Interactions between Lead on Nutrients (Ca$^{2+}$, K$^+$, N), and their Consequences on Growth in Sesuvium portulacastrum and Brassica juncea

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Abstract

Lead is one of the most toxic metals in the environment and causes drastic morphological and physiological deformities in plants. Growth restriction, chlorosis and necrosis are usually accompanied with a large disturbance of the uptake of essential elements. The aim of this work is to study the effects of lead on mineral nutrient acquisition (Ca$^{2+}$, K$^+$, N), and their consequences on growth in two different species: halophytic (Sesuvium portulacastrum) and glycophytic (Brassica juncea). Seedlings were grown for 21 days in split-root conditions. One half of the root system was immersed in complete nutrient solution supplemented with 400 µM Pb(NO$_3$)$_2$, and the other half was immersed in a Pb$^{2+}$-free medium, containing all nutrients or deprived of potassium or calcium or nitrogen. Using this approach, we demonstrated that lead interfere mainly with the absorption of Ca$^{2+}$ by the roots, thus limiting the growth of plants and their ability to accumulate this metal. We propose that the increase of Ca$^{2+}$ availability in soils could improve the growth of both species in the presence of lead. This would be essential for improving their utility for phytoremediation of this metal in contaminated soils.

Keywords: Halophytes; Glycophyte; Lead accumulation; Nutritional disturbance; Phytoremediation

Introduction

Soil pollution by metals has attracted considerable public attention in recent decades [1]. So, human activities have continuously increased the level of heavy metals circulating in the environment. This pollution by heavy metal in the biosphere has accelerated rapidly since the onset of the industrial revolution posing major environmental problems [2]. Lead contamination has resulted from mining, smelting activities, Pb-containing paints, gasoline, explosives, as well as from the disposal of municipal sewage sludge enriched in Pb [3,4]. The toxic effects on human populations and potential health hazard induced by the consumption of Pb-contaminated food have been extensively studied [5,6]. Results of these studies demonstrated that Pb could be implicated in several diseases such as liver and kidney alterations [7]. In the environment, heavy metals cannot be degraded neither by microbial nor by chemical process, and tend to accumulate in soils or to be transported by streaming water and contaminate surface water and ground water [8]. For these reasons, it would be interesting to develop techniques for heavy metal extraction from soils. Much research has been conducted on remediation of metal-contaminated soils by employing chemical and physical techniques [9,10]. However, environmentalists and governments do not encourage contaminated soils by employing chemical and physical techniques from soils. Much research has been conducted on remediation of metal-contaminated soils. In a previous study, we showed that the halophyte spece S. portulacastrum accumulated Pb contaminated nutrient solution, S. portulacastrum accumulated

main toxic effects of Pb include interference with other nutrients uptake and translocation [17], growth retardation [18], decrease in dry weight of different plant parts [19], disturbed respiration [20] and a decline in the total chlorophyll and photosynthetic activity [21-23]. Hence, it is known also, that lead reduces the uptake of essential macro and micronutrients. Ca, K, N, P, Mg, Fe and Zn absorption and translocation in several plant species are affected by the presence of Pb in the culture substrate leading to mineral deficiency in tissues [24,25]. This indirect toxic effect of Pb in plant could be associated to direct toxic effect governed by the interaction of ions Pb$^{2+}$ with several enzymes, metabolical processes and nucleic acids alteration [26]. Both direct and indirect effects are responsible to the global effect of lead on plant development such as chlorosis and growth reduction. The research of new plant species having a high potential of metals tolerance and accumulation is highly encouraged by botanists and environmentalists. In this context, it has been suggested that salt-tolerant plants would be better adapted to coping with environmental constraints, including heavy metals [27-31] than salt-sensitive (glycophytic) crop plants (Zea mays L., Brassica. juncea L., Pisum sativum L.) commonly chosen for metals phytoextraction studies. In a previous study, we showed that the halophyte spece S. portulacastrum is more tolerant to lead than the glycophyte one B. juncea. When cultivated under the same conditions on Pb contaminated nutrient solution, S. portulacastrum accumulated
Materials and Methods

Plant material and growth conditions

S. portulacastrum L. (Aizoaceae), a dicotyledonous halophyte commonly known as sea purslane, was propagated by cutting. Three cm long-stem segments with one node and two opposite leaves were taken from mother plants cultivated in greenhouse, on a mixture of sandy soil and organic matter, and irrigated with tap water. Cuttings were disinfected for 5 min in saturated calcium hypochlorite solution, thoroughly washed with distilled water, and placed for 7 days in an aerated solution diluted 10 times, supplemented with Fe EDTA and micronutrients [32,33]. Rhizogenesis took place after 1 week. Seeds of B. juncea L. (Acc PI 173874) were kindly provided by the North Central Regional Plant Introduction Station (NCRPIS-USDA-USA). They were sterilized in a 10% H2O2 solution during 20 min, washed with distilled water, and placed for 7 days in an aerated solution supplemented with Fe EDTA; pH was adjusted to pH 4.8 adjusted with HCl. For split-root experiments, each plant was placed between two 750 mL plastic containers filled with aerated solutions, with one half of the root system plunging in each container. Split-root experiment aimed at determining the implication of Pb2+ on K+, Ca2+ and N acquisition by plants with filter-paper. The fresh weight (FW) was measured immediately, and the dry weight (DW) after 48 h of desiccation in an oven at 60°C.

Water content

The tissue water content (TWC) was determined as TWC (ml g-1 DW) = (FW – DW)/DW.

Cations concentration

Dried samples (ca. 100 mg) were grounded to a fine powder using a porcelain mortar and a pestle and digested in 4:1 (v/v) HNO3/HClO4 (20 ml) mixture at 100°C. After total evaporation, 30 ml of HNO3 0.5% was added and Pb2+ and Ca2+ concentrations were determined by atomic absorption spectrometry (Spectra AA 220 FS). Potassium and nitrogen contents were determined in the same homogenate by flame spectrometry (Corning photometer). Reduced nitrogen was measured according to Kjeldahl method.

Analysis of results

For the period between the initial and final harvests the following indexes were calculated.

The relative growth rate (RGR) based on whole-plant dry weight production, as RGR = ln(Wi) – ln(Wf)/(t2 – t1), where Wf and Wi were the dry matter at the beginning and the end of the treatment period and (t2 – t1) was the duration of the period [36].

Statistical analysis

Analyses of variance (ANOVA) with orthogonal contrasts and mean comparison procedures were used to detect differences between treatments. Mean separation procedures were conducted using the multiple range tests with Fisher’s least significant difference (LSD) (P<0.05).

Results

Plant morphology and growth

After 8 days of treatment, chlorosis was visible in the young leaves of Pb/Pb treated plants of B. juncea. One week later, chlorosis increased and necrosis appeared in oldest leaves, with a subsequent falling of these senescing leaves. In contrast, S. portulacastrum plant morphology was not significantly modified in the presence of Pb2+ (Pb/Pb). Both species almost produced similar biomasses in the absence of Pb2+ (Figure 2a). However, Pb/Pb treatment reduced the biomass production as compared to control B/B (treatment). So, this effect is more pronounced in B. juncea than in S. portulacastrum. Nevertheless, lead significantly reduced the dry weight of Pb/Pb treated plants of B. juncea, this reduction reaches 69% compared to control (T/T) but does not exceed 35% in S. portulacastrum. With the exception of (B-Ca)/Pb, all the other treatments: B/Pb, (B-K)/Pb, and (B-N)/Pb, produced plants without visual toxicity symptom on their leaves, and biomass comparable to that of control plants. For both species, (B-Ca)/Pb plants showed reduction of dry weight plants, without visual toxicity symptoms compared to the control plants. The change in the growth

Figure 1: Schematic representation of different treatment used in this experiment. B: Basal medium; Pb: basal medium supplied with 400 µM Pb(NO3)2; B-K: B-Ca and B-N: basal medium deprived of K+; Ca2+ or N, respectively. For each treatment, eight replicates corresponding to single plants are used for both species.

Figure 2: Effects of Pb2+ on biomass production and tissue cation content in B. juncea and S. portulacastrum. Each value is the mean ± SD of eight replicates. Significant differences (P < 0.05) are indicated by different letters. 

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of different parts (roots and aerial parts) showed the same aspects as that of the whole plant (Figure 2b and 2c). Figure 2c showed that only the Pb/Pb and (B-Ca)/Pb treatments significantly limited the root system growth and this effect is more pronounced in *B. juncea* than *S. portulacastrum*. Furthermore, the roots were less sensitive to Pb$^{2+}$ than the shoots. Thus, in Pb/Pb treatment the root-biomass was reduced by 30% and 50% of the control respectively in *S. portulacastrum* and *B. juncea*, whereas in the shoots, this decrease was 41% and 70%. Table 1 compares the relative growth rate (RGR) of *S. portulacastrum* and *B. juncea* species. In unstressed conditions, the perennial halophyte species have a low RGR (0.06 day$^{-1}$) as compared with *B. juncea* (0.12 day$^{-1}$). For both species, the RGR values in the Pb/Pb and (B-Ca)/Pb treatments significantly reduced and this effect are more pronounced in *B. juncea* than *S. portulacastrum*. Therefore, in Pb/Pb treatment the RGR value was reduced by 33% and 50% of the control respectively in *S. portulacastrum* and *B. juncea*, whereas in the shoots, this decrease was 17% and 41%. For all the other treatments: B/Pb, (B-K)/Pb, and (B-N)/Pb, the RGR values does not show a significant difference compared to that of control plants and it even remains unchangeable for *S. portulacastrum*. For both species, lead significantly reduced the shoot
water content of Pb/Pb and (B-Ca)/Pb treated plants, but this reduction is more significant in B. juncea than in S. portulacastrum (Figure 3). For Pb/Pb treated plants, this reduction reaches 57% compared to control (T/T) but does not exceed 30% in S. portulacastrum. The shoot water content for all the other treatments: B/Pb, (B-K)/Pb, and (B-N)/Pb, does not show a difference compared to the control (T/T).

Lead accumulation

Figure 4 showed that S. portulacastrum accumulated significantly more Pb²⁺ in the shoot than B. juncea. For all treatments, lead concentration in shoots was significantly higher in S. portulacastrum than in B. juncea (Figure 4). Hence, the concentration of sequestered Pb²⁺ in shoots of Pb/Pb plants were 1623 and 720 µg g⁻¹ DW for S. portulacastrum and B. juncea respectively (Figure 4). For both species, the Pb/Pb treated have the higher Pb²⁺ concentration in their shoots than other treatments (B/Pb, (B-N)/Pb, (B-Ca)/Pb and (B-K)/Pb). B/Pb, (B-N)/Pb, (B-Ca)/Pb and (B-K)/Pb Pb S. portulacastrum plants showed a similar Pb²⁺ concentration in their shoots. (B-Ca)/Pb and Pb/Pb B. juncea plants accumulated significantly more Pb²⁺ in the shoots as compared to B/Pb, (B-N)/Pb and (B-K)/Pb plants. The phytoextraction potential of plants is estimated by the determination of the total amounts of metals accumulated in the shoots which represents the product of shoot biomass by its metal concentration. This parameter given in Table 2, demonstrates that S. portulacastrum extracted more Pb²⁺ than B. juncea. However, the Pb²⁺ amounts differed between the treatments within species: for S. portulacastrum, the highest extraction was obtained with Pb/Pb treatment. However, in B. juncea, B/Pb, (B-K)/Pb and (B-N)/Pb plants extracted more Pb²⁺ as compared to Pb/Pb and B-Ca/Pb. The reduction of shoot amount of Pb²⁺ accumulated in the plants (Pb/Pb and (B-Ca)/Pb), in spite of the higher Pb²⁺ concentrations, was essentially the consequence of the low shoot biomass production showed in these plants under the effect of lead.

Calcium nutrition

Independently of treatments, the glycophyte species B. juncea showed higher Ca²⁺ tissue concentrations than S. portulacastrum. So, in both species, Ca²⁺ concentrations in the shoots of Pb/Pb plants decreased that of control (B/B) plants (Figure 5a), but this effect is more distinct in B. juncea than in S. portulacastrum. B/Pb, (B-K)/Pb and (B-N)/Pb treatments did not affect Ca²⁺ status. However, in (B-Ca)/Pb plants, Ca²⁺ concentrations were reduced in shoot tissues, this reduction reaches 51% and 29% of the control in B. juncea and S. portulacastrum respectively. In addition, for both treatments, Pb/Pb and (B-Ca)/Pb the reduction in Ca²⁺ shoot concentrations was accompanied by a growth inhibition, suggesting that Pb²⁺ impaired Ca²⁺ uptake by the roots. The shoot Ca²⁺ amounts were strongly diminished in plants subjected to Pb/Pb and (B-Ca)/Pb in both species. However they remained unchanged in the shoots of other treated plants as compared to control plants (B/B) (Table 1). Hence, Ca²⁺ uptake in B/Pb, (B-K)/Pb and (B-N)/Pb/plants was essentially assured by the part of root system developed in free Pb²⁺ basal medium containing Ca²⁺.

Potassium nutrition

For both species, the lowest shoot K⁺ concentrations were observed in (B-K)/Pb and Pb/Pb plants (Figure 5b), this effect is more pronounced in B. juncea than in S. portulacastrum. This data suggest that Pb²⁺ disturbed K⁺ uptake by the roots. Indeed, when a part of the root system was maintained in a medium containing K⁺ and free of Pb²⁺ (B/ Pb, (B-Ca)/Pb and (B-N)/Pb), K⁺ shoot concentration was not modified as compared to control (B/B). The amounts of K⁺ accumulated in the shoots of B. juncea, were decreased significantly in Pb/Pb (Table 1) as compared to S. portulacastrum. For both species, plants subjected to Pb/Pb and (B-Ca)/Pb accumulated the lowest amount of K⁺. These results were deeply related to a decrease in biomass comitant to reduced K⁺ concentration in the shoots in Pb/Pb plants (Figure 2b and 5b). However in (B-Ca)/Pb plants of both species, the reduction of K⁺
shoot-amounts was essentially due to the decrease in the shoot biomass production.

Nitrogen nutrition

In both species, the reduced nitrogen concentration in the shoots was not modified by different treatments (Figure 5c). In the other hand, (B-N/Pb) plants accumulated more reduced N than Pb/Pb plants (Table 1) in spite of the similar shoot N concentration showed in these plants. This difference in N amounts was essentially due to the elevated biomass production in (B-N)/Pb plants. These data suggest that in (B-N)/Pb plants, the half of root system developed in medium containing Pb2+ is able to absorb NO3-. So, in Pb/Pb plants, growth was not limited through the effect of Pb2+ on N uptake by the roots.

Discussion

Depending on their concentration in the environment, heavy metals, including micronutrients like Zn, Ni, Co, Cu necessary for plant growth and those such as Pb, Cd or Hg, for which no function in living organisms has yet been demonstrated, exert a toxic influence on plant metabolism. So, when present in excess within plant tissues, lead interferes with proper enzymatic functions and inhibits overall plant growth [16,30]. However, interspecific variability was showed in plants responses to this metal [37,38]. Based in a previous study, we showed that the halophyte species S. portulacastrum is more tolerant and accumulated more lead in the shoot than glycophyte species B. juncea [30,31]. However, this accumulation was accompanied by a growth reduction and mineral nutrition disturbances especially in B. juncea. The present study aims at determining whether Pb2+ limits the growth of these species through impairment of the acquisition of some essential nutrients (Ca2+, K+, N) or through toxic effect related to an excessive metal accumulation in the shoots. Plant growth upon controlled conditions showed no statistical difference between the two tested species. However, Pb/Pb treatment induced growth inhibition particularly in B. juncea. On the other hand, for both species, (B/Pb), (B-K)/Pb, and (B-N)/Pb plants showed no visual toxicity symptom and maintained normal growth in spite of the relatively high Pb2+-concentration in their shoots. In addition (B/Pb), (B-K)/Pb and (B-N)/Pb plants do not show a significant difference in biomass production compared to that of control plants (B/B). Thus, large accumulation of lead in the shoots of both species is compatible with high growth when a part of the roots was maintained in the medium free of Pb2+. We suggest that the growth reduction induced by Pb2+ in Pb/Pb plants was partially due to Pb2+-induced nutritional disturbances, as has been described in different plant species [10,39,40]. In addition the (Pb/Pb) and (B-Ca)/Pb plants showed a significant reduction of their biomass production compared to that other treatments. We hypothesize therefore, that Pb2+ inhibited growth essentially through limitation of Ca2+ uptake by roots and/or transport to shoots. The total dry matter measured at the final harvest depended on the initial size of the plant (before the beginning of treatments) and on its growth during the treatment. Relative growth rate (RGR) is a recommended parameter to evaluate the specific effect of the constraints on the growth activity during the period of treatment [36]. Indeed, the rate of biomass production (RGR) was strongly correlated with the amount of Ca2+ accumulated in the shoots (Figure 6). This correlation presents two contrasting behaviors: Pb/Pb and (B-Ca)/Pb plants had the lowest RGR values and Ca2+ accumulation in their shoots, and the other plants [(B/B), (B/Pb), (B-K)/Pb and (B-N)/Pb] presented normal growth activity concomitantly with seemingly adequate Ca2+ amounts in shoots. The similar behavior of Pb/Pb and (B-Ca)/Pb plants suggests that the restriction of Ca2+ uptake in the presence of lead is an important factor that limiting plant growth. Based on the RGR values, Table 1 showed in the Pb/Pb and (B-Ca)/Pb treatments significantly reduced and this effect is more pronounced in B. juncea than S. portulacastrum. For all the other treatments: B/Pb, (B-K)/Pb, and (B-N)/Pb, the RGR values does not show a significant difference compared to that of control plants and it even remains unchanged for S. portulacastrum in spite of a large shoot Pb2+ accumulation. These data demonstrated that the halophyte species exhibited a higher tolerance to accumulated toxic ions as compared to B. juncea which have nevertheless been frequently used for metal phytoextraction [37,41]. Thus, due to the importance of Ca in many aspects of plant cell biology [42], tolerance to low Ca conditions could therefore represent an important aspect of tolerance to lead in plants, as is the case in the higher plant species [25,43]. Restriction of growth concomitant to a perturbation of Ca2+ uptake induced by lead has been reported in several plant species, such as rye, maize, tomato and mustard varieties [25] and could result from the inhibition of Ca transporters by toxic lead ions [43,44] and/or replacement of Ca ions with Pb ions due to the high affinity of the latter for Ca binding-sites on biological structures [42,45]. Numerous studies report an ameliorating effect of calcium on heavy-metal toxicity [46,47].

Figure 6: Relationship between the amounts of Ca2+ accumulated in the shoots (µg plant-1) and the relative growth rate (RGR. d-1). B: Basal medium; Pb basal medium supplied with 400 µM Pb(NO3)2; B-K; B-Ca and B-N: basal medium deprived of K+; Ca2+ or N, respectively. Means of eight replicates.

Table 1: Variations of the RGR values in the shoots of S. portulacastrum and B. juncea grown for 21 days in various split-root configurations. B: Basal medium; Pb basal medium supplied with 400 µM Pb(NO3)2; B-K; B-Ca and B-N: basal medium deprived of K+; Ca2+ or N, respectively.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B. juncea</th>
<th>S. portulacastrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/B</td>
<td>0.12 ± 0.017 (d)</td>
<td>0.06 ± 0.008 (b)</td>
</tr>
<tr>
<td>B/Pb</td>
<td>0.11 ± 0.015 (d)</td>
<td>0.06 ± 0.008 (b)</td>
</tr>
<tr>
<td>Pb/Pb</td>
<td>0.06 ± 0.008 (b)</td>
<td>0.04 ± 0.005 (a)</td>
</tr>
<tr>
<td>(B-K)/B</td>
<td>0.11 ± 0.015 (d)</td>
<td>0.06 ± 0.008 (b)</td>
</tr>
<tr>
<td>(B-N)/B</td>
<td>0.11 ± 0.015 (d)</td>
<td>0.06 ± 0.008 (b)</td>
</tr>
<tr>
<td>(B-Ca)/B</td>
<td>0.07 ± 0.008 (bc)</td>
<td>0.05 ± 0.007 (ab)</td>
</tr>
</tbody>
</table>
Table 2: Pb, Ca²⁺, K⁺ (µg plant⁻¹), and reduced nitrogen (mg plant⁻¹) contents in the shoots of S. portulacastrum and B. juncea as affected by the different treatments. B: Basal medium; B-Pb basal medium supplied with 400 μM Pb(NO₃)₂; B-K: B-Ca and B-N: basal medium deprived of K⁺; Ca²⁺ or N, respectively.

<table>
<thead>
<tr>
<th>Element</th>
<th>S. portulacastrum</th>
<th>B/Pb</th>
<th>B/Pb/Pb</th>
<th>(B-K)/B</th>
<th>(B-N)/B</th>
<th>(B-Ca)/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb²⁺</td>
<td>11.72 ± 1.67a</td>
<td>480.62 ± 68.66d</td>
<td>425.07 ± 60.72d</td>
<td>427.57 ± 61.08d</td>
<td>419.43 ± 60d</td>
<td>402.23 ± 57.46d</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.148 ± 0.021b</td>
<td>0.043 ± 0.0061a</td>
<td>0.150 ± 0.021b</td>
<td>0.152 ± 0.021b</td>
<td>0.161 ± 0.023b</td>
<td>0.070 ± 0.007a</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.460 ± 0.065e</td>
<td>0.180 ± 0.025b</td>
<td>0.359 ± 0.051d</td>
<td>0.316 ± 0.048d</td>
<td>0.380 ± 0.054de</td>
<td>0.241 ± 0.034c</td>
</tr>
<tr>
<td>N</td>
<td>27.17 ± 3.88 b</td>
<td>10.74 ± 1.53a</td>
<td>23.04 ± 3.29b</td>
<td>25.63 ± 3.66b</td>
<td>22.48 ± 3.21b</td>
<td>15.56 ± 2.22ab</td>
</tr>
</tbody>
</table>

B. juncea

<table>
<thead>
<tr>
<th>Element</th>
<th>B/Pb</th>
<th>B/Pb/Pb</th>
<th>(B-K)/B</th>
<th>(B-N)/B</th>
<th>(B-Ca)/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb²⁺</td>
<td>22.88 ± 3.26a</td>
<td>141.81 ± 20.25b</td>
<td>217.49 ± 31.07d</td>
<td>214.78 ± 30.68c</td>
<td>224.64 ± 32.09c</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.673 ± 0.096c</td>
<td>0.103 ± 0.014ab</td>
<td>0.591 ± 0.084c</td>
<td>0.547 ± 0.078c</td>
<td>0.569 ± 0.081c</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.563 ± 0.084f</td>
<td>0.087 ± 0.012 a ±</td>
<td>0.493 ± 0.070e</td>
<td>0.218 ± 0.031c</td>
<td>0.435 ± 0.062e</td>
</tr>
<tr>
<td>N</td>
<td>65.95 ± 9.42e</td>
<td>13.51 ± 1.93a</td>
<td>50.671 ± 7.23d</td>
<td>45.70 ± 6.52c</td>
<td>41.26 ± 5.89 c</td>
</tr>
</tbody>
</table>

For example, a higher calcium concentration in a medium was also reported to abolish the toxic effects of both Cd⁺² [48,49] and Pb⁺² [50] on the activity of photosystem II. In addition, high Ca-status and a high level of tolerance to Ca-deficit accompanied enhanced Zn, Pb, Cu and Al tolerance [51-53]. Such a result points to new, interesting possibility, that calcium plays a role in the regulation of lead detoxification by influencing the formation of lead containing precipitates in cell walls. Antosiewicz and Hennig [44], suggest that in the physiological range of Ca⁺ concentrations LCT1 could contribute to regulating the activity of the plant cadmium and lead detoxification system. Numerous authors have described the phenomenon of calcium mitigating heavy metal toxicity [47-49]. Pb2+ has no chemical similarity with Pb²⁺. 

For all treatments, lead concentration in shoots was significantly higher in Pb/Pb treated have the higher Pb ²⁺ concentration in their shoots than other treatments (Table 2). This behavior is explained by the production of biomass amount deposited in the shoots, which is the product of shoot biomass by shoot Pb²⁺ concentration. Based in this parameter, we demonstrate that S. portulacastrum extracted more Pb²⁺ than B. juncea. However, the Pb²⁺ amounts differed between the treatments within species: for S. portulacastrum, the highest extraction was obtained with Pb/Pb treatment. Nevertheless, in B. juncea, B/Pb, (B-K)/Pb and (B-N)/Pb plants extracted more Pb²⁺ as compared to Pb/Pb and (B-Ca)/Pb plants (Table 2). This behavior is explained by the production of biomass concomitant with a relatively elevated Pb⁺² concentration in the shoots. The reduced biomass showed especially in B. juncea, (B-Ca)/Pb plants limited significantly their phytoextraction capacity in spite of the high Pb⁺² concentration in shoots. The disturbances of Ca⁺ uptake induced by lead contributed largely to the growth restriction and therefore reduced Pb⁺²-phytoextraction capacities in both species mainly in B. juncea.

Conclusion

Our results showed that the restriction of growth in plants grown in the presence of 400 μM Pb(NO₃)₂, related to nutritional disturbances rather than to toxic effects of lead. So, the reduction of nutrient uptake by Pb²⁺ contributes largely to the growth restriction. Neither K⁺ nor N seem to be limiting for growth when absorbed from Pb⁺²-contaminated solution, but the restriction of Ca⁺ uptake by lead is an important factor of growth reduction, which limits Pb-phytoextraction capacities of these species. We propose that the increase of Ca⁺² availability in soils could improve the growth of both species especially for B. juncea that is more sensitive than S. portulacastrum in the presence of lead. This would be essential for improving their utility for phytoremediation.
of this metal in contaminated soils. So, the supply of this nutrient might be cheaper, more feasible and more efficient than the addition of synthetic chelators, presenting risk of negative effects on plant growth.

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