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Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: Tolerance and accumulation

Hanen Zaier[a,*,1], Tahar Ghnaya[a,1], Abelbasset Lakhdar[a], Rawdha Baioui[a], Rim Ghabriche[a], Majda Mnasri[a], Souhir Sghair[a], Stanley Lutts[b], Chedly Abdelly[a]

[a] *Laboratoire d'Adaptation des Plantes aux Stress Abiotiques, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisia*
[b] *Groupe de Recherche en Physiologie Végétale (GRPV), Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium*

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ABSTRACT

Lead phytoextraction from salty soils is a difficult task because this process needs the use of plants which are able to tolerate salt and accumulate Pb^{2+} within in their shoots. It has recently been suggested that salt-tolerant plants are more suitable for heavy metals extraction than salt-sensitive ones commonly used in this approach.

The aim of this study was to investigate Pb-phytoextraction potential of the halophyte *Sesuvium portulacastrum* in comparison with *Brassica juncea* commonly used in Pb-phytoextraction. Seedlings of both species were exposed in nutrient solution to 0, 200, 400, 800 and 1000 μM Pb^{2+} for 21 days. Lead strongly inhibited growth in *B. juncea* but had no impact on *S. portulacastrum*. Exogenous Pb^{2+} reduced nutrients uptake mainly in *B. juncea* as compared to *S. portulacastrum*. Lead was preferentially accumulated in roots in both species. *S. portulacastrum* accumulated more Pb^{2+} in the shoot than *B. juncea*. Hence, the amounts of Pb^{2+} translocated at 1000 μM Pb^{2+} were 3400 $\mu g\, g^{-1}$ DW and 2200 $\mu g\, g^{-1}$ DW in *S. portulacastrum* and *B. juncea*, respectively. These results suggest that *S. portulacastrum* is more efficient to extract Pb^{2+} than *B. juncea*.

1. Introduction

Several heavy metals such as lead, cadmium and mercury are not essential for living organisms and may be toxic even at low concentrations. Over the past centuries, numerous anthropogenic activities have contributed to extensive soil contamination by these heavy metals [1,2]. Lead (Pb) is considered as one of the heavy metals of environmental concern and several remediation researches focus on this pollutant [3,4]. Lead accumulates as a result of industrial and mining activities: paints, battery manufacture and disposal, gasoline, explosives and anti-spark linings as well as disposal of lead-containing municipal sewage sludge contributing to dissemination of this toxic compound in the environment [5,6].

Soil contamination by lead may cause a variety of environmental disturbances and its high phytotoxicity is a major problem in cultivated areas [7–9]. Its transport by streaming water may also lead to the contamination of ground and surface waters. The consumption of contaminated foods and waters represents the major source of Pb-accumulation in animals and humans [10,11].

Although Pb has not been shown to be essential in plant metabolism, its presence at low concentrations in all plants tissues has no deleterious effects [12]. However, high Pb concentration in plants interferes with metabolic components and inhibits physiological processes [13,14]. Most of the Pb taken up by plants accumulates in roots and only very small amount is translocated to the shoots [15,16]. Nevertheless, plant leaves may show obvious symptoms of Pb toxicity consisting in reduction in growth and rate of leaf appearance in relation to changes in photosynthetic pigments concentration. Indeed, photosynthesis is considered as one of the most sensitive metabolic processes to Pb toxicity. Lead toxicity is also known to induce oxidative stress through overproduction of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (OH·) and hydrogen peroxide (H_2O_2) [17–19]. Lead-induced nutritional disturbances have also been reported since Pb^{2+} competes with essential cations such Ca^{2+}, Mg^{2+}, K^+, Fe^{2+} for transporter in plasmalemma [20–22].

Several physicochemical options, including stabilization/solubilization in the soil, soil washing or extraction, capping, *in situ* immobilization or *ex situ* vitrification and reclamation were tested for treating lead-contaminated sites. These techniques are rather expensive and could have deleterious effects on biological and physicochemical soil proprieties [23]. Hence, biological treatment, especially phytoremediation could appear as a promising method contributing to the remediation of Pb contaminated soils.

* Corresponding author. Tel.: +216 71 430 215; fax: +216 71 430934.
E-mail address: hanen_zaer@yahoo.fr (H. Zaier).
[1] Both authors Hanen Zaier and Tahar Ghnaya equally contributed in this work.

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This approach, based on the ability of some plant species to take up and to concentrate heavy metals in their shoots, is an environmentally friendly and relatively cheap technique comparatively to physicochemical methods [24–27].

Efficiency of phytoextraction is, however, limited by the low mobility and bioavailability of some heavy metals (especially Pb) in polluted soils [28]. Moreover, since the use of heavy metal-hyperaccumulating plants (e.g., *Thlaspi* sp.) is associated with slow plant growth and low biomass yields and since a true Pb-hyperaccumulator has not yet been identified, most of the recent Pb-phytoextraction studies focused on the use of fast growing crops (e.g., *Brassica juncea*, *Zea mays*, *Helianthus annuus*) with high biomass yields combined with the enhancement of heavy metal mobility and bioavailability through addition of synthetic chelators like EDTA [29–31]. Lead hyperaccumulating plant species are able to concentrate 0.1% or more of this metal in their dry leaves without suffering stress or toxic consequences [32]. Indian mustard, *B. juncea*, is a dry-land species which accumulates several metals (Pb, Cu, and Zn) from contaminated soils together with reasonable biomass yields [33]. These plants are typical glycophytes lacking salt-tolerance mechanisms and can therefore not be used to extract metals from salt-affected soils. Halophytes, such as *Atriplex nummularia*, *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* tolerate saline soils and accumulate large amounts of salts in the aboveground tissues. These plant species can achieve biomass yields of 20–30 t ha^{-1} and have been shown to accumulate up to 40% NaCl on a dry weight basis [33]. Recent studies have provided evidence suggesting that halophytes may be useful for phytoremediation, particularly for saline soils [34–36]. It is also suggested that salt-tolerant plants would be better adapted to cropping with environmental stresses, including heavy metals [33,32,37–39]. Halophyte species are naturally present in environments characterized by an excess of toxic ions, mainly sodium and chloride. These plants are able to sequester Cl^- and Na^+ in their vacuoles or accumulate them in trichomes, thus preventing their toxicity for the metabolism in cytoplasm. Several studies demonstrated that some tolerance mechanisms operating at the whole-plant level are not always specific to sodium and that other toxic element such as copper, zinc, or cadmium may accumulate in salt glands or trichomes in tamaris (*Tamarix aphylla* (L.) Karst, and marshdaisy [*Armeria maritima* (Mill.) Willd] [40,35]. Accordingly, halophytes show high potential to tolerate and accumulate metal in their tissues by triggering mechanisms for toxic metals detoxification such adequate compartimentation in the vacuole or in cell wall and peptide detoxification with phytochelatines [38,41–44]. Nevertheless, Pb-phytoextraction by halophyte plants receives only little attention. In this work, we seek to assess the potential of Pb-accumulation in the halophyte *S. portulacastrum* compared with *B. juncea*, a typical glycophyte species commonly used for this purpose. A particular attention was paid to parameters involved in plant growth and to Pb^{2+} distribution between roots and shoots.

2. Material and methods

2.1. 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 [45–47]. 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% 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 [48] containing different concentrations of Pb (0, 200, 400, 800 and 1000 μM) provided as $Pb(NO_3)_2$. The Hoagland's solution consisted of 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50 lM 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 FeEDTA; pH was adjusted to pH 4.8 adjusted with HCl. The total volume of the solution was kept constant by adding deionised water to compensate for water lost through plant transpiration, sampling and evaporation. The test solutions were changed every 3 days and pH was readjusted daily to 4.8.

Two harvests were performed, at the beginning of treatment and 21 days later. At the harvests, shoots and roots developed in free-Pb^{2+} medium were successively rinsed three times in cold water and blotted between two layers of filter-paper. Treated roots were dipped in a 0.01 M HCl cold solution to eliminate external heavy metal adsorbed at the root surface according to Aldrich et al. [49], 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.

2.2. Chlorophyll concentration

A hundred milligrams of small discs from fresh apical leaves were incubated in 5 ml 80% acetone in darkness at 4 °C during three days (until complete chlorophyll extraction) Chlorophylls were determined according to Arnon [50].

2.3. Water content and osmotic potential

The tissue water content (TWC) was determined as TWC (ml g^{-1} DW) = (FW − DW)/DW.

For osmotic potential determination, 100 mg of leaves were immediately frozen in liquid nitrogen, and centrifuged at 15,000 × *g* for 15 min at 4 °C. The supernatant was analysed for Ψs estimation. Osmolarity (*c*) was assessed with a vapour pressure osmometer (OSMOMAT 030) and Ψs was calculated using the formula: Ψs (MPa) = −RTC (osmoles kg^{-1}) [51].

2.4. 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 Pb^{2+}, Mg^{2+} and Ca^{2+} concentrations were determined by atomic absorption spectrometry (Spectra AA 220 FS). Potassium concentrations were determined in the same homogenate by flame spectrometry (Corning photometer).

2.5. 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,

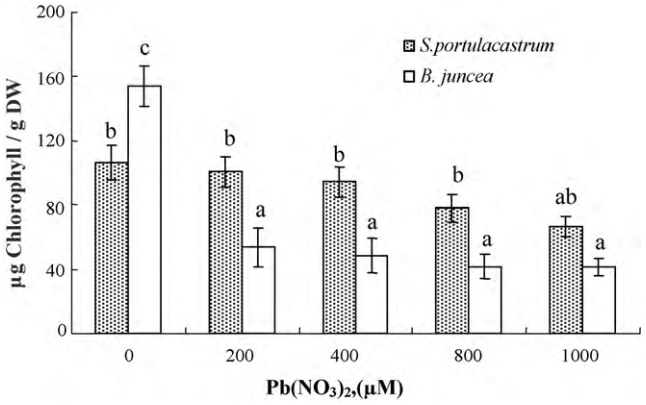

Fig. 1. Variation of total chlorophyll concentration in leaves of *S. portulacastrum* and *B. juncea* treated during 21 days by various $Pb(NO_3)_2$ concentrations. Means of eight replicates. Bars marked with same letter are not significantly different at $P = 0.05$.

and $(t_2 - t_1)$ was the duration of the period [52]. The bioconcentration factor (BCF) was calculated as the following ratio:

BCF = [Pb] in shoot/[Pb] in solution [53].

2.6. 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) ($P < 0.05$).

3. Results

3.1. Plant morphology and growth

After 7 days of treatment, chlorosis was visible in young leaves of *B. juncea* exposed to Pb^{2+}. One week later, chlorosis increased and necrosis appeared in oldest leaves, with a subsequent falling of these senescing leaves at the highest Pb^{2+} concentrations (800 and 1000 µM). In contrast, *S. portulacastrum* plant morphology was not significantly modified in the presence of Pb^{2+} as compared to control, even at the highest concentration (1000 µM).

The analysis of total chlorophyll concentrations in apical leaves (Fig. 1) confirmed that *B. juncea* was more sensitive to lead nitrate than *S. portulacastrum*. Indeed the presence of 1000 µM Pb^{2+} in the medium culture induced a significant decrease in chlorophyll concentrations in the apical leaves of *B. juncea* while they remained unaffected in the apical leaves of *S. portulacastrum*.

Both species produced similar biomasses in the absence of Pb^{2+} (Fig. 2). The addition of lead to the culture solution differently affected the growth of the two species. Lead significantly decreased dry matter in *B. juncea* plants even at the lowest concentration (200 µM) and such an effect increased with increasing concentration in the medium; reaching 88% of reduction at 1 mM $Pb(NO_3)_2$ as compared to control. In contrast, the depressive effect of Pb^{2+} on whole-plant biomass production in the halophyte species *S. portulacastrum* becomes significant only at the highest external metal concentration (1000 µM) and the recorded reduction never exceeded 34% as compared with control plants (Fig. 2).

Fig. 3 compares the relative growth rate (RGR) of *S. portulacastrum* and *B. juncea* species. In unstressed conditions, the perennial halophyte species have a low RGR (0.05 day^{-1}) as compared with *B. juncea* (0.17 day^{-1}). The addition of Pb^{2+} to the medium, however, significantly reduced growth rate in *B. juncea* but not in *S. portulacastrum*.

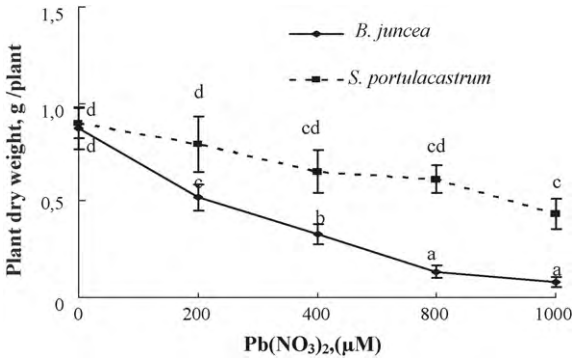

Fig. 2. Changes in whole-plant dry matter (g $plant^{-1}$) produced by *S. portulacastrum* and *B. juncea* treated by various $Pb(NO_3)_2$ concentrations. Means of eight replicates. Bars marked with same letter are not significantly different at $P = 0.05$.

The shoot water content (Table 1) remained unchanged in *S. portulacastrum* until 800 µM Pb^{2+}, while *B. juncea* exhibited a decrease in water content even at 200 µM Pb^{2+}. In this latter species, shoots of plants exposed to 1000 µM Pb^{2+} appeared drastically dehydrated. This species exhibited a decrease in osmotic potential while Ψs was marginally affected in *S. portulacastrum* leaves: only plants exposed to 1000 µM Pb^{2+} exhibited a significant decrease in Ψs values.

3.2. Lead accumulation

As expected, no traces of Pb^{2+} were detected in control plants, whatever the considered species. In treated plants, roots and shoots Pb^{2+} concentrations increased with increasing Pb^{2+}concentration in the culture solution (Fig. 4).

For both *S. portulacastrum* and *B. juncea*, Pb^{2+} concentration in the roots tissues was higher than in the shoots (Fig. 4), the differences being more pronounced in *B. juncea* than in S. *portulacastrum*. As shown in Fig. 4, *S. portulacastrum* accumulated significantly more Pb^{2+} in the shoot than *B. juncea* while a reverse trend was noticed for the roots. Hence, the concentration of sequestered Pb^{2+} in shoots at 1000 µM were 3400 and 2200 µg g^{-1} DW for *S. portulacastrum* and *B. juncea* (Fig. 4), respectively. 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 Fig. 5, demonstrates that *S. portulacastrum* extracted more Pb^{2+} than *B. juncea* at all exogenous Pb^{2+} concentrations. In fact, at 800 µM

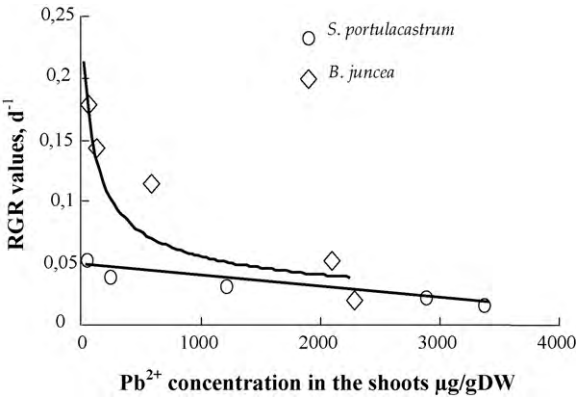

Fig. 3. Relationship between the variations of the RGR values and Pb^{2+} concentration in the shoots of *S. portulacastrum* and *B. juncea*. RGR measures the quantity of biomass deposited by 1 g of biomass per unit of time. It was estimated as $\Delta \ln(\Delta W)/\Delta t$; where ΔW is the dry weight, ln stands for natural logarithm and Δ represents the difference between final and initial value [52].

Table 1
Variation of shoot water content and osmotic potential in leaves of *S. portulacastrum* and *B. juncea* treated during 21 days with various $Pb(NO_3)_2$ concentrations. Data are the means of eight replicates. Means followed by the same letters are not significantly different at $P \leq 0.05$.

	$Pb(NO_3)_2$ (μM)				
	0	200	400	800	1000
Shoot water content (ml/gDW)					
S. portulacastrum	14 ± 0.96d	13.14 ± 0.87d	13 ± 0.93d	13.1 ± 0.83d	9.40 ± 0.65c
B. juncea	13.66 ± 0.92d	9.30 ± 0.83c	5.30 ± 0.65b	3.30 ± 0.67ab	1.80 ± 0.48a
Osmotic potential (MPa)					
S. portulacastrum	−1.17 ± 0.02a	−1.25 ± 0.13a	−1.067 ± 0.11a	−1.36 ± 0.23a	−4.19 ± 0.32b
B. juncea	−0.66 ± 0.07a	−2.13 ± 0.17ab	−3.31 ± 0.008b	−6.16 ± 0.012c	−8.54 ± 0.012d

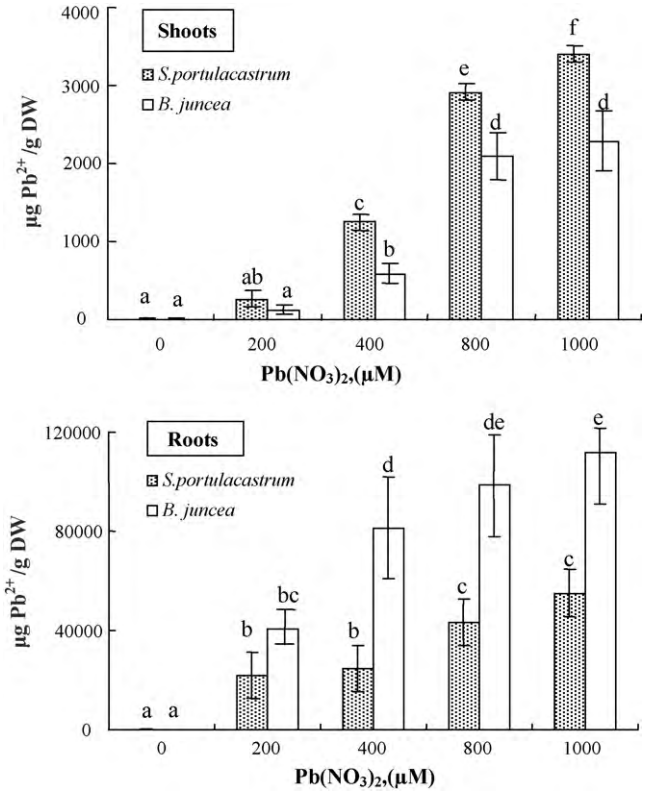

Fig. 4. Changes in Pb shoot and root concentrations (ppm) of *S. portulacastrum* and *B. juncea* treated by various $Pb(NO_3)_2$ concentrations. Means of eight replicates. Bars marked with same letter are not significantly different at $P = 0.05$.

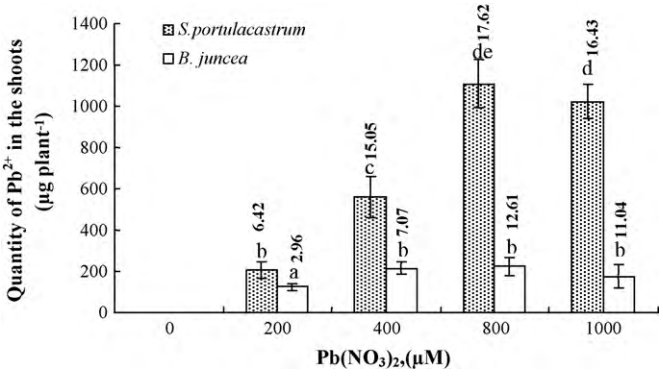

Fig. 5. Changes in lead amounts (μg plant^{-1}) accumulated in the shoots of *S. portulacastrum* and *B. juncea* treated by various $Pb(NO_3)_2$ concentrations. For each treatment this parameter is the product of Pb shoot concentrations (μg g^{-1} DW) by shoot DW (g plant^{-1}). Means of eight replicates. Bars marked with same letter are not significantly different at $P = 0.05$. The values contiguous to histograms correspond to bioconcentration factors (BCF). BCF = [Pb] in shoot/[Pb] in solution.

Pb^{2+} in the nutrient solution, the amounts of Pb^{2+} accumulated in the shoots were 1200 and 270 μg plant^{-1} in *S. portulacastrum* and *B. juncea*, respectively.

3.3. Potassium nutrition

Addition of Pb^{2+} to the nutrient solution significantly reduced shoot K^+ concentration in *B. juncea*. This effect becomes more pronounced with increasing external Pb^{2+} concentration. In *S. portulacastrum*, K^+ accumulation in the shoots was not significantly affected by Pb^{2+} in the nutrient solution, except at the highest lead concentration.

The addition of Pb^{2+} had no impact on root K^+ concentration in *S. portulacastrum* while the highest stress intensities slightly decreased root K^+ concentration in *B. juncea* (Table 2).

3.4. Calcium nutrition

Independently of Pb^{2+} supply, the glycophyte species *B. juncea* showed higher Ca^{2+} tissue concentrations than *S. portulacastrum* (Table 2). Lead treatment resulted in a large decrease in *B. juncea* shoot and root Ca^{2+} concentrations. In plants of *B. juncea* exposed to 1000 μM Pb^{2+}, shoot and root Ca^{2+} concentrations represent respectively 55% and 70% of those measured in control plants. In the halophyte *S. portulacastrum*, Pb^{2+} did not significantly affect shoot Ca^{2+} concentrations. Lead induced a significant decrease of root Ca^{2+} concentrations and this effect was more pronounced in *B. juncea* than in *S. portulacastrum*.

3.5. Magnesium nutrition

For both species, the shoot Mg^{2+} concentration was higher in plants treated with Pb^{2+} than in untreated ones. An opposite behaviour was observed in roots, where a significant reduction in Mg^{2+} concentrations was observed in the presence of Pb^{2+} (Table 2). Once again, Pb-induced modifications in Mg^{2+} shoot and root concentrations were more pronounced in *B. juncea* than in *S. portulacastrum*.

4. Discussion

When present in excess within plant tissues, lead interferes with proper enzymatic functions and inhibits overall plant growth. However, interspecific variability was showed in plants responses to this metal [15,21]. The establishment of plants to extract heavy metals from a contaminated soil remains a difficult task due to the high toxicity of these pollutants which frequently hampers plant growth, even at low concentrations.

In the present study, the two tested species showed a different pattern in response to the addition of Pb^{2+} in the nutrient solution. Results showed that the halophyte (salt-tolerant) species *S. portulacastrum* was more tolerant to Pb^{2+} than the glycophyte (salt-sensitive) *B. juncea* (Fig. 2).

Table 2
Changes in potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in shoots and roots of *S. portulacastrum* and *B. juncea* subjected to increasing $Pb(NO_3)_2$ concentrations in culture medium. Data are the means of eight replicates. Means followed by the same letters are not significantly different at $P \leq 0.05$.

		Pb $(NO_3)_2$ (μM)				
		0	200	400	800	100
K^+ (mmol/g DW)						
Sesuvium	Shoot	0.95 ± 0.1e	0.84 ± 0.1e	0.81 ± 0.08de	0.79 ± 0.09e	0.75 ± 0.07d
	Root	1.27 ± 0.1bc	1.23 ± 0.1bc	1.28 ± 0.098c	1.29 ± 0.09c	1.18 ± 0.09bc
Brassica	Shoot	0.77 ± 0.08de	0.80 ± 0.08de	0.47 ± 0.05c	0.19 ± 0.04b	0.03 ± 0.01a
	Root	1.26 ± 0.078bc	1.24 ± 0.068bc	1.20 ± 0.063bc	1.02 ± 0.068a	0.98 ± 0.07a
Ca^{2+} (μmol/g DW)						
Sesuvium	Shoot	343.69 ± 62a	218.81 ± 58a	214.08 ± 46a	206.47 ± 35a	241.32 ± 38a
	Root	103.90 ± 12b	65.1 ± 8.6c	59.36 ± 7.3c	56.97 ± 8.6c	49.20 ± 8.9c
Brassica	Shoot	1092.51 ± 68.7d	617.17 ± 95.3c	548.27 ± 94.6c	516.44 ± 56.4c	489.87 ± 65.9c
	Root	197.41 ± 10.6e	173.08 ± 9.6cd	154.05 ± 10.8c	70.11 ± 10.3a	58.17 ± 9.5a
Mg^{2+} (μmol/g DW)						
Sesuvium	Shoot	98.93 ± 18bc	126.19 ± 14c	130.08 ± 18.3cd	131.77 ± 18.9cd	157.16 ± 12.2d
	Root	45.18 ± 5.9e	37.66 ± 4.6d	27.77 ± 4.8c	26.31 ± 4.09c	25.65 ± 3.3c
Brassica	Shoot	48.02 ± 9.7a	76.59 ± 7.5b	115.37 ± 10.8c	120.71 ± 15.6c	122.73 ± 11.05c
	Root	36.20 ± 4.3d	26.88 ± 3.7c	12.74 ± 3.9b	7.49 ± 2.9ab	5.04 ± 1.2a

Plant growth upon controlled conditions showed no statistical difference between the two tested species. However, under Pb^{2+} treatment, *S. portulacastrum* produced much more dry matter as compared to the glycophyte *B. juncea*. 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 [38]. Based on the RGR values, Fig. 3 showed that the growth reduction in *B. juncea* could be the consequence of the increase in the internal pool of lead within the photosynthetic organs: in this species, the accumulation of Pb^{2+} in shoots was concomitant and correlated with the slowdown of RGR. However, in *S. portulacastrum*, the rate of biomass production was only slightly reduced (Fig. 3) in spite of a large shoot Pb^{2+} 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 [15,16].

The superiority of the halophyte to maintain its growth potential and to tolerate lead could be, at least partly, linked to the maintenance of an adequate nutrients uptake. It is known that, in sensitive plants, lead and other toxic heavy metals interfere with essentials nutrients uptake and translocation, which adversely affect the acquisition of macro and micronutrients in plants, thus leading to nutrients deficiencies [21,22,54,55]. Reduced calcium contents in lead-exposed plants (Table 2) has previously been observed in other species, such as rye, maize, tomato and mustard [20] and could result from the inhibition of Ca^{2+} transporters by toxic lead ions [56,57] and/or replacement of Ca^{2+} ions with Pb^{2+} ions due to the high affinity of the latter for Ca^{2+} binding-sites on biological structures [58,59]. Several studies have indeed shown the poor molecular-specificity of some Ca transporters [58]. It is therefore possible that the more abundant Pb^{2+} ions competed with Ca^{2+} ions [56,57]. Resistant plants are able to selectively absorb essential nutrients from contaminated substrates and to maintain appropriate nutrition of their photosynthetic organs, which undoubtedly was the case of *S. portulacastrum* while Pb^{2+} induced a significant reduction in nutrients concentration in *B. juncea*.

On the other hand, high Pb^{2+} concentrations induced water stress in several plant species as a result of a decrease in root water absorption leading to significant root and shoot dehydrations [60]. *S. portulacastrum* was only slightly dehydrated in the presence of Pb^{2+} in the medium culture, while Pb^{2+} drastically reduced shoots water content in *B. juncea* (Table 1). Hence, in the presence of Pb^{2+} in the medium, the maintenance of tissue hydration allowed *S. portulacastrum* to keep its osmotic potential unchanged even at 800 μM Pb^{2+} suggesting that this metal did not induce water stress in this halophyte plant species. Conversely, in *B. juncea*, Pb^{2+} obviously induced a decrease in water uptake. In order to overcome this Pb^{2+}water stress, *B. juncea* plants react through a decrease in the cell osmotic potential in response to all Pb^{2+} treatments. 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 [60–62]. The hypothesis that Pb-induced decrease in Ψs values was a consequence of a dehydration process rather than an active process of osmotic adjustment could, however, not be ruled out.

Both species accumulated Pb^{2+} in shoots at high concentrations, reaching 3400 and 2200 ppm, respectively in *S. portulacastrum* and *B. juncea* (Fig. 4). Lead hyperaccumulation is a rare phenomenon in plants [63] and until now, only two species were reported to accumulate lead at high concentration in the shoots: *Tlaspi rotundifolium* (8200 ppm) from a lead/zinc mining area of Cave del Predil, northern Italy [64] and *T. caerulescens* (2740 ppm) colonizing a lead mine district in Pennines, England [65]. Considering the present study, we suggest that *S. portulacastrum* could be classified as Pb "accumulator" species because it tolerates by far more than 1000 ppm Pb^{2+} in the shoots with only marginal growth inhibition. However, the second criteria defining a hyperaccumulating species, which consists in a higher concentration of the considered heavy metal in the shoots than in the roots, was not fulfilled by *S. portulacastrum*. Although *B. juncea* accumulated more than 2000 ppm Pb in the shoots, endogenous Pb^{2+} induced a strong growth inhibition (Fig. 2). Nevertheless, owing to high annual biomass production, *B. juncea* was selected as valuable species for phytoextraction of several heavy metals but it was also demonstrated that the addition of metal chelators, such EDTA, significantly increased metals accumulation in Indian mustard and suggested the effectiveness of this species in assisted phytoextraction of heavy metals [66–69]. Considering the cost of chelating agents, the opportunity to use more efficient plant species for continuous phytoextraction could be considered as a cost-effective promising strategy.

Beside concentrations, a total amount of metals accumulated in the shoots is considered as the most important parameter to evaluate the potential of phytoextraction in plants. We demonstrated that the halophyte species, *S. portulacastrum*, sequestrated much more lead in the shoots as compared with salt-sensitive plants (*B. juncea*) (Fig. 5). In fact, in the presence of 800 μM Pb^{2+} in the medium, the amount of extracted Pb in shoots was

1200 and 270 μg plant^{-1} in *S. portulacastrum* and *B. juncea* respectively. *S. portulacastrum* may produce 17 t ha^{-1} year^{-1} of dry matter [70,38]. On the basis of our data, this species would enable to extract up to 51 kg Pb ha^{-1} year^{-1}. The potential of Pb extraction of *S. portulacastrum* would therefore exceed those of *B. juncea* (36 kg Pb ha^{-1} year^{-1}) determined in previous work which considered that *B. juncea* could produce 18 t ha^{-1} year^{-1} of dry matter [71].

The bioconcentration factor is a common index used to estimate plant's ability to pump heavy metals from the substrate and to compare species for phytoextraction potentials. Analysis of BCF values (Fig. 5), demonstrated that, in hydroponics conditions, both *S. portulacastrum* and *B. juncea* were able to absorb Pb^{2+} and to translocate it towards their shoots. However, it was apparent that *S. portulacastrum* exhibited greater ability to bioaccumulate Pb^{2+} than *B. juncea*. BCF values were 15.05 and 7.07 respectively in *S. portulacastrum* and *B. juncea* exposed to 400 μM Pb^{2+}. This is consistent with the result of Niu et al. [72] working on *B. juncea* cultivated hydroponically in the presence of 200 mg Pb^{2+} l^{-1}. For *S. portulacastrum*, no previous work related to Pb^{2+} accumulation was found in the literature. The works of Ghnaya et al. [38,39] already underlined the high potential of this halophyte to accumulate cadmium in the shoots without growth retardation. The ability to tolerate both Cd^{2+} and Pb^{2+} accumulation in the shoots without deleterious effects on growth suggests an efficient protection of the cellular biochemical machinery against free metal ions (Cd^{2+} and Pb^{2+}) and could be of crucial interest for phytomanagement of polluted areas which are frequently contaminated by several heavy metals**.** Accordingly, a similar study comparing the response to Cu stress between *Messembryanthemum crystallinum* (halophyte) and *Arabidopsis thaliana* (a glycophyte) demonstrated enhanced Cu uptake and higher Cu tolerance by *M. crystallinum* [37]. Similarly, Jordan et al. [33] showed that the halophyte (*Atriplex nummularia*) may have greater potential to selectively phytoextract metals from contaminated soils than glycophyte (*Zea mays*) despite their lower growth rate. The use of halophytes to extract several toxic metals has received increasing attention since a few years [35,38,43,73–75]. It has been postulated that halophytes species recruit non-selective salt-resistance mechanisms to sequester toxic ions in the vacuole and/or salt glands or trichomes [35]. Metal deposit in the cell walls as a result of binding to pectic compounds could be also considered as an important mechanism for metal detoxification in halophyte species, as demonstrated in *Halimione portulacoïdes* [43]. Both sequestrations in cell walls and in foliar trichomes enable halophyte to avoid toxic accumulation of heavy metals in the cytoplasm of mesophyll cells [76,43].

Toxic ion accumulation could be associated with other mechanisms of ions sequestering and detoxification, such as the biosynthesis of phytochelatins which are able to detoxify and chelate metals. Further researches are therefore necessary to unravel the biochemical basis of Pb tolerance in *S. portulacastrum*.

5. Conclusion

Our results indicated that, in hydroponics culture, the halophyte species *S. portulacastrum* is more tolerant to lead than *B. juncea* and that such a tolerance is associated with a high potential of Pb^{2+} accumulation in the shoots. BCF and the amounts of extracted Pb^{2+} values indicated that *S. portulacastrum* is more efficient to extract lead from contaminated solution than *B. juncea*. The specific behaviour of the halophyte plant species could be related to its ability to maintain adequate mineral and water supply in the presence of Pb^{2+}. The halophyte *S. portulacastrum* could be therefore considered as a promising species for Pb^{2+} phytoextraction.

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