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RESEARCH ARTICLE

# Lead uptake and translocation pathways in soybean seedlings: the role of ion competition and transpiration rates

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## Abstract

*Glycine max* (L.) Merr. (soybean) crop plants have been found to have high lead (Pb) levels in aerial organs; however, knowledge about the processes involved in the incorporation, and subsequent translocation and accumulation of the metal in the plants is scarce. Considering the toxicity of this heavy metal, the aim of the present study was to evaluate Pb uptake and translocation, and their toxic effects on soybean seedlings via experiments of ionic competition with $Ca^{2+}$ (2.5 mM, Ca:Pb 1:1) and alteration of the transpiration flow [0.25 mM $Pb(NO_3)_2$]. The following variables were analyzed: biomass, leaf area (morphological parameters), photosynthetic efficiency, biochemical response (considered physiological stress markers: antioxidant power, chlorophylls, carotenoids, starch, proteins, sugars, and malondialdehyde), and Pb content. Results showed that soybean seedlings can accumulate high Pb concentration in its organs; however, in general, no morpho-physiological Pb stress symptoms were observed, except for lipid peroxidation and antioxidant power. The treatment with Ca ions was not effective in reducing Pb entry into root over time when both Ca and Pb where present in the grow solution. Alteration of the transpiration rate in soybean showed that the air flow increased the consumption of solutions, regardless of the treatments. However, Pb accumulation was lower in seedlings exposed to air flow, indicating a selective exclusion of the metal in the solution. In both experiments, soybean seedlings showed to be tolerant to high Pb concentrations.

**Keywords** *Glycine max* · Pb · Ionic competition · Transpiration flow · Toxicity · Plant tolerance

## Introduction

In recent decades, the concentrations of pollutants in different environmental compartments, such as air, water, and soil, have rapidly increased due to industrial activities. This is the case of lead (Pb), which has been used for more than 5000 years and is currently widely employed in numerous industrial processes due to its low corrosion, high density, ductility, and malleability (Liu et al. 2018; Pourrut et al. 2013; Zhang et al. 2015). Therefore, Pb can be found as a pollutant in all environmental compartments, affecting living organisms. The main problems related to this heavy metal lie in its persistence, accumulation, and toxicity in the environment and trophic levels, since it has no biological function in living organisms (Carocci et al. 2016; Maestri et al. 2010). Thus, exposure to Pb can produce toxic effects, even at low concentrations, due to the absence of adequate defense mechanisms to mitigate their toxic effects (Nakata et al. 2017; Pourrut et al. 2013).

Contamination of agricultural soils with Pb has been widely reported, with indications of a potential toxicological risk through the consumption of crops grown in these soils. Thus, food consumption has been identified as the main source of exposure to metals in humans (El-Kady and Abdel-Wahhab 2018), and its accumulation in agricultural crops is of great interest in terms of both environment and food security. In this sense, high levels of Pb have been reported in different crops, such as *Glycine max* (L.) Merr. (soybean, fabaceae family),

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which has been mentioned as a crop plant that accumulates Pb and other toxic elements in its aerial organs, particularly in seeds (Blanco et al. 2017; Rodriguez et al. 2011; Rodriguez et al. 2014; Salazar et al. 2012; Vergara Cid et al. 2020; Zhao et al. 2014). Knowledge about the processes that involve the uptake of Pb into plants is scarce and under constant revision. Some studies indicate that Pb can passively enter the roots and then move through the apoplastic pathway for water transport. This process, however, is limited by the barrier generated by the endodermis (Pourrut et al. 2011). This passive mechanism would be the main Pb uptake route from radical hairs, to be accumulated later in the cell wall (Kabata-Pendias 2011). However, the molecular mechanism by which Pb enters the roots is still unknown. Pb might enter the roots through several pathways, with the ionic channels having been proposed as the most important (Kohli et al. 2020). Several investigations indicate the $Ca^{2+}$ ionic channels as the main pathway of Pb uptake in roots (Pourrut et al. 2011). The competition with $Ca^{2+}$ and $Mg^{2+}$ was found to significantly reduce Pb concentration in rice roots (*Oryza sativa* L.), especially at the root tip (Kim et al. 2002). Wang et al. (2007) demonstrated that the use of $Ca^{2+}$ channel blockers (such as lanthanum) significantly reduces the entry of Pb into corn roots (*Zea mays* L.). In addition, the voltage-dependent $Ca^{2+}$ channels were found to be the main Pb uptake pathway through the cell membrane (Sharma and Dubey 2005). Considering the toxicity of Pb and the scarce knowledge of the uptake processes in soybean crops, further studies are necessary to help elucidate these topics.

On the other hand, once heavy metals enter the plant, one part can be stored and fixed in different sites of the root, whereas another part of the metals can cross the root and be transported to higher organs, in a process called translocation. Thus, translocation of toxic metals from roots to higher organs (leaves, seeds, fruits) is one of the processes of the greatest concern in edible plants, due to the toxicological risk associated with food security, either by direct or indirect consumption (Salazar et al. 2012). In this sense, the Casparian strip in the roots has been frequently proposed as the main barrier to the transport of metals to the upper vessels of plants (Sharma and Dubey 2005). The transport of toxic metals would be driven by negative pressures of transpiration flow (Liao et al. 2006; Sheoran et al. 2016). Thus, with the entry of water, Pb could also enter the roots and then be translocated by mass flow to the aerial organs. In addition, the rate of transpiration is a climatic-dependent parameter that could affect the absorption (Sheoran et al. 2016). Rucińska-Sobkowiak (2016) indicates that the transport of water from roots to the aerial organs occurs through the xylem in more than 99.5% of plants, being driven mainly by the negative pressures originated in the transpiration process. Thus, these authors suggest that the inclusion of an external air flow to a plant could increase the transpiration rate, which could be accompanied by an increase of the uptake of metals (including contaminants), as proposed in the present study. Therefore, to elucidate the efficiency of a plant to transfer the metal, the translocation factor is calculated, i.e., the relationship between accumulation in upper organs (stem, leaves, seeds, etc.) and lower organs (roots).

The aim of the present study was to evaluate Pb uptake and translocation in soybean seedlings via experiments of ionic competition with Ca and alteration of the transpiration flow by assessing physiological and biochemical variables.

## Materials and methods

### Plant material and growth conditions

Seeds of *G. max* Alim 3.14 GM III, provided by the Instituto Nacional de Tecnología Agropecuaria (INTA - Argentina) seed bank, were surface sterilized with 1% NaClO for 10 min and then washed with ultrapure water. Then, the seeds were germinated for 4 days in vermiculite under controlled conditions of temperature (28 °C) and daily watering with ultrapure water. After this period, seedlings were grown in a culture chamber under controlled hydroponic conditions of light-dark period of 16–8 h respectively (luminance > 300 PAR, MASTER TL5 HO 54 W/830 SLV/40 Philips), 70% relative humidity, and an average temperature of 28 °C; in a modified Hoagland nutrient solution containing the following nutrients: $Ca(NO_3)_2$ 5 mM, $KNO_3$ 5 mM, $KH_2PO_4$ 1 mM, $MgSO_4 \cdot 7H_2O$ 2 mM, $H_3BO_3$ 47 μM, $ZnSO_4 \cdot 7H_2O$ 0.2 μM, $CuSO_4 \cdot 5H_2O$ 0.32 μM, $Na_2MoO_4 \cdot 2H_2O$ 0.09 μM, $MnCl_2 \cdot 4H_2O$ 10 μM, Fe-EDTA 24 μM. At V1 development stage according to Fehr and Caviness (1977), the seedlings were transplanted to hydroponic cultivations under different treatments (see below).

### Competition between calcium and lead ions ($Ca^{2+}$–$Pb^{2+}$)

Soybean seedlings were grown in dark 750-mL containers with the corresponding culture solution at pH 5.5, with three replicates for each of the four treatments: (1) control ($NH_4NO_3$ 2.5 mM); (2) control + Ca ($NH_4NO_3$ 2.5 mM + $CaCl_2$ 2.5 mM); (3) Pb ($Pb(NO_3)_2$ 2.5 mM); and (4) Pb + Ca ($Pb(NO_3)_2$ 2.5 mM + $CaCl_2$ 2.5 mM). A high concentration of Pb, and consequently of Ca, was selected according to previous studies in polluted sites cultivated with soybean (Blanco et al. 2017; Vergara Cid et al. 2020). In addition, three plants per pot, which was considered an experimental unit, were cultivated in order to increase the plant biomass. Finally, seedlings were harvested on days 3 and 6 of exposure.

Plant growth was monitored at the different stages of development by periodically measuring quantum efficiency of PSII photochemistry (ΦPSII) under ambient light conditions

(Maxwell and Johnson 2000), using pulse of amplitude modulated fluorometer (FMS2, Hansatech Instruments, Pentney King's Lynn, United Kingdom).

ΦPSII was calculated as follows:

$$\Phi PSII = \frac{(Fm' - Ft)}{Fm'}$$

where Fm' is the maximum fluorescence in the light, and Ft is the steady-state fluorescence yield.

After each harvest, roots, stems, and leaves were separated, fresh weight (FW) was recorded, and plant samples were oven-dried at 50 °C until constant dry weight (DW) for biomass assessment. Moreover, leaf area (LA) was measured from photographs using the ImageJ software at the beginning and end of the exposure treatments on each harvest day to calculate the percentage of leaf growth (% LG), as follows:

$$\%LG = \frac{(LAf - LAi)}{LAi} * 100$$

where LAi and LAf are the initial and final leaf area, respectively, measured at the beginning and end of the treatment exposure.

In addition, three disks (1 cm of diameter) of the first trifoliate leaf were obtained using a round punch hole toll and stored at − 80 °C until biochemical analysis.

During this experiment, the solutions were not replenished during cultivation in order to compute the difference in consumption among treatments.

## Alteration of transpiration rate

To assess if the alteration of the transpiration rate could change the Pb uptake and translocation, soybean seedlings were transplanted to dark containers, which were placed on a lid support that also prevented evaporation of the solution. Each container had 8 seedlings and the experiment was performed in triplicate. Seedlings were subjected to the following treatments with or without air flow (AF) with the culture solutions at pH 5.5: control ($NH_4NO_3$ 0.25 mM), Pb ($Pb(NO_3)_2$ 0.25 mM), control + AF ($NH_4NO_3$ 0.25 mM + air flow), Pb + AF ($Pb(NO_3)_2$ 0.25 mM + air flow) (Fig. 1). In this experiment, a lower concentration of Pb was selected with the purpose to not saturate the accumulation of Pb in the seedlings, but to stimulate a differential incorporation with the air flow, as mentioned in other studies (Liao et al. 2006). The experiments with or without air flow were carried out under controlled hydroponic conditions (light-dark period of 16–8 h respectively, 70% relative humidity, and an average temperature of 28 °C) in consecutive weeks. The air flow was generated with two fans (each with an air flow of 58.39 $m^3$ min) located on each side of the plant growth chambers during the first two days of treatment. Then, during the 2 days later (4 days from the start of the experiment), the rest of the plants did not receive air flow so that they could potentially recover from stress, and were then harvested according to Liao et al. (2006). Four plants per container were collected in each harvest. On each collection day, samples of roots, stems, and leaves were separated, and FW and LA were measured. In this case, specific leaf area was calculated as a ratio between LA and leaves DW. Moreover, first trifoliate leaf samples were also collected for further biochemical analysis.

## Pb determination in plants

Lead content was analyzed according to Arshad et al. (2008) and Rosenkranz et al. (2017). Briefly, each sample was ground in a stainless mill, and 1 g was washed at 430 °C for 8 h and then digested using $H_2O_2$ and $HNO_3$ 65% (1:4 v/v) for 24 h, with the digestion being completed in Hach® tubes by heating over a hot plate at 120 °C. Finally, the digested samples were filtered (Whatman no. 542) and the volume was adjusted to 10 mL with ultrapure water. The Pb concentrations were measured by atomic absorption spectrophotometer (AAS; Perkin-Elmer AA3110). As a quality control, blanks and samples of the standard reference material (hay IAEA-V-10, and beech leaf WEPAL -IPE 2012.1) were prepared as described above. The quality control resulted in recoveries of over 93% of the certified material value. The amount of Pb accumulated by each plant organ was expressed as a concentration value (μg Pb $g^{-1}$ DW), and as absolute Pb content (μg Pb), taking into account the biomass for the latter. The absolute Pb content allows to evaluate the accumulation of the metal in relation to the total quantity of biomass of that organ. This measure is useful to evaluate the potentiality of a species as a phytoextractor and phytoaccumulator of heavy metals (Salazar et al. 2016).

## Biochemical analyses

### Ferric-reducing ability of plasma, chlorophylls, carotenoids, malondialdehyde, and soluble sugars

The ferric-reducing ability of plasma (FRAP), chlorophylls, carotenoids, and malondialdehyde (MDA), as well as total leaf soluble sugars, were measured from a single plant extract, following a sequential extraction technique (Miranda Pazcel et al. 2018; Ergo et al. 2018). Briefly, first trifoliate leaf samples were ground using liquid nitrogen with a mortar and pestle, and then suspended in 750 μL of ethanol (80% v/v). This homogenate was centrifuged at 14,000 RPM at 4 °C for 10 min. Subsequently, an aliquot of the supernatant was used to calculate the FRAP value (Robert et al. 2014), by diluting

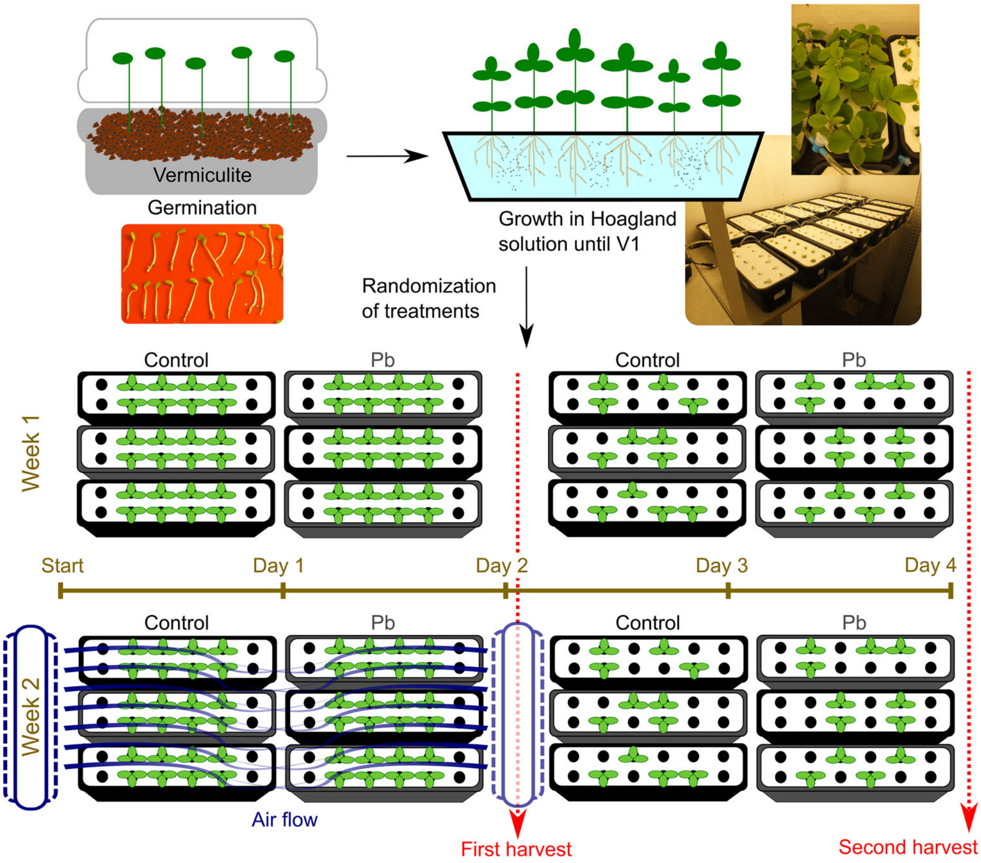

**Fig. 1** Design of the experiment incorporating air flow to alter the transpiration rate

the sample in a reaction mix consisting of a 0.3 M sodium acetate buffer (pH 3.6), 2,4,6-tri-2-pyridyl-*s*-triazine 10 mM (TPTZ), and $FeCl_3$ 200 mM. Then, a calibration curve was performed with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as standard reference; finally, the samples were measured by spectrophotometry in microplates at 600 nm (Thermo Scientific-Multiskan Spectrum).

The pellet was re-suspended with the remaining supernatant and incubated at 80 °C for 20 min; then, it was stabilized at room temperature and centrifuged again at 14,000 RPM for 10 min to be used for measurement of the remaining parameters.

MDA was quantified according to Heath and Packer (1968), using a digestion of the supernatant with 0.5% TBA and 20% TCA at 90 °C for 20 min. Then, it was centrifuged at 14,000 RPM for 5 min, and the absorption was read at 532 and 600 nm, being MDA concentration calculated as follows:

$$\mathrm{MDA} = \frac{(\mathrm{Abs}_{532} - \mathrm{Abs}_{600})}{\varepsilon * l}$$

where $\varepsilon$ is the extinction coefficient of 155 $\mathrm{mM}^{-1}\,\mathrm{cm}^{-1}$, $l$ is the length of the optical path, and Abs is the absorbance at a certain wavelength.

Chlorophyll (Chl) and carotenoid determination was performed using the same supernatant as that used for the determination of MDA content; absorbance was measured at 470, 645, 654, and 663 nm, and calculated according to Lightenthaler (1987) and Palta (1990), as follows:

$$\begin{aligned}
\mathrm{Chl}_{\mathrm{total}} &= (1000 \times \mathrm{Abs}_{654})/39.8 \\
\mathrm{Chl}_{a} &= 13.36 \times \mathrm{Abs}_{664} - 5.19 \times \mathrm{Abs}_{649} \\
\mathrm{Chl}_{b} &= 27.43 \times \mathrm{Abs}_{649} - 8.12 \times \mathrm{Abs}_{664} \\
\mathrm{Carotenoids} &= (1000 \times \mathrm{Abs}_{470} - 2.13 \times \mathrm{Chl}_{a} - 97.64 \times \mathrm{Chl}_{b})/209
\end{aligned}$$

For the measurement of total soluble sugars, the supernatant was oven-dried for 24 h and then resuspended in water and mixed with antrone reagent. Then, it was placed at 80 °C for 30 min, and measured at 620 nm, using a glucose calibration curve (Leyva et al. 2008).

### Starch and proteins

Starch content was determined as described by Guan and Janes (1991), and protein content according to Lowry et al. (1951), with some modifications; in both cases, the supernatant was the same as that used for the determination of the previous parameters (2.5.1.). The pellet was oven-dried at 60 °C for 24 h and then partitioned equally for total starch and protein determinations. Regarding total starch content, the pellet was re-suspended by shaking it in hot water (100 °C) for 30 min. Then, a digestion with the enzyme amyloglucosidase was performed overnight at 55 °C.

Finally, it was centrifuged at 12,000 RPM and the sample was suspended in summer reagent (NaOH, NaK tetrahydrate, 3,5-dinitrosalicylic acid) to be immediately read at 550 nm.

For the total protein content, the pellet was re-suspended by shaking it in water containing 0.1% v/v tween 20 (Sigma-Aldrich, St. Louis, USA) and heated at 100 °C for 5 min, and then centrifuged at 14,000 RPM for 10 min at room temperature. Finally, the supernatant was mixed with a solution composed of 10 parts of 2% sodium carbonate, 0.1% sodium hydroxide, and 1% tween 20; 0.25 part of 1% sodium potassium tartrate (aqueous); and 0.25 parts of 1% Cu sulfate (aqueous). The total leaf protein value was calculated using bovine serum albumin (Sigma-Aldrich, St. Louis, USA) as the standard reference. The samples were incubated for 15 min and then the reaction was stopped with Folin-Ciocalteu reagent. Finally, it was measured by spectrophotometry in microplates at 578 nm.

### Relative water content

The leaf relative water content (RWC) was calculated as follows (Kholodova et al. 2011):

$$\mathrm{RWC} = \frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{SW}-\mathrm{DW}} \times 100$$

where FW is fresh weight, DW is dry weight, and SW is the saturated water weight of a leaf (obtained after immersing a leaf in ultrapure water for 24 h).

## Data analyses

### Translocation factor

The translocation factor (TF) was calculated using the ratio of Pb concentration in aerial organs (stem and/or leaves) and roots or stems, according to the following formula (Rodriguez et al. 2011):

$$\mathrm{TF} = \frac{\text{Pb content in : stem}-\text{leaf}-\text{aerial part}}{\text{Pb content in : root}-\text{stem}}$$

TF values higher than 1 indicate that the metal was easily translocated (effective translocation).

### Statistical analysis

Data were analyzed using the R 3.3.3 software (R Core Team 2017), Tydiverse package (Wickham 2017). A principal component analysis (PCA) was performed using the calcium treatments as the classification criteria in order to assess the relationship among them, the accumulation of Pb, and morpho-biochemical parameters in *G. max*. The assumptions of PCA were met (with continuity of the variables and the number of elements observed being greater than the number of original variables). Moreover, multifactorial analysis of variance, and a Tukey post-hoc test for multiple means comparisons, was employed. Normality assumptions were corroborated, and for cases without homogeneity, the results were adjusted using the Infostat ® Software (Di Rienzo et al. 2019).

## Results and discussion

### Lead calcium competition assays

#### Lead accumulation

The results of Pb accumulation in different soybean organs showed great differences between control and Pb treatments, as expected, since control values were below the detection limit of Pb measurement by AAS (< 0.01 mg $L^{-1}$). Therefore, in order to evaluate the effect of $Ca^{2+}$ as a competitor ion, only Pb treatments were compared (Fig. 2). The presence of Ca significantly decreased Pb accumulation in roots after 3 days of exposure. This result agrees with a decrease in Pb concentration in rice roots after 24 h of exposure to 10 μM of $Pb(NO_3)_2$ and $Ca(NO_3)_2$ reported by Kim et al. (2002). A partial recovery of Pb accumulation was found after 6 days of treatment. Thus, the result indicates a decrease in Pb absorption by roots in the presence of $Ca^{2+}$ at the beginning of treatment, but an increase of Pb over longer exposure periods, probably due to the effect of depletion of the competitor ion (Fig. 2). A significant reduction of Pb accumulation was observed in roots and shoots of *Typha latifolia* L. exposed to three times more $Ca^{2+}$ than $Pb^{2+}$, without a reversion of Pb incorporation after 10 days of exposure (Rodriguez-Hernandez et al. 2015), which was probably due to the higher concentration of $Ca^{2+}$ ions in the nutrient solution. Antosiewicz (2005) showed a consistent decrease of Pb accumulation, especially in roots, in four species treated with Ca ions for 7 days.

Pb accumulation in stems and leaves did not vary significantly (Fig. 2), indicating that Pb translocation to the aerial organs remained unchanged. Thus, our findings suggest that the ability of soybean roots to translocate Pb to aerial organs could be saturated at low concentrations of the metal; Pb would be accumulated mainly in the roots, probably as a protective mechanism, as previously reported (Fahr et al. 2013; Khan et al. 2015; Tangahu et al. 2011).

In agreement with the results of Pb accumulation, the TF between roots and aerial parts of soybean seedlings indicates ineffective translocation (TF < 1) (supplementary material Fig. S1).

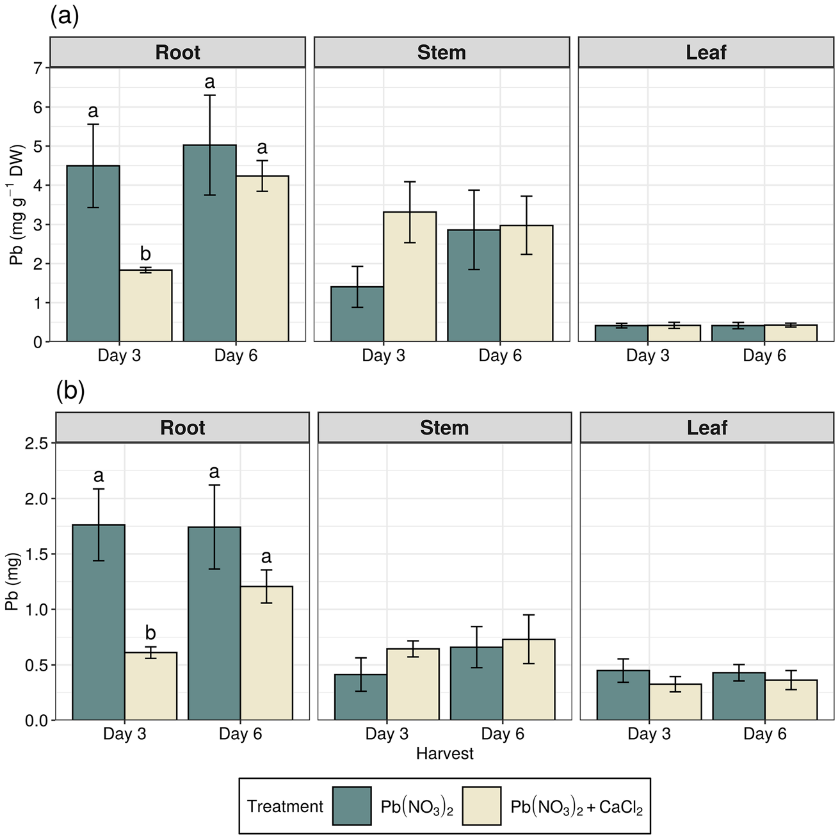

**Fig. 2** **a** Pb concentration (mg g$^{-1}$) and **b** absolute Pb content per plant (mg) (mean ± SE) in different organs of *G. max* treated with Pb with and without the addition of Ca on two harvest days (3 or 6 days of exposure). Different letters indicate significant differences at $p < 0.05$

## Biomass, %LG, and ΦPSII

To evaluate if the incorporation of Ca$^{2+}$ reverted the stress symptoms due to Pb exposure, different morpho-physiological parameters were analyzed. Table 1 shows the biomass values of soybean organs and the %LG. Results of the accumulated biomass (DW) between harvests (3 or 6 days) showed no differences for any treatments with Pb, with a significant increase being observed only in the control (Table 1). Regarding leaf growth (%LG), although the development was not altered by the addition of Ca ions, a decrease in the treatments with Pb on the second harvest day was observed (Table 1). By contrast, Wojas et al. (2007) demonstrated a significant effect of Ca on tolerance of tobacco plants measured as root elongation, using 750 more times Ca than Pb in the culture solution.

On the other hand, the analysis of ΦPSII did not show significant differences among treatments (data not shown). In contrast, there is much evidence of the deleterious effect of Pb on photosynthesis, in particular on PSII efficiency

**Table 1** Mean ± SE of biomass (g DW) of roots, stems, and leaves, and percentage of leaf growth (%LG) of *G. max* under different treatments with Pb with/without the addition of Ca at different harvests

| Treatment | Harvest (day) | Root | Stem | Leaf | % LG |
|---|---|---|---|---|---|
| NH$_4$NO$_3$ 2.5 mM | 3 | 0.81 ± 0.08 b | 0.59 ± 0.06 b | 1.54 ± 0.07 c | 3.07 ± 4.45 a |
| | 6 | 1.3 ± 0.08 a | 0.97 ± 0.07 a | 2.58 ± 0.23 ab | 0.4 ± 0.83 a |
| NH$_4$NO$_3$ + CaCl$_2$ 2.5 mM | 3 | 0.71 ± 0.08 b | 0.57 ± 0.05 b | 1.42 ± 0.17 c | 1.29 ± 1.39 a |
| | 6 | 1.39 ± 0.08 a | 1.05 ± 0.05 a | 2.73 ± 0.21 a | 3.29 ± 0.49 a |
| Pb(NO$_3$)$_2$ 2.5 mM | 3 | 0.81 ± 0.06 b | 0.59 ± 0.01 b | 2.11 ± 0.22 abc | − 2.21 ± 3.81 a |
| | 6 | 0.7 ± 0.02 b | 0.48 ± 0.04 b | 2.09 ± 0.05 abc | − 34.04 ± 3.63 b |
| Pb(NO$_3$)$_2$ + CaCl$_2$ 2.5 mM | 3 | 0.67 ± 0.04 b | 0.41 ± 0.04 b | 1.55 ± 0.19 c | − 1.65 ± 2.15 a |
| | 6 | 0.57 ± 0.05 b | 0.47 ± 0.06 b | 1.67 ± 0.26 bc | − 12.17 ± 6.92 ab |
| ANOVA significance | | *** | *** | ** | * |

Different letters indicate significant differences at $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

(Zhou et al. 2018). Thus, soybean would seem to have a higher tolerance to Pb stress than other species.

## Principal component analyses

The results of the biochemical parameters (FRAP, MDA, Chl, proteins, starch, soluble sugars, RWC, ΦPSII), morphological parameters (biomass, LA, %LG), and Pb accumulation (in roots and leaves) were analyzed by a PCA (Fig. 3; supplementary material Table S1). Results of the first harvest showed a strong association between control treatments, with or without Ca, with Chl, accumulation of starch, RWC, and growth leaf (Fig. 3a). Heavy metal effects in Chl content has been widely reported, since it can replace $Mg^{2+}$ in the molecule (Rai et al. 2016). This effect on Chl impacts on less effective photosynthesis, being starch one of the main primary products. Pb could also generate a drop in RWC and growth, by a reduction in the plasticity of the cell wall (Kohli et al. 2020).

Our results showed that Pb treatments were associated with Pb content in roots and leaves (as was discussed in the "Lead accumulation" section), leaf biomass, and MDA concentration, indicating lipid peroxidation (Fig. 3a, Table S2). Indeed, the increase of MDA has been widely reflected in studies of plants exposed to Pb, even at low concentrations (Kumar et al. 2017; Malar et al. 2016). Moreover, a weak association with FRAP, proteins, and soluble sugars was observed in Pb + Ca treatment, showing the ability of the plant to respond to oxidative stress (Fig. 3a). Plants can remobilize starch in order to generate sugar as available energy source in conditions of abiotic stress (Thalmann and Santelia 2017). This process could explain an increase in soluble sugar content under Pb + Ca treatment (Table S2). FRAP values are generally linked to the presence of reducing substances, which donate hydrogen protons to free radicals and thereby break their chains and destabilize them (Meriño-Gergichevich et al. 2015). FRAP increased in Pb treatment, which was expected, but also increased even more in Pb + Ca treatments (Table S2). Similar results were reported for blueberry leaves treated with Al + Ca after a longer exposure time (15 days) by Meriño-Gergichevich et al. (2015). Several studies indicate an increase in the calmodulin proteins bound to calcium ions and as a signal transduction in response to toxic metals, which control the activation of genes associated with detoxification of these metals (Jain et al. 2018).

On the second harvest day (Fig. 3b), a decrease in the protective effect of Ca was observed, with the differences between treatments with and without Ca being less evident (Fig. 3b; Table S1). MDA content was higher in Pb + Ca treatment, showing a tendency of more stress. Moreover, a decrease of the photosynthetic pigments, and the consequent consumption of starch as energy storage, was observed for Pb + Ca treatment (Table S2), This behavior could be a response of the plant to ensure a normal physiological function as mentioned by Santos et al. (2015). In summary, our results showed that the inefficiency of the competitor ion was directly related to the exposure time, causing it to deplete, while the Pb availability increased being the stress effect more significant.

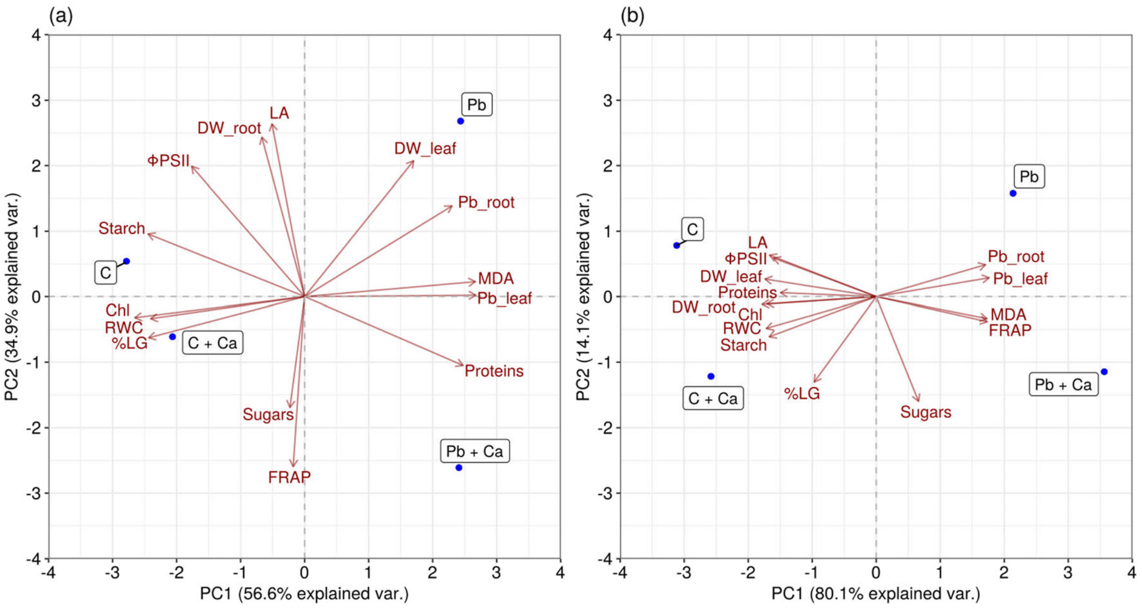

**Fig. 3** Biplot based on the first two components of the principal components analysis for Pb in leaves and biochemical parameters measured in *G. max* leaves exposed to different treatments (control and Pb with/without Ca) on two harvest days: **a** 3 days of exposure; **b** 6 days of exposure. PC, principal component; var., variance; C, control; LA, leaf area; % LG, percentage of leaf growth; MDA, malondialdehyde; Chl, total chlorophyll; Pb_root, Pb concentration in roots; Pb_leaf, Pb concentration in leaves; ΦPSII, quantum efficiency of PSII; RWC, relative water content in leaves; DW_root, biomass dry weight of root; DW_leaf, biomass dry weight of leaf

## Alteration of transpiration rate

### Consumption of culture solutions

To evaluate if the treatment with external air flow could modify the transpiration rate of soybeans and, consequently, the consumption of the culture solutions [control, $NH_4NO_3$; and Pb, $Pb(NO_3)_2$], the volume of each experimental container was measured every day during the experiment. The results showed that the air flow increased the solution consumption by the plant, with approximately 25% higher reduction of the volume than in the containers corresponding to the treatments without air flow (Fig. 4). Thus, our results suggest a higher consumption of solutions by plants exposed to air flows. In addition, plants grown in Pb + air flow treatments absorbed less solution than those exposed to the control treatment + air flow. Similar results were observed by Mensah et al. (2008), who reported a significant decrease in transpiration in cabbage, carrot, and lettuce crops grown in solutions with Pb. Heavy metals were frequently reported to produce an effect on water relations, with a negative influence on water consumption in root and, therefore, in the hole plant (Rucińska-Sobkowiak 2016).

### Pb concentration in soybean

Results of Pb concentration and absolute Pb content in roots and aerial organs (stem and leaves) for the Pb treatments are shown in Fig. 5. Regarding Pb concentration, an earlier accumulation in the roots after 2 days of exposure was observed; however, this accumulation was significantly lower in the treatment with air flow (Fig. 5a). In the aerial organs, the earlier concentration of Pb was also lower at higher transpiration rate (air flow), but a recovery in the concentration after 4 days of treatment was observed (Fig. 5b). Similar results were obtained for absolute Pb content, but in this case, it was increased by a significant rise in biomass on the second harvest day (see the "Biomass, %LG, and ΦPSII" section). However, a very low translocation factor was observed between roots and aerial organs and between stems and leaves in soybeans (< 1) (supplementary material Fig. S2).

In summary, the application of air flow generated a decrease in Pb concentration, whereas during the recovery period, the accumulation of Pb increased again in the aerial organs. Indeed, the effect of Cd on transpiration rate was widely studied (Kaznina et al. 2011; Kaznina and Titov 2014; Mensah et al. 2008), but few studies have evaluated other toxic metals such as Pb. In this context, based on a study carried out with three species, Ahkter and Macfie (2012) concluded that the dependence or not on the xylematic pathway for metal translocation (in particular for Cd) would be species-specific.

On the other hand, the analysis of the accumulation in the whole plant on the final harvest day (4 days) showed that Pb content in the treatments with air flow was lower (Fig. 6). This

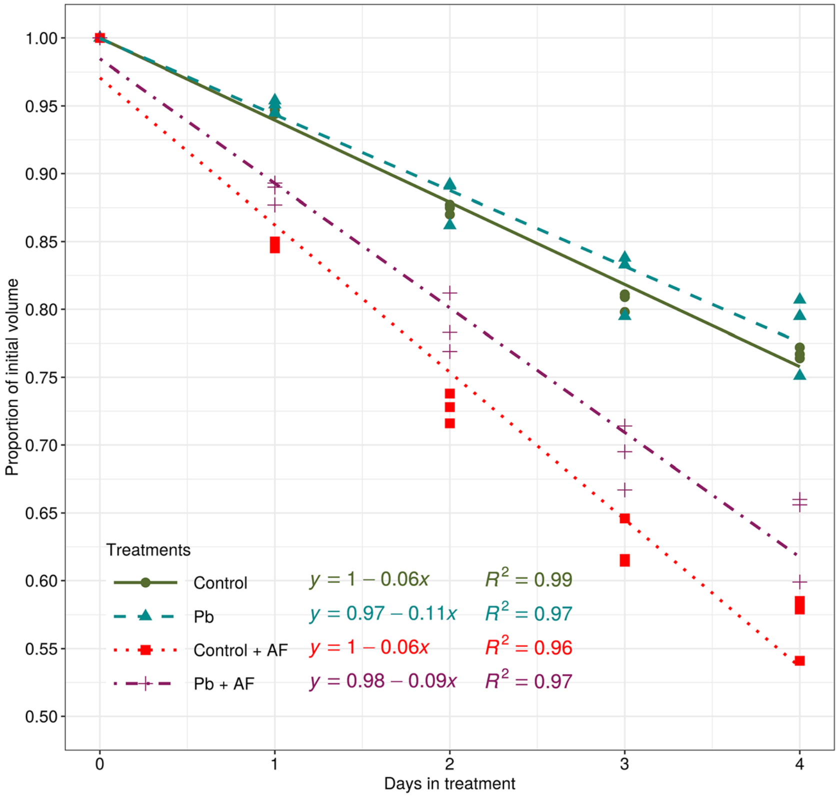

**Fig. 4** Proportional decrease in relation to the initial volume content of the culture solutions. Control, 0.25 mM $NH_4NO_3$; control + AF, 0.25 mM $NH_4NO_3$ + air flow; Pb, 0.25 mM $Pb(NO_3)_2$; Pb + AF, 0.25 mM $Pb(NO_3)_2$ + air flow

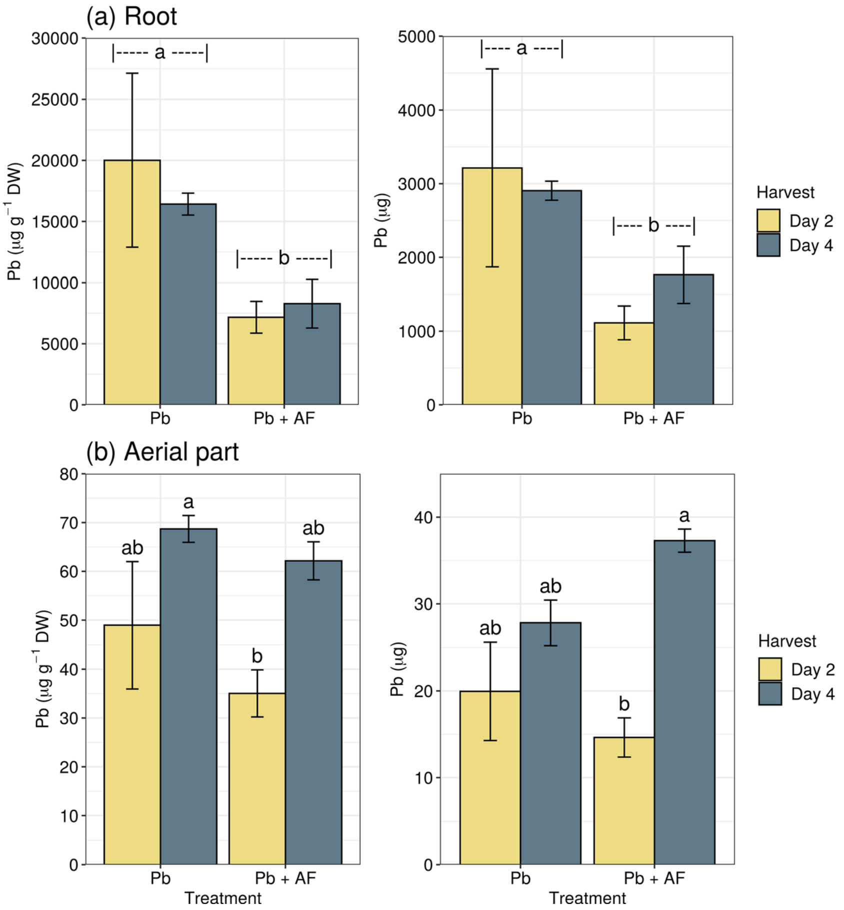

**Fig. 5** Pb concentration (left), and absolute Pb content per plant (right) (mean ± SE) corresponding to roots (**a**) and aerial organs (**b**) of *G. max* plants exposed to treatments with Pb [0.25 mM $Pb(NO_3)_2$] with and without air flow (AF) on two harvest days (2 or 4 days of exposure). Different letters indicate significant differences at $p < 0.05$

behavior could be explained either by a limitation in the absorption of the roots (saturation of Pb), or by a selective absorption of ions in the culture solution, with Pb ions being excluded. The latter assumption was supported by the final Pb concentration in the remaining solution, which was significantly higher in the treatments with air flow (Fig. 6). It is important to note that if the plants had absorbed the culture solutions equally, the volume would have decreased, with the concentrations in the solutions being the same. However, the Pb concentration remaining in the culture solution of the

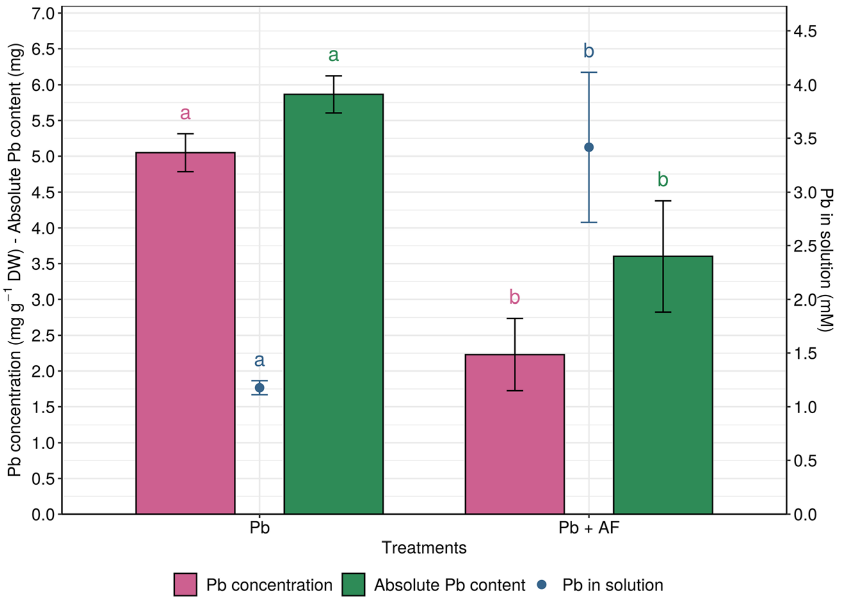

**Fig. 6** Mean ± SE of Pb concentration, absolute content in *G. max* plants, and concentration in the remaining culture solutions on the last harvest day. Pb, 0.25 mM $Pb(NO_3)_2$; Pb + AF, 0.25 mM $Pb(NO_3)_2$ + air flow. Different letters indicate significant differences at $p < 0.05$ for each variable

**Table 2** Mean ± SE of DW of roots, stems and leaves, LA and SLA measured in *G. max* plants exposed to different treatments (control, Pb with/without air flow) and exposure time (2 or 4 days)

| Treatment | Harvest (days) | Root (g) | Stem (g) | Leaf (g) | LA (cm$^2$) | SLA (cm$^2$ g$^{-1}$) |
|---|---|---|---|---|---|---|
| Control | 2 | 0.26 ± 0.01 b | 0.12 ± 0.01 c | 0.52 ± 0.05 b | 115.7 ± 10.8 | 452.39 ± 1.33 a |
| Control + AF | 2 | 0.43 ± 0.01 a | 0.18 ± 0.01 b | 0.66 ± 0.03 ab | 159.07 ± 7.3 | 460.88 ± 6.87 a |
| Pb | 2 | 0.32 ± 0.01 ab | 0.18 ± 0.01 b | 0.69 ± 0.02 ab | 135.2 ± 11.23 | 415.58 ± 15.6 ab |
| Pb + AF | 2 | 0.41 ± 0.06 a | 0.28 ± 0.02 a | 0.9 ± 0.13 a | 145.06 ± 9.24 | 453.27 ± 8.18 a |
| Control | 4 | 0.31 ± 0.02 ab | 0.16 ± 0.01 bc | 0.65 ± 0.07 ab | 138.3 ± 5.88 | 447.8 ± 17.93 a |
| Control + AF | 4 | 0.36 ± 0.03 ab | 0.19 ± 0 b | 0.62 ± 0.04 ab | 158.57 ± 17.14 | 366.62 ± 20.2 b |
| Pb | 4 | 0.31 ± 0.02 ab | 0.19 ± 0.01 b | 0.65 ± 0.05 ab | 117.38 ± 12.37 | 380.36 ± 13.74 b |
| Pb + AF | 4 | 0.42 ± 0.01 a | 0.29 ± 0.01 a | 0.92 ± 0.03 a | 161.06 ± 6.33 | 351.02 ± 11.61 b |

Different letters indicate significant differences at $p < 0.05$ in ANOVA

*Control* $NH_4NO_3$ 0.25 mM, *Pb* $Pb(NO_3)_2$ 0.25 mM, *AF* air flow, *DW* dry weight, *LA* leaf area, *SLA* specific leaf area

treatment without air flow was lower than the one with air flow, indicating a Pb exclusion mechanism.

### Morpho-physiological response of soybean

In presence of air flow, both control and Pb treatments showed an increase in the biomass of the aerial organs, mainly on the second harvest day (4 days) (Table 2). However, the biomass of roots remained constant among treatments. These results suggest that the plants would be consuming other nutrients of the solutions, with the consequent increase in the aerial biomass. However, in contrast to our results, Liao et al. (2006) did not observe an increase in aerial biomass of lettuce exposed to a high rate of transpiration, with values between 0.3 mM and 1 mM of $Pb(NO_3)_2$ in the culture solution.

On the other hand, the tolerance of soybean seedlings to Pb concentration (0.25 mM) is notable, since they did not show differences in biomass compared to the controls (results not shown). Regarding LA, a tendency to an increase in plants exposed to air flow was observed (Table 2). However, when the specific leaf area (SLA) was analyzed, considering the leaf DW, a decrease in all treatments was observed in the second harvest, except for control (Table 2). These results indicate a reduction of leaf thickness due to the effect of Pb and air flow, as reported in other studies involving plants exposed to high Cd levels (Nazarian et al. 2016; Yilmaz et al. 2009).

Results of biochemical parameters (MDA, FRAP, sugars, starch, proteins, chlorophylls, carotenoids, and RWC) are shown in Table 3. Significant differences among treatments were found only for starch, FRAP, MDA, and carotenoid concentrations. However, only FRAP increased as a consequence of the air flow, regardless of the treatment, and no clear results were observed for the remaining parameters. These results are mainly related to the fact that the seedlings excluded the incorporation of Pb when air flow was increased, as a protective response.

## Conclusions

Our findings indicate that the concentrations of the competitor ion $Ca^{2+}$ (1:1, Ca:Pb) were not high enough to reverse Pb

**Table 3** Biochemical parameters (mean ± SE) measured in leaves of *G. max* plants exposed to treatments with/without Pb and air flow, and exposure time (2 or 4 days)

| T | E | Starch (mg g$^{-1}$) | Protein (mg g$^{-1}$) | FRAP (μmol g$^{-1}$) | MDA (μmol g$^{-1}$) | Sugar (mg g$^{-1}$) | Chl_tot (mg g$^{-1}$) | Chl_a (mg g$^{-1}$) | Chl_b (mg g$^{-1}$) | Carotenoids (mg g$^{-1}$) | RWC (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 2 | 95.05 ± 31.11 ab | 11.10 ± 5.62 | 20.71 ± 4.65 abc | 361.02 ± 51.07 ab | 76.21 ± 12.36 | 9.22 ± 0.98 | 7.12 ± 0.70 | 0.85 ± 0.31 | 1.68 ± 0.30 a | 93.86 ± 0.94 |
| C + AF | 2 | 63.99 ± 42.59 b | 66.55 ± 18.61 | 39.48 ± 10.21 a | 376.25 ± 64.73 ab | 50.44 ± 9.81 | 10.37 ± 3.70 | 7.81 ± 2.66 | 1.26 ± 0.65 | 1.03 ± 0.28 ab | 90.35 ± 0.68 |
| Pb | 2 | 195.5 ± 41.49 ab | 92.97 ± 26.51 | 23.64 ± 6.32 abc | 342.08 ± 51.37 ab | 68.19 ± 19.62 | 7.42 ± 1.11 | 5.88 ± 0.78 | 0.61 ± 0.29 | 1.46 ± 0.28 ab | 96.01 ± 1.63 |
| Pb + AF | 2 | 322.28 ± 104.6 a | 66.56 ± 9.20 | 25.9 ± 8.28 ab | 243.8 ± 41.62 b | 48.35 ± 10.54 | 8.66 ± 2.02 | 6.37 ± 1.43 | 1.08 ± 0.43 | 1.03 ± 0.17 ab | 92.29 ± 0.72 |
| C | 4 | 367.11 ± 99.71 a | 65.6 ± 17.85 | 7.59 ± 1.27 c | 185.09 ± 16.53 b | 40.3 ± 3.59 | 5.23 ± 0.49 | 3.84 ± 0.37 | 0.69 ± 0.12 | 0.6 ± 0.06 b | 84.22 ± 4.02 |
| C + AF | 4 | 339.45 ± 67.79 a | 53.17 ± 3.85 | 30.53 ± 8.06 a | 498.21 ± 66.95 a | 95.44 ± 10.69 | 10.76 ± 2.67 | 8.23 ± 1.83 | 1.2 ± 0.55 | 1.3 ± 0.24 ab | 89.93 ± 1.26 |
| Pb | 4 | 426.59 ± 94.24 a | 85.66 ± 13.09 | 10.54 ± 1.8 bc | 261.15 ± 36.02 b | 44.68 ± 3.87 | 5.73 ± 0.67 | 4.22 ± 0.40 | 0.8 ± 0.20 | 0.69 ± 0.05 ab | 91.52 ± 1.80 |
| Pb + AF | 4 | 191.06 ± 37.18 b | 53.43 ± 6.32 | 43.7 ± 13.99 a | 380.03 ± 43.12 ab | 96.57 ± 14.92 | 9.42 ± 2.90 | 6.81 ± 1.93 | 1.42 ± 0.64 | 1.11 ± 0.27 ab | 91.62 ± 1.57 |

Different letters indicate significant differences at $p < 0.05$ in ANOVA

*T* treatment, *E* exposure time, *C* control $NH_4NO_3$ 0.25 mM, *Pb* $Pb(NO_3)_2$ 0.25 mM, *AF* air flow, *MDA* malondialdehyde, *FRAP* ferric-reducing antioxidant power, *RWC* relative water content, *Chl_tot* total chlorophylls, *Chl_a* chlorophyll a, *Chl_b* chlorophyll b accumulation in soybean seedlings over time, although the greatest accumulation was recorded in roots, with a minimal translocation to the aerial organs. This result is attributed to a decrease of the $Ca^{2+}$ ion in the culture solution over time, which is used by the plant, as opposed to the Pb ions, which have no essential function. This behavior was demonstrated by assessing morpho-physiological and biochemical parameters, which showed a protective effect of calcium at the beginning of exposure where the symptoms of physiological stress decreased but were reversed over time.

On the other hand, the alteration of the transpiration rate in soybean seedlings showed that at a higher air flow, the consumption of all the culture solutions increased, followed by an increase in biomass, regardless of the Pb treatment, whereas Pb accumulation in seedlings exposed to air flow was even lower. This indicates that soybean seedlings exposed to Pb and air flow selectively excluded the metal in the solution, absorbing only water and nutrients, which was also confirmed by the absence of physiological stress.

In summary, soybean seedlings showed to be highly tolerant to Pb; further research is necessary to elucidate the processes and factors that influence the translocation of toxic metals such as Pb from roots to the aerial organs.

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**Authors' contributions** All authors contributed to the study conception and design. AB, MJS, and JHR performed material preparation, data collection and analysis. JHR, MLP, and HRL performed the experimental design, and supervision of experiments. The first draft of the manuscript was written by AB and JHR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Compliance with ethical standards

**Competing interests** The authors declare that they have no competing interests.

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

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