Research Article

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$_2$)$_3$, 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 implied in several diseases such 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 their use because they are expensive, soils disturbing and applicable only to small areas. More recently, increasing attention has been given to the development of a plant-based technology (phytoremediation) to remediate heavy metal contaminated soils [11,12]. In the phytoremediation process, several sequential crops of selected plant species can be cultivated to reduce the concentrations of heavy metals in upper layers of contaminated soils to environmentally acceptable levels [13,14]. After a period of metals accumulation in shoots, the metal-rich plant material may be safely harvested and removed from the site without extensive excavation and then incinerated or stocked in controlled areas [15,16]. However, due to the high toxicity to plants, rare are the species able to accumulate largely these pollutants. Hence, Pb which considered as non essential metal for plants is known to cause adverse physiological and biochemical deleterious effects. The

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, metabolic processes and nucleic acids alteration [26]. Both direct and indirect effects are responsible to the global effect of lead on plant development such 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 specie *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

more lead in the shoot than *B. juncea*. Hence, the amounts of Pb²⁺ translocated at 1000 μM Pb²⁺ were 3400 μg g⁻¹ DW and 2200 μg g⁻¹ DW in *S. portulacastrum* and *B. juncea*, respectively [30]. However, this accumulation was accompanied by a growth reduction and mineral nutrition disturbances essentially in *B. juncea*. This study aims at determining whether Pb²⁺ limits the growth of these species through impairment of the acquisition of some essential nutrients (Ca²⁺, K⁺ and N) or through toxic effect related to an excessive metal accumulation in the shoots. Thus, we conducted a split root experiment to separate the two factors.

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 5min 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-USDAUSA). They were sterilized in a 10% H_2O_2 solution during 20 min, washed with distilled water, sown on perlite imbibed with distilled water and incubated in the dark at 25°C for 5 days. The rooted cuttings (*S. portulacastrum*) and the seedlings (*B. juncea*) were transferred for 21 days to aerated Hoagland's nutrient solution [34] containing different treatments. The Hoagland's solution consisted of 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50 μM H_3BO_3, 1 mM $MgSO_4$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.1 mM $(NH_4)_6Mo_7O_{24}$ and 10 μM 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 nutritional disruptions in growth inhibition under Pb²⁺ stress, and to compare the effects of Pb²⁺ on K⁺, Ca²⁺ and N acquisition by plants and their corresponding consequences on growth. Six treatments were applied (Figure 1). In the first and second treatments, the two halves of root system were immersed either in basal medium free of Pb²⁺ (B/B) or in basal medium added with 400 μM $Pb(NO_3)_2$(Pb/Pb). In the third treatment, one half of the roots was immersed in basal medium, and the other half in the same medium supplemented with 400 μM $Pb(NO_3)_2$ (B/Pb treatment). For the three other treatments, one half of the root system was immersed in basal medium supplemented with 400 μM $Pb(NO_3)_2$ and the other part in basal medium deprived of potassium ((B-K)/Pb) or of calcium ((B-Ca)/Pb) or of nitrogen ((B-N)/Pb).

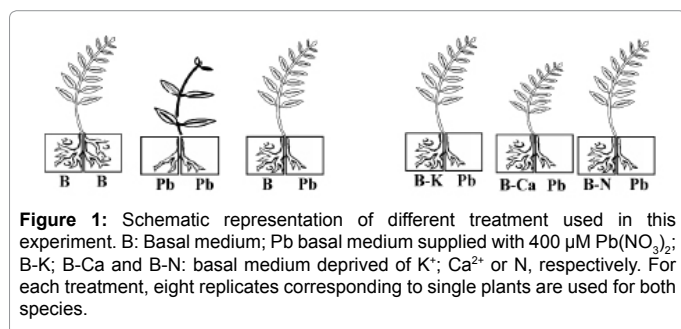**Figure 1:** Schematic representation of different treatment used in this experiment. B: Basal medium; Pb basal medium supplied with 400 μM $Pb(NO_3)_2$; B-K; B-Ca and B-N: basal medium deprived of K⁺; Ca²⁺ or N, respectively. For each treatment, eight replicates corresponding to single plants are used for both species.

The culture solutions were renewed every 3 days. Eight plants grown individually were used for each treatment. Two harvests were made, at the beginning of treatment and 21 days later. At the harvests, shoots and roots developed in free-Pb²⁺ medium were successively rinsed three times in cold water and blotted between two layers of filter-paper. Treated roots were dipped in a 0.01M HCl cold solution to eliminate external heavy metal adsorbed at the root surface according to Aldrich et al. [35] then rinsed three times with cold distilled water and blotted 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 (c.a. 100 mg) were grounded to a fine powder using a porcelain mortar and a pestle and digested in 4/1 (v/v) $HNO_3/HClO_4$ (20 ml) mixture at 100°C. After total evaporation, 30 ml of HNO_3 0.5% were 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= $\ln W_2 - \ln W_1/(t_2 - t_1)$, where W_1 and W_2 were the dry matter at the beginning and the end of the treatment period and ($t_2 - t_1$) 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
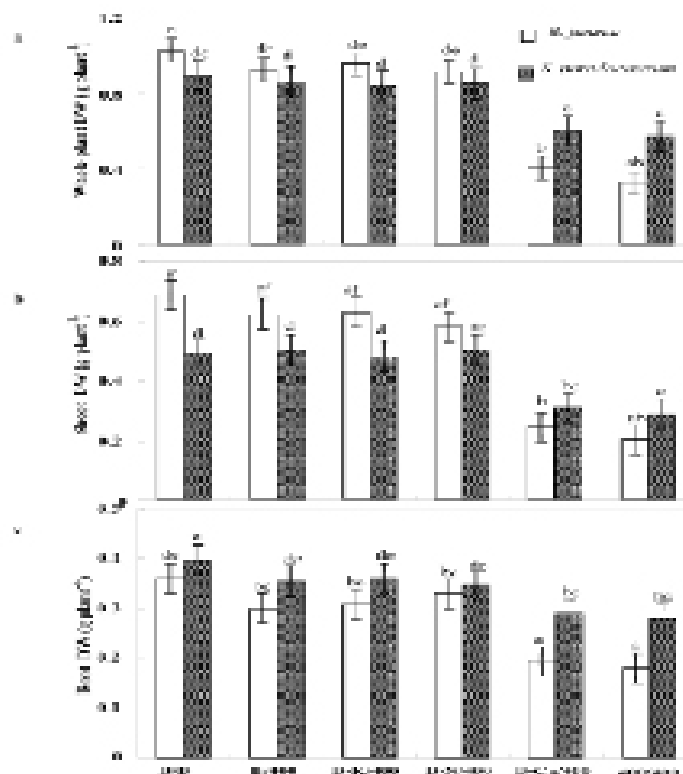
Figure 2: Changes in (a) whole plant, (b) shoot-biomass and (c) root-biomass in plants grown for 21 days in a split-root system. B/B: whole root system in basal medium; Pb/Pb: whole root system in basal medium supplemented with 400 µM Pb(NO$_3$)$_2$; B-K; B-Ca and B-N: basal medium deprived of K^{+}; Ca^{2+} or N, respectively. Means of eight replicates. Bars marked with same letter are not significantly different at *P = 0.05*.
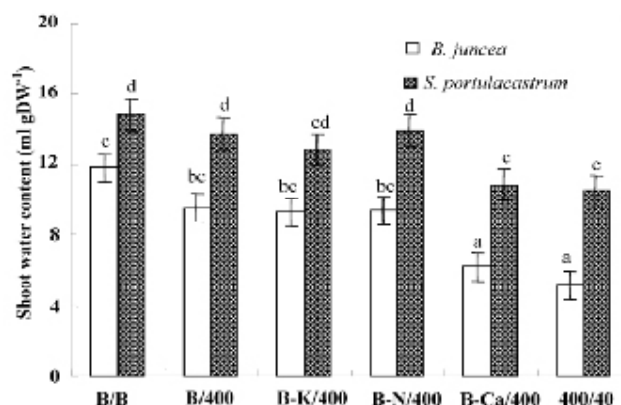
Figure 3: Variation of shoot water content in leaves of *S. portulacastrum* and *B. juncea* treated during 21 days in a split-root system. B/B: whole root system in basal medium; Pb/Pb: whole root system in basal medium supplemented with 400 µM Pb(NO$_3$)$_2$; B-K; B-Ca and B-N: basal medium deprived of K^{+}; Ca^{2+} or N, respectively. Means of eight replicates. Bars marked with same letter are not significantly different at *P = 0.05*.

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*, and for (B-Ca)/Pb treatment, 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

Figure 4: Changes in Pb²⁺ concentration (µg g⁻¹ DW) in shoots of *S. portulacastrum* and *B. juncea* grown for 21 days in various split-root system. B/B: whole root system in basal medium; Pb/Pb: whole root system in basal medium supplemented 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. Bars marked with same letter are not significantly different at *P = 0.05*.
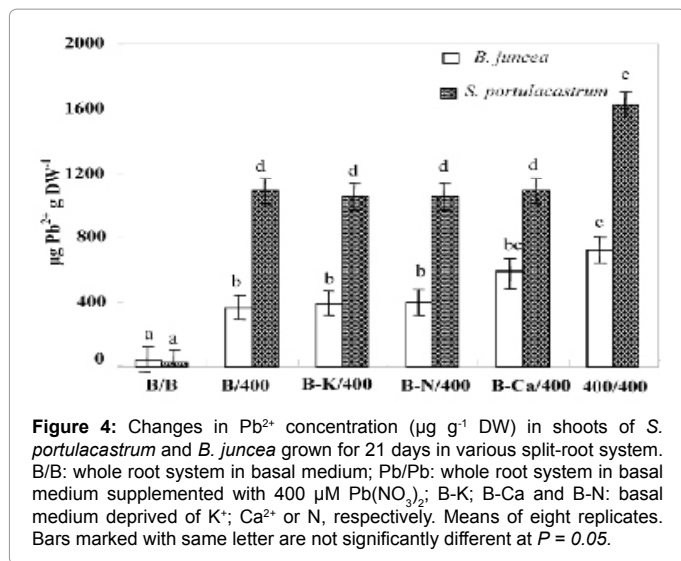

Figure 5: Changes in nitrogen and mineral composition of shoots of *S. portulacastrum* and *B. juncea* grown for 21 days in various split-root configurations. (a) Ca²⁺ (µmol g⁻¹ DW), (b) K⁺ (mmol g⁻¹ DW), (c) reduced N (µmol g⁻¹ DW). B: Basal medium; Pb basal medium supplied with 400 µM Pb(NO₃)₂; B-K; B-Ca and B-N: basal medium deprived of K⁺; Ca²⁺ or N, respectively. Means of eight replicates. Bars marked with same letter are not significantly different at *p = 0.05*.

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).
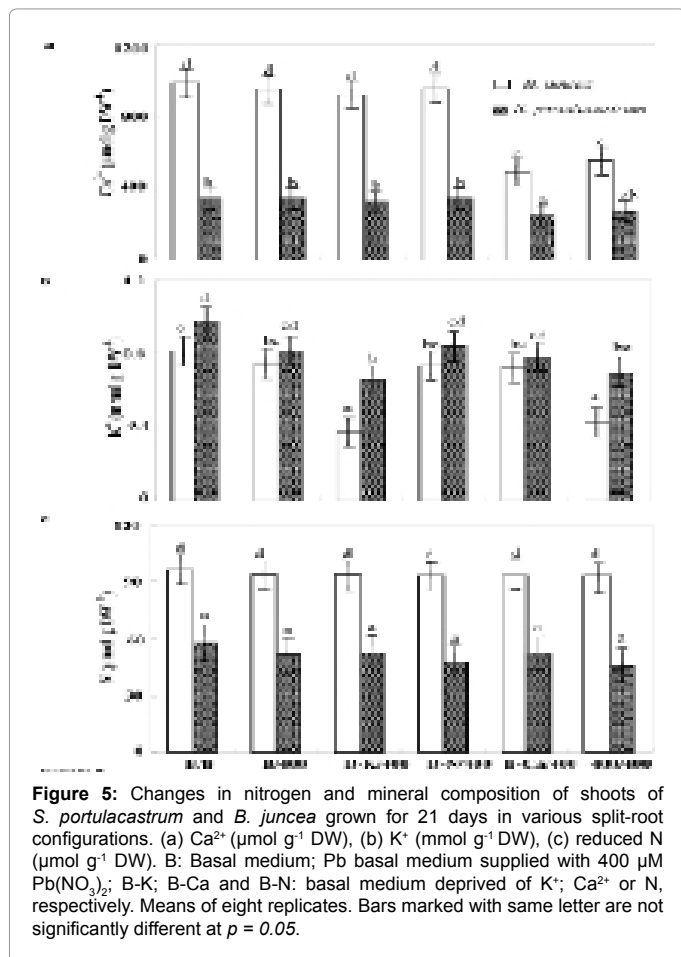
Lead accumulation

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 *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 concomitant 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

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.

Treatments	*B. juncea*	*S. portulacastrum*
B/B	0.12 ± 0.017 (d)	0.0 6 ± 0.008 (b)
B/Pb	0.11 ± 0.015 (d)	0.06 ± 0.008 (b)
Pb/Pb	0.06 ± 0.008 (b)	0.04 ± 0.005 (a)
(B-K)/B	0.11 ± 0.015 (d)	0.06 ± 0.008 (b)
(B-N)/B	0.11 ± 0.015 (d)	0.06 ± 0.008 (b)
(B-Ca)/B	0.07 ± 0.008 (bc)	0.05 ± 0.007 (ab)

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.

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 specie *S. portulacastrum* is more tolerant and accumulated more lead in the shoot than glycophyte specie *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⁺ and 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 does 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 unchangeable 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].
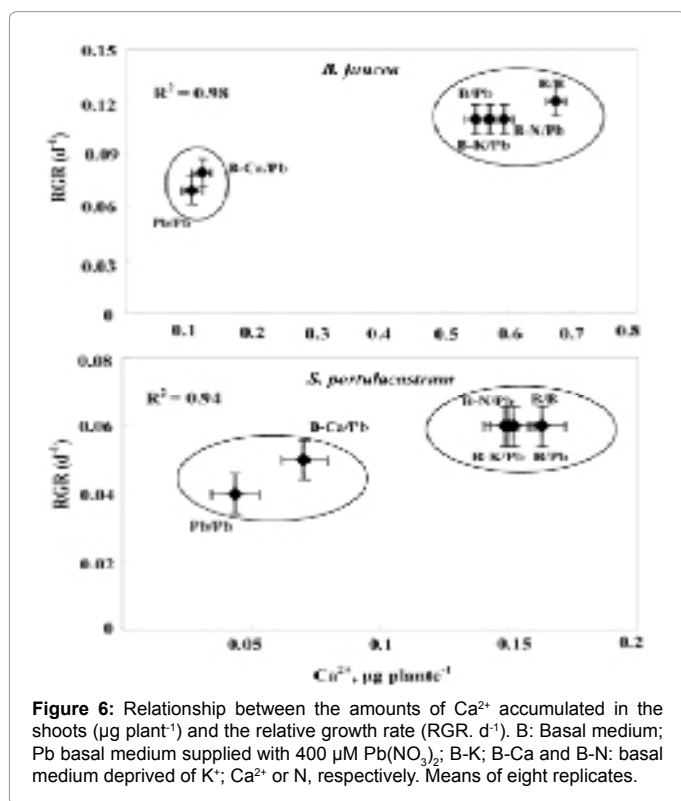
Split-root configuration						
Element	*S. portulacastrum*					
	B/B	Pb/Pb	B/Pb	(B-K)/B	(B-N)/B	(B-Ca)/B
Pb²⁺	11.72 ± 1.67a	480.62 ± 68.66e	425.07 ± 60.72d	427.57 ± 61.08d	419.43 ± 60d	402.23 ± 57.46d
Ca²⁺	0.148 ± 0.021b	0.043 ± 0.0061a	0.150 ± 0.021b	0.152 ± 0.021b	0.161 ± 0.023b	0.070 ± 0.007a
K⁺	0.460 ± 0.065e	0.180 ± 0.025b	0.359 ± 0.051d	0.316 ± 0.048d	0.380 ± 0.054de	0.241 ± 0.034c
N	27.17 ± 3.88 b	10.74 ± 1.53a	23.04 ± 3.29b	25.63 ± 3.66b	22.48 ± 3.21b	15.56 ± 2.22ab
	B. juncea					
Pb²⁺	22.88 ± 3.26a	141.81 ± 20.25b	217.49 ± 31.07d	214.78 ± 30.68c	224.64 ± 32.09c	107.28 ± 15.32b
Ca²⁺	0.673 ± 0.096c	0.103 ± 0.014ab	0.591 ± 0.084c	0.547 ± 0.078c	0.569 ± 0.081c	0.118 ± 0.016ab
K⁺	0.563 ± 0.084f	0.087 ± 0.012 a ±	0.493 ± 0.070e	0.218 ± 0.031c	0.435 ± 0.062e	0.165 ± 0.023b
N	65.95 ± 9.42e	13.51 ± 1.93a	50.671 ± 7.23d	45.70 ± 6.52cd	41.26 ± 5.89 c	17.76 ± 2.5ab

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; 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 example, a higher calcium concentration in a medium was also reported to abolish the toxic effects of both Cd²⁺ [48,49] and Pb²⁺ [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 Ca²⁺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 [47-49,54]. It is known that calcium is involved in the regulation/control of the secretion activity of Golgi apparatus vesicle movement and their fusion with the plasmalemma [55]. Since lead is to be extruded by Golgi vesicles out of the symplast to the cell wall, the theoretical hypothesis could be forwarded that under low calcium this process could be disturbed, which might cause less efficient lead immobilisation in the cell wall. In turn, this could lead to the formation of smaller precipitates and effectively to higher Pb²⁺ toxicity. Consequently, based on the study of Behling et al. [56] indicating higher level of soluble calcium in all parts of plants of the Ca-efficient tomato line relative to the Ca-inefficient, one may expect less disordered Ca-dependent Pb-detoxification in Ca-dT plants due to higher availability of Ca²⁺ for metabolic processes. The K⁺ shoot concentration was significantly reduced in Pb/Pb plants suggesting that Pb²⁺ impaired the uptake of K⁺ by roots (Figure 5b). This result was consistent with several previous studies [30,38,39]. Since Pb²⁺ has no chemical similarity with K⁺, we suggest that it exerts an indirect effect on K⁺-uptake, perhaps by complexing ATP and reducing energy availability [57]. (B-K)/Pb plants expressed a normal growth activity in spite of the reduced K⁺ shoot concentrations. It's known that, potassium is essential for growth in higher plants. Its absorption is mainly by ATPases that hydrolyze ATP to provide energy for the transport of K⁺ in the interior of the cell root. The effect of Pb²⁺ on the uptake of K⁺ could not be due to direct competition at the sites of absorption for the lack of homology between these cations (Pb²⁺ on one hand and the other K⁺). So, in an indirect way that is the inhibition of the uptake of potassium, probably by the binding of metal cations on the ATP molecules thus preventing hydrolysis and consequently reducing the energy required for the absorption of K⁺ [57]. The uptake of K⁺ in the presence of heavy metals could also be decreased due to a restriction of passive absorption of this cation and an increase in efflux in the medium. The reduced N concentration was not significantly modified by Pb²⁺ treatments in the leaves of both species. The same result was found by Paivoke [39]. The different N amounts in shoots observed in response to different treatments (Table 2) were probably a consequence of treatment influence on biomass production. Thus, we conclude that Pb²⁺ did not limit growth through the restriction of N root uptake. On the other hand, (Pb/Pb) and (B-Ca)/Pb plants showed a significant decreased in root water absorption leading to significant shoot compared to that other treatments. This effect is more pronounced in *B. juncea* than in *S. portulacastrum*. In fact, several data demonstrated that heavy metal affect severely water status of sensitive-metal species by affecting transpiration, osmotic potential of cell sap, and water content [58,59]. For all treatments, lead concentration in shoots was significantly higher in *S. portulacastrum* than in *B.juncea*. 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. 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). The evaluation of the Pb²⁺-phytoextraction capacity is based on the lead 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 Pb²⁺ 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 Pb²⁺ concentration in shoots. The disturbances of Ca²⁺ uptake induced by lead contributed largely to the growth restriction and therefore reduced Pb²⁺-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 Pb²⁺ contaminated solution, but the restriction of Ca²⁺ uptake by lead is an important factor of growth reduction, which limits Pb-phytoextraction capacities of these species. We propose that the increase of Ca²⁺ 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 nutriment 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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