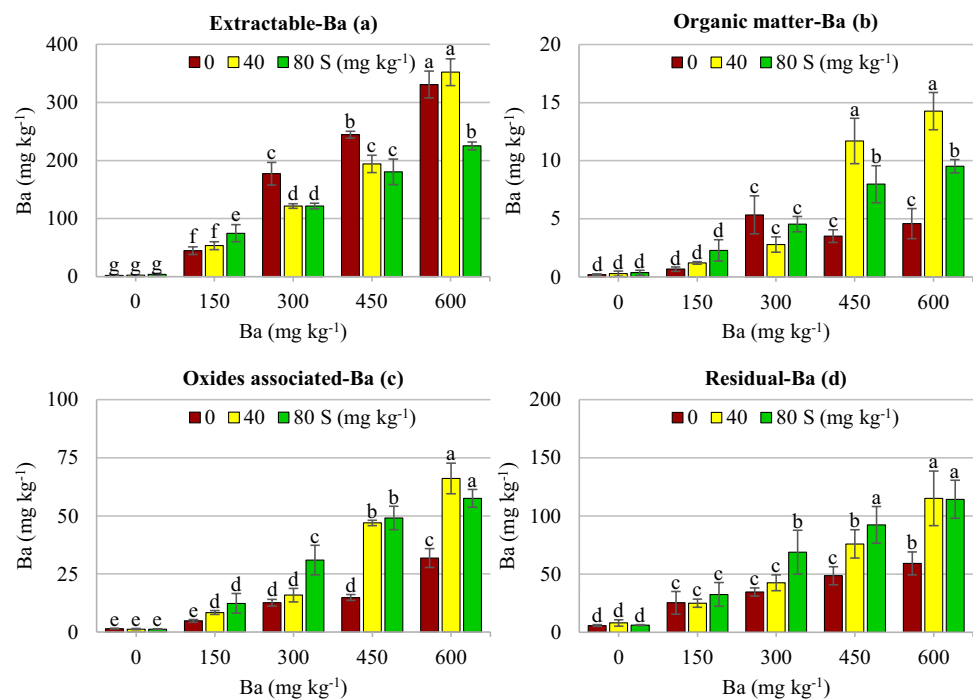
### Relationships between Ba in soil, Ba in plants, and lettuce growth

We observed strong negative correlations between Ba concentrations in lettuce tissues and shoot and root productions (Fig. 6). Regarding Ba concentrations in plant tissues and Ba fractions in soil, we observed strong positive correlations between the extractable-Ba fraction and Ba in shoots and roots (Fig. 7), while moderated positive correlations were observed between organic matter-Ba, oxides associated-Ba, and residual-Ba fractions and Ba concentrations in lettuce tissues.

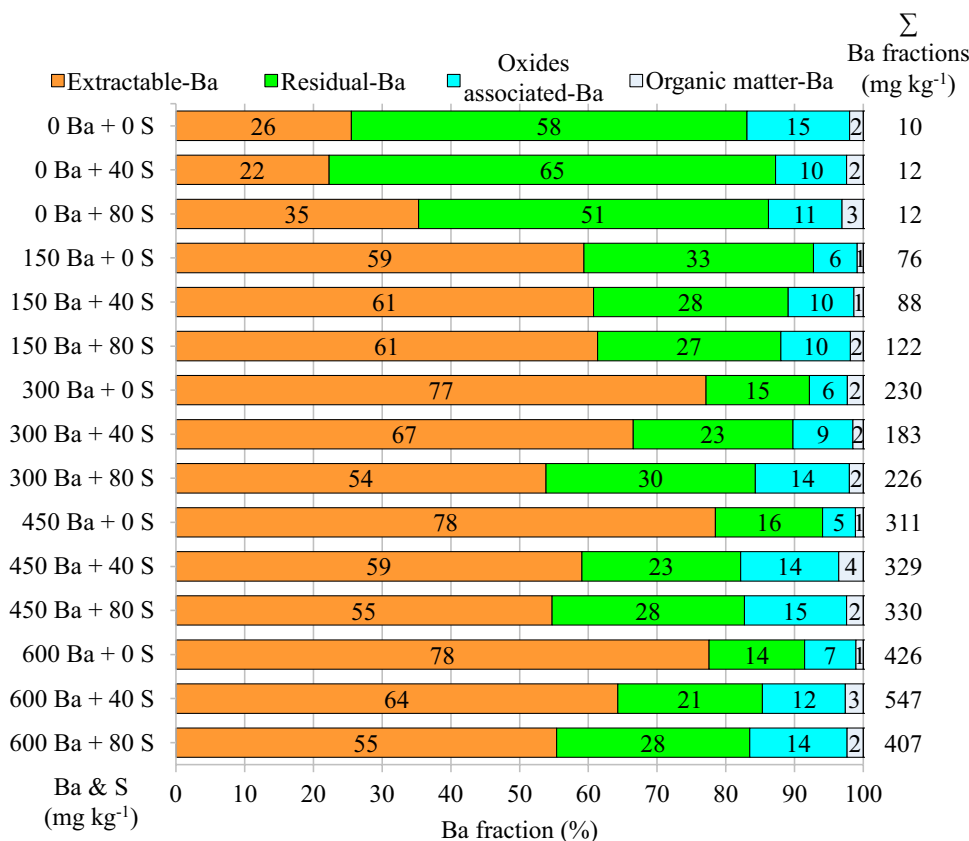
### Discussion

Sulfur supply protected lettuce plants from Ba toxicity (Fig. 1), mainly at the higher S dose (80 mg kg<sup>-1</sup> S), which represents twice the standard amount for plant cultivation in soils under greenhouse conditions (Huang et al. 2021; Cardoso et al. 2022). Since we did not observe growth inhibition in plants cultivated without S addition, it seems the S naturally available in soil supplied this nutrient for proper lettuce growth (Table S1). However, the growth inhibition in plants cultivated without S addition under Ba exposure suggests that plants supplied just with S naturally available in soil are more susceptible to Ba's harmful effects, like inhibition

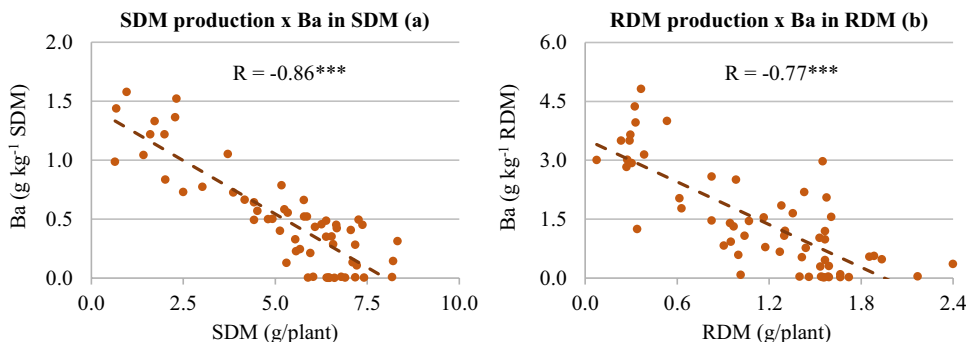
**Fig. 4** Ba chemical fractions in soil under Ba and S doses. Ba in extractable fraction (a), Ba bound to organic matter (b), Ba bound to Al, Fe, and Mn oxides (c), and Ba in residual fraction (d). Columns with the same letters were grouped by the Scott-Knott test ( $p < 0.05$ )



**Fig. 5** Relative distribution of Ba chemical fractions in soil under Ba and S doses. Extractable-Ba: Ba in extractable fraction. Residual-Ba: Ba in residual fraction. Oxides associated-Ba: Ba bound to Al, Fe, and Mn oxides. Organic matter-Ba: Ba bound to organic matter



**Fig. 6** Correlations between Ba concentrations and growth of lettuce cultivated under Ba and S doses in soil. Correlation between shoot dry matter (SDM) and Ba in SDM (a). Correlation between root dry matter (RDM) and Ba in RDM (b). \*\*\**p* < 0.001



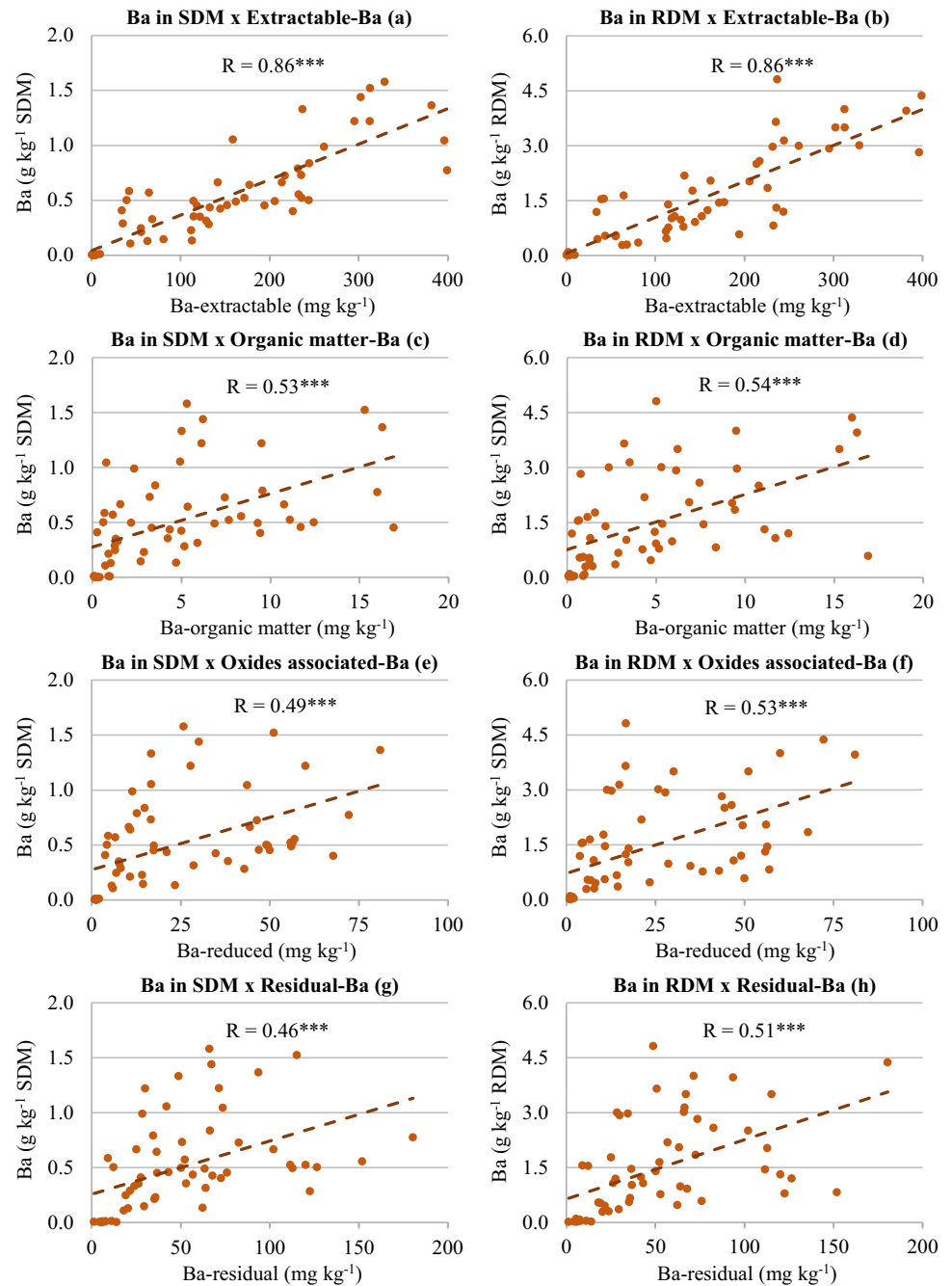
in sulfur and magnesium uptake (Fig. 2(c, d), S1d). These results indicate that high S availability can enhance plant resistance to Ba toxicity, probably due to its role in plant growth and influence in Ba availability in soil.

Sulfur is a nutrient for all organisms and plays several functions in plants, mainly due to its structural role in cysteine and methionine amino acids and other compounds related to plant growth, development, and stress resistance (Capaldi et al. 2015; Kopriva et al. 2019). Thus, many studies have reported that S can enhance plant tolerance to contaminants toxicity (Rabêlo et al. 2018; Siddiqui et al. 2019). Regarding Ba, de Souza Cardoso and Monteiro (2021a) suggested that S can reduce its phytotoxicity by immobilization in roots as barium sulfate, which was a mechanism that

probably contributed to lettuce resistance in this study, considering the higher Ba accumulation in roots under S supply (Fig. 3(b, c)). In addition, de Souza Cardoso and Monteiro (2021b) demonstrated that S could enhance the antioxidant system of plants to oxidative stress caused by Ba, which possibly contributed to toxicity alleviation in this study, considering the higher S tissues concentrations in plants grown under S supply (Fig. 2(c, d)).

The protective effect of S supply in lettuce under Ba exposure can also be related to its influence on metal availability in soil. Our results indicated that S decreases the Ba availability in soil, as suggested by Melo et al. (2014). The S addition decreased the Ba bioconcentration factor and Ba concentrations in shoots and roots (Figs. 2(a, b) and 3(c)).

**Fig. 7** Correlations between Ba fractions in soil and Ba concentrations in shoot (SDM) and root (RDM) dry matter of lettuce grown under Ba and S doses. Extractable-Ba and Ba in SDM (a) and RDM (b); organic matter-Ba and Ba in SDM (c) and RDM (d); oxides associated-Ba and Ba in SDM (e) and RDM (f); and residual-Ba and Ba in SDM (g) and RDM (h). \*\*\* $p < 0.001$



Furthermore, S supply decreased the Ba accumulation in shoots until  $300 \text{ mg kg}^{-1}$  Ba and in roots at  $150 \text{ mg kg}^{-1}$  Ba (Fig. 3(a, b)). The lower Ba availability under S supply is also indicated by the reduction in the extractable-Ba fraction (Figs. 4(a) and 5), which was the Ba fraction better correlated with Ba uptake (Fig. 7(a, b)) and growth inhibition (Fig. S2a, b). Thus, the S supply decreased Ba availability and uptake by lettuce plants, protecting the plants from Ba toxicity since the increasing Ba tissue concentrations were related to growth inhibition (Fig. 6(a, b)). Furthermore, the lower Ba uptake by S supply can decrease the Ba toxicity

risk for humans and animals by ingesting lettuce cultivated in soil with low Ba concentrations (Fig. S3c).

Many studies have demonstrated the high affinity between  $\text{Ba}^{2+}$  cation and  $\text{SO}_4^{2-}$  anion in aqueous solutions (Fig. S4), producing barium sulfate or barite ( $\text{BaSO}_4$ ), a very-low solubility compost (around  $2.5 \text{ mg L}^{-1}$  at  $25^\circ\text{C}$  and 1 bar) useful for industrial purposes (He et al. 2014; Corrêa et al. 2022; Ketegenov et al. 2022). This affinity probably was responsible for the decrease in  $\text{Ba}^{2+}$  availability, indicated by a reduction of extractable-Ba under S supply, while the  $\text{BaSO}_4$  produced was probably detected in the less soluble



Ba-fractions, mainly the residual-Ba and the oxides associated-Ba (Figs. 4 and 5). Furthermore, the results indicate the potential of  $\text{CaCl}_2$  0.1 M or similar solutions to evaluate  $\text{Ba}^{2+}$  cation or Ba-exchangeable in soils, a fraction that plays a major role in the retention of Ba in soils of tropical climates (Gong et al. 2020). Thus, the extractable-Ba fraction can be used as a Ba toxicity risk indicator, considering that the  $\text{Ba}^{2+}$  cation is the main absorbable and toxic Ba form to plants (Llugany et al. 2000; Kabata-Pendias 2010) and the strong positive correlation between extractable-Ba and Ba uptake and toxicity (Fig. 7(a, b), S2a, b).

Although S reduced Ba availability, plants grown at Ba doses higher than  $300 \text{ mg kg}^{-1}$  under S supply presented higher Ba accumulations (Fig. 3). This effect is possibly related to the lower Ba tissue concentration due to the higher biomass production in these plants (Fig. 2(a, b)), which resulted in higher Ba accumulations, even presenting lower Ba tissue concentrations and less severe toxicity (Fig. 1). Thus, S supply increased the Ba critical levels in soil and shoots for lettuce growth (Fig. S3a, b). These results indicate the potential of S addition to enhance Ba phytoextraction and phytoremediation through higher plant biomass production due to the S roles in plant growth and development. Thus, S supply can be a suitable low-cost and environmentally friendly strategy to manage Ba-contaminated areas, like the one studied by Viana et al. (2021), due to Ba availability reduction or Ba phytoextraction enhancement.

## Conclusions

The extractable-Ba fraction was the main one responsible for Ba bioavailability and phytotoxicity, probably corresponding to the  $\text{Ba}^{2+}$  exchangeable cation in the soil. The S supply reduced extractable-Ba at higher Ba doses while it increased the other fractions. Thus, S supply protected the lettuce plants from Ba toxicity by reduction of Ba availability in soil, in addition to plant growth enhancement. Our findings suggest that S supply can be a suitable low-cost and environmentally friendly strategy to manage Ba-contaminated areas due to Ba availability reduction or Ba phytoextraction enhancement. Furthermore, future studies can focus on S doses higher than  $80 \text{ mg kg}^{-1}$  S since the results indicate the potential of higher S addition in plant tolerance and Ba availability reduction. However, studies about the S effect in Ba-contaminated soils at field conditions are still necessary.

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**Data Availability** All data generated or analyzed in this research are included in this article.

## Declarations

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Competing interests** The authors declare no competing interests.

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