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Physiological responses and tolerance mechanisms to Pb in two xerophils: *Salsola passerina* Bunge and *Chenopodium album* L.

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ABSTRACT

Lead (Pb) has great toxicity to human beings and other livings. Although there are varied ways to rehabilitate the Pb contaminated area, phytoremediation of Pb pollution in arid lands is still a difficult task, it is therefore urgent to find and identify Pb tolerant plants in arid areas. The physiological responses and tolerance mechanisms to Pb stress (expressed as the Pb concentration, e.g., 0, 50, 150, 300, 600, 800, 1000 mg/L) were investigated for the xerophils *Salsola passerina* Bunge and *Chenopodium album* L. Results indicated that *S. passerina* exhibited higher Pb tolerance than *Ch. album* in terms of the seed germination rate, bio-activities of SOD and POD, and lower MDA production. There were two ways for *S. passerina* to reduce Pb toxicity in organism level, e.g., cell wall precipitation and state transfer of free Pb into anchorage. These findings demonstrate that *S. passerina* is a Pb tolerant species and may have potential application in phytoremediation of Pb contaminated arid lands.

1. Introduction

The heavy metals from mine exploitation, vehicle emissions and irrational use of chemical fertilizer seriously contaminate soil and environment. Lead (Pb) is one of the most widespread heavy metals in the world and could remain in the soil for a long time. Its high toxicity affects the growth of crop (or plant) and the human health [1].

Vast areas of Pb mine tailings in arid Western China have become the main source of pollution. To reduce the environmental risks of Pb contamination, phytoremediation has been considered as the most promising and a relative new method for cleanup of polluted environments [2].

Phytoremediation comprises two technologies: phytoextraction and phytostabilization [3,4]. Phytoextraction uses the hyperaccumulator plants to take up and translocate Pb to their tissue, then harvests the plant to reduce the contamination. However, it has certain limitations, these plants were not suitable for every polluted site and the disposal of the metal-enriched biomass has not been well-defined yet [5], while phytostabilization mainly focus on utilizing the tolerant plant to stabilize the metal and the ecesis of plants can reduce aeolation and water erosion of the bare contaminated tail. It has great practical significance in the ecological restoration of mining tailings and remediation of soil polluted by heavy metals. Therefore, it is important to choose more tolerant plants for phytostabilization activities. These plants have a defense system composed of a variety of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), which can neutralize, convert and scavenge ROS (reactive oxygen species) induced by Pb stress [6]. Recent studies have reported that tolerant plants, such as *Paulownia fortunei* [7], *Avicennia marina* [8], *Athyrium wardii* [5], *Atriplex lentiformis* (Torr.) [9], *Lygeum spartum* and *Piptatherum miliaceum* [10] can grow normally in Pb mining tailings or Pb contaminated places, implying a potential value for the purpose of rehabilitating the degraded land. However, there is a big disadvantage that these plants are not drought resistant which restricts the application in restoring the metal polluted soil in arid regions.

Chenopodiaceae plants dominate in arid and semi-arid regions. Previous studies have focused on their distribution [11] and response to salt and drought stress [12]. Little information exists on their physiological and biochemical mechanisms under heavy metal stress. In fact, Chenopodiaceae plants grow in a complicated environment including Pb polluted sites. Hence, it is necessary to study the correlation between Chenopodiaceae plants and heavy metals for the arid land conservation.

Salsola passerina and *Chenopodium album* are the major species of Chenopodiaceae in semiarid environment. Due to their fast growth, large biomass, drought tolerance and universal

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adaptability, they grow in similar habitats and distribute worldwide, including the extremely harsh environments. Therefore, they may be useful species in environmental remediation of Pb pollution. However, there were little research on their responses and tolerance to Pb stress and the differences between these two species in resistance to Pb stress were not well known. Furthermore, previous studies were only conducted with adult Chenopodiaceae plants [13]. It is obvious that tolerance mechanism of a specific drought resistant species to Pb stress remains equally important during all the growing periods.

This study aimed to (1) investigate the tolerance to Pb stress of the two Chenopodiaceae plants from stage of seed germination to seedling growth, (2) evaluate the physiological and biochemical effects of Pb stress, and (3) identify a more Pb tolerant plant based on its tolerance mechanisms.

2. Materials and methods

2.1. Lead concentration and seed germination

The effects of Pb stress (in terms of the Pb concentrations in $Pb(NO_3)_2$ solution, e.g., 50, 150, 300, 600, 800 and 1000 mg/L) on seed germination were evaluated by assessing the seed germination rate, germination energy and germination index.

Seeds of the two xerophils species, *S. passerina* and *Ch. album* were collected at North Mountain of Lanzhou, Gansu, China. The seeds were surface-sterilized by 0.1% (v/v) sodium hypochlorite for 5–6 min and rinsed 4–5 times with distilled water. Then 50 seeds of each species were sown in Petri dish with two layers of filter paper that was moistened by 4 mL of aqueous Pb solution. Controls were obtained by moistening the filter papers with 4 mL of deionized water. The seeds were cultured in incubator without light at 24 °C. Each treatment was carried out in triplicate. The experiment lasted for 10 days. The germinated seeds were counted on the sixth day, and the germination energy was calculated on the third day [14].

2.2. Seedling growth and biomass assays

Plump seeds selected from the two Chenopodiaceae plants were surface-sterilized by 2% (v/v) sodium hypochlorite for 30 min and rinsed 4–5 times with distilled water. Then 15 seeds of each species were sown in MS culture medium in the incubator at 24 °C. Each sample was illuminated for 12 h per day. The experiment lasted for one month. Then the seedlings were transplanted to sterilized beakers with 16 layers of gauzes inside, and filled with $Pb(NO_3)_2$ solutions for 10 days. Each treatment was carried out in triplicate. The elongation of roots and shoots was measured with a ruler. The fresh seedling biomass was determined with an electronic balance, and the number of lateral roots was counted on each seedling.

2.3. Enzyme extraction and assays

The superoxide dismutase (SOD) activity was assayed according to the method utilized by Beauchamp and Fridovich [15], by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The enzyme extract was prepared at 4 °C. Half gram of tissues in an ice cold mortar was homogenized with potassium phosphate buffer (50 mmol/L, pH 7.8), and was centrifuged at 20,000 × *g* for 20 min to get the supernatant for SOD determination. An aliquot of 3 mL reaction mixture consisted of 50 mmol/L potassium phosphate buffer (pH 7.8), 75 mol/L NBT, 13 mmol/L L-methionine, 0.1 mmol/L EDTA, 0.002 mmol/L riboflavin, and 50 μL of enzyme extract was set at 25 °C, under cool white fluorescent light for 30 min. One unit of SOD was defined as the enzyme amount causing 50% inhibition reduction of NBT, and the enzyme activity was expressed in units per mg of protein.

The peroxidase (POD) activity was assayed in accordance with the method of Rao et al. [16]. The extraction was processed at 4 °C. One gram of tissues in an ice cold mortar was homogenized with 50 mmol/L potassium phosphate buffer (pH 7.0), and then centrifuged at 15,000 × *g* for 15 min to get the supernatant for determination of POD. A 50 μL reaction mixture consisted of 20 mmol/L guaiacol, 2.8 mL of 10 mmol/L potassium phosphate buffer (pH 7.0), and 50 μL of enzyme extract was set at 25 °C. To initiate the reaction, 20 μL H_2O_2 of 40 mmol/L concentration was added. Changes in absorbance at 470 nm were recorded at an interval of 30 s. One unit of POD activity was defined as 1 μmol of tetraguaiacol formed per minute, and the enzyme activity was expressed as units per min and per mg of protein.

Proteins were determined according to Bradford [17]. Bovine serum albumin was used as the standard protein.

Malondialdehyde (MDA) is a major decomposition product of lipid peroxidation and is regarded as an index for the status of lipid peroxidation. Thiobarbituric acid reactive substances that represent the lipid peroxidation product were extracted by homogenizing 0.5 g fresh plant samples in 5 mL solution containing 20% (w/v) of trichloroacetic acid and 0.5% of 2-thiobarbituric acid. This mixture was heated at 95 °C for 30 min, then, it was quickly moved into an ice bath to prevent any further reaction. This cooled mixture was centrifuged at 5000 × *g* at 25 °C for 10 min, and the absorbance of the supernatant was recorded at 532 and 600 nm. After subtracting the nonspecific turbidity at 600 nm by spectrophotometer, MDA concentration was calculated using its molar extinction coefficient of 155 mmol/(L cm) [18].

2.4. Determination of photosynthetic pigment

Approximately 0.2 g of leaves with little quartz and calcium carbonate was rubbed in 3 mL of 96% alcohol. To determine the content of Chla, Chlb and total carotenoid, respectively, by using extinction coefficients of the clear supernatant and following the method proposed by Lichtenthaler [19].

2.5. Pb tolerant and mechanism analysis

The fresh frozen specimens were rinsed with deionized water 4–5 times before the biochemical analyses were conducted. The specimens were digested by the mixture of HNO_3–$HClO_4$ (3:1, v/v) [20,21], and then the Pb concentrations were measured using the flame atomic absorption spectrophotometer (Z2000, Hitachi, Japan).

The Pb distribution at the subcellular level, e.g., in the stems and leaves of *S. passerina*, was determined by the differential centrifugation at 4 °C. An aliquot of 0.2 g fresh frozen tissues in ice cold mortar was homogenized with 10 mL Tris–HCl (0.1 mol, pH 8.5) to get a supernatant, this supernatant was then centrifuged at 200 × *g* for 2 min, 600 × *g* for 10 min, 1000 × *g* for 30 min, respectively. Subsequently, the sediment from the centrifuge was labeled as components F1 (cell wall and residual), F2 (karyon and chloroplast) and F3 (mitochondria) by sequence. The last clear supernatant was labeled as F4 (cytosolic contained ribosomal protein). Each component was then digested to determine the Pb concentrations.

The Pb concentrated in roots and stems of *S. passerina* was studied with the stepwise extraction. Plant samples of 0.5 g each were cut into slices of 1–2 mm width. We added 35 mL extracting agent in an oscillator for 2 h and then transferred the sample into the incubator for 18 h. The extract was retrieved and added the same volume of extraction solvent in the next day. The process for each extracting agent was repeated twice, and then merged into one sample. Four kinds of extracting agents were used in the experiment, (1) 80% (v/v) alcohol and 2% (v/v) acetic acid for extracting insoluble salt of heavy metals, (2) deionized water was used to extract the

soluble salt, (3) 0.6 mol/L hydrochloric acid for extracting the salt in oxalate form, and (4) 1 mol/L sodium chloride for extracting the salt in pectate form. These extracts were digested for calculating Pb concentrations.

2.6. Statistical analyses

All the statistical analyses were performed using SPSS version 13.0 for Windows. The data was all presented as means ± standard error. Differences between Pb treatments were quantified using analysis of variance (one-way ANOVA). Graphical work was carried out using Origin 8.0.

3. Results

3.1. Effect of Pb on seed germination

The Pb toxicity was evaluated by its influence on seed germination (Table 1). Seed germination rate of the two plants was somewhat inhibited as Pb concentrations increased, while *S. passerina* was more tolerant. The rate of seed germination of the two plants was notably unaffected under the treatment of 50 mg/L compared to the control. When the Pb concentration increased to 150 mg/L, the germination rate, germination energy and germination index of *Ch. album* were significant inhibited ($p < 0.05$), whereas, such inhibition occurred at Pb concentration of 300 mg/L for *S. passerina*. Under the highest Pb stress (e.g., 1000 mg/L), *S. passerina* demonstrated more tolerance to Pb. The germination rate of *S. passerina* and *Ch. album* was decreased by 27.4% and 33.3% respectively, as compared to control. In addition, the germination energy was reduced by 28% and 31.3% for *S. passerina* and *Ch. album*, respectively. The seed germination of *Ch. album* was more severely inhibited under Pb stress as compared to that of *S. passerina*.

3.2. Effect of Pb on seedling growth

Two seedlings of each species were cultured in MS medium for one month, and then incubated in Pb solutions for 10 days. The addition of lead to the culture solution affected the growth of the two species differently. The fresh weight of *Ch. album* was significant inhibited, declined by 0.09 g when compared to control, which was more greatly affected than that of *S. passerina* when the Pb concentration was at 150 mg/L of Pb solution. Lead inhibited ($p < 0.05$) the root length, number of lateral roots and the shoot growth significantly in *Ch. album* even at the lower concentration (300 mg/L), reaching 65%, 29% and 35% of reduction, respectively, as compared to control. While, the inhibition of root length and the number of lateral roots in *S. passerina* was not significant until 600 mg/L of Pb solution, the inhibited rate was 28% and 46%, respectively. The shoot length was significantly declined by 45% at 800 mg/L of Pb solution compared to control. However, it is evidenced that the *S. passerina* was still alive even at the highest Pb concentration (e.g., 1000 mg/L), implied more tolerance than *Ch. album* in seedling growth (Table 2).

3.3. Antioxidative enzymes activities and oxidative damage

The activity of SOD was increased under Pb stress for both plants. At 1000 mg/L of Pb solution, activity of SOD increased roughly 3 times for *S. passerina* compared to control. In contrast, the activity of SOD reached its highest value of 29.7 U/mg protein for *Ch. album* at the Pb concentration of 300 mg/L (Fig. 1).

The activity of POD of *S. passerina* increased with elevated Pb concentrations and reached its maximum value at the highest Pb solution (e.g., 1000 mg/L), which was 2.93 times higher

Table 1
Effects of Pb stress on seed germination of two experimental plants.

Growth parameter	Species	Pb concentration (mg/L)					
		0	50	150	300	600	800	1000
Germination rate (%)	*Salsola passerina*	68.7 ± 1.3[a]a	65.3 ± 1.3ab	63.3 ± 1.8ab	60.0 ± 2.3bc	53.3 ± 1.8 cd	50.7 ± 4.4d	41.3 ± 2.4e
	Chenopodium album	62.0 ± 2.0a	61.3 ± 2.7a	50.0 ± 3.1b	46.7 ± 2.9b	43.3 ± 2.9b	33.3 ± 1.8c	28.7 ± 1.3c
Germination energy (%)	*Salsola passerina*	63.3 ± 3.3a	58.0 ± 1.2ab	53. ±0.7abc	49.3 ± 6.4bc	46.7 ± 4.7 cd	43.3 ± 1.3 cd	35.3 ± 1.8d
	Chenopodium album	51.3 ± 4.1a	50.0 ± 6.0ab	40.0 ± 2.3bc	36.7 ± 1.8 cd	33.3 ± 2.9 cd	28.7 ± 1.3de	20.0 ± 2.3e
Germination index	*Salsola passerina*	5.7 ± 0.1a	5.4 ± 0.1ab	5.3 ± 0.2ab	5.0 ± 0.2bc	4.5 ± 0.2 cd	4.2 ± 0.4d	3.4 ± 0.2e
	Chenopodium album	5.2 ± 0.2a	5.1 ± 0.2a	4.2 ± 0.3b	3.9 ± 0.2b	3.6 ± 0.2b	2.8 ± 0.1c	2.4 ± 0.1c

[a] Means ± standard error; letters represent $p < 0.05$ levels; data followed by different letters is significantly different within a row, according to Duncan test.

Table 2
Seedling properties and fresh biomass of the two experimental plants cultured in Pb solutions for 10 days.

Growth parameter	Species	Pb concentration (mg/L)						
		0	50	150	300	600	800	1000
Root length (cm)	*Salsola passerina*	2.92 ± 0.12[a]a	2.78 ± 0.35ab	2.75 ± 0.01ab	2.40 ± 0.27ab	2.10 ± 0.05b	1.05 ± 0.33c	0.91 ± 0.20c
	Chenopodium album	5.31 ± 0.84a	5.02 ± 0.52a	4.91 ± 0.26a	1.84 ± 0.21b	1.49 ± 0.15b	1.4 ± 0.26b	1.24 ± 0.31b
Shoot length (cm)	*Salsola passerina*	3.01 ± 0.15a	2.53 ± 0.04ab	2.49 ± 0.28ab	2.41 ± 0.17abc	2.38 ± 0.69abc	1.67 ± 0.16bc	1.89 ± 0.51c
	Chenopodium album	5.39 ± 0.36a	5.38 ± 0.56a	3.92 ± 0.53ab	3.51 ± 0.92bc	3.00 ± 0.61bcd	1.98 ± 0.44cd	1.64 ± 0.20d
Number of lateral roots	*Salsola passerina*	14.58 ± 2.57a	13.80 ± 1.85a	10.8 ± 1.19ab	10.00 ± 1.12ab	7.93 ± 1.91b	6.90 ± 1.90b	5.50 ± 1.15b
	Chenopodium album	23.32 ± 1.07a	23.20 ± 0.87a	20.60 ± 0.84ab	16.57 ± 2.07bc	16.00 ± 2.86bc	14.50 ± 2.23c	13.70 ± 0.72c
Fresh weight (g)	*Salsola passerina*	0.81 ± 0.12a	0.75 ± 0.09a	0.62 ± 0.55ab	0.52 ± 0.42ab	0.30 ± 0.03c	0.23 ± 0.02c	0.25 ± 0.05c
	Chenopodium album	0.28 ± 0.05a	0.20 ± 0.03ab	0.19 ± 0.01bc	0.17 ± 0.03bc	0.15 ± 0.01bc	0.13 ± 0.01bc	0.12 ± 0.01c

[a] Means ± standard error; letters represent $p < 0.05$ levels; data followed by different letters is significantly different within a row according to Duncan test.

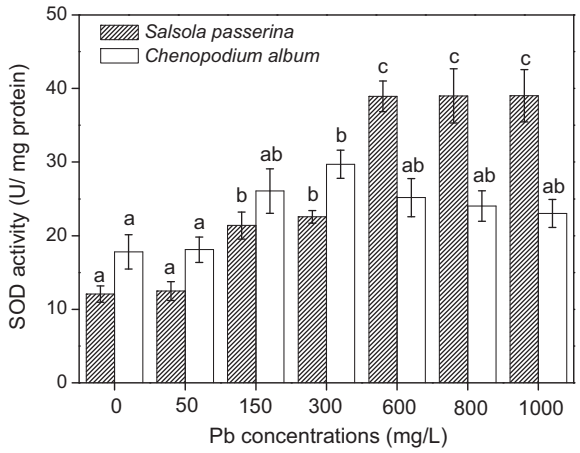

Fig. 1. Effects of Pb concentrations on SOD activity of two plants. Results are means ± SE of three independent replicates. Different letters in the same plant are significant at $p < 0.05$ according to Duncan test.

compared to control. For *Ch. album*, POD activity increased in low-concentrations of Pb treatment, reached its maximum value of 43.4 U/mg proteins at 300 mg/L of Pb treatment, and then the POD activity decreased (Fig. 2).

The level of lipid peroxidation of *S. passerina* and *Ch. album* was determined by MDA content (Fig. 3). A notable increase in MDA content for *Ch. album* and *S. passerina* was detected in treatments of 150 mg/L and 600 mg/L Pb solution, which were then enhanced by increasing the Pb concentration at a rate of 168–240%, 51–64%, respectively, compared to control, a dose-dependent free radical generation was evidenced. The lipid peroxidation of *Ch. album* was more remarkable in response to Pb stress.

3.4. Effect of Pb on photosynthetic pigment content

Pigment contents of *S. passerina* and *Ch. album* showed a consistent decrease as the concentration of Pb increased (Figs. 4 and 5). There was a significant decrease of Chla when *S. passerina* grew at a lower concentration (e.g., 150 mg/L), whereas under the higher stress (≥600 mg/L), it remained constant ($p > 0.05$), which was contrary to Chla of *Ch. album*. The decrease of Chlb in *Ch. album* was more sensitive compared to that of *S. passerina* under the same Pb treatments. The carotenoid of both plants decreased remarkably when the Pb solution exceeds 300 mg/L.

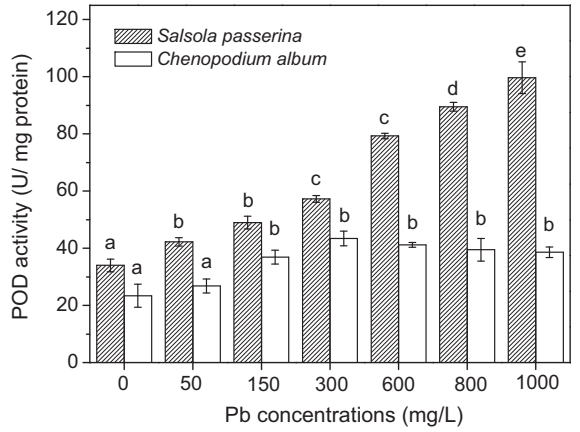

Fig. 2. Effects of Pb concentrations on POD activity of two plants. Results are means ± SE of three independent replicates. Different letters in the same plant are significant at $p < 0.05$ according to Duncan test.

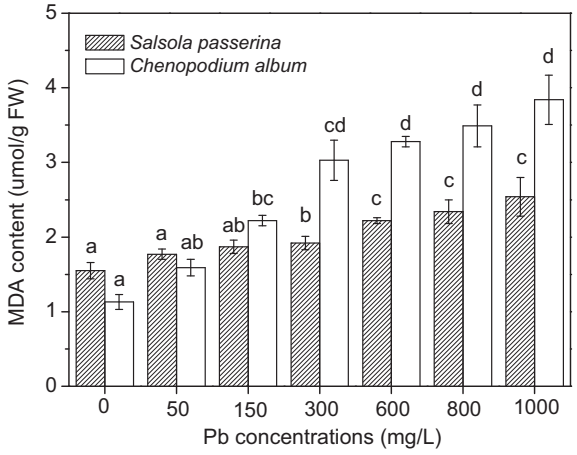

Fig. 3. Effects of Pb concentrations on MDA content of two plants. Results are means ± SE of three independent replicates. Different letters in the same plant are significant at $p < 0.05$ according to Duncan test.

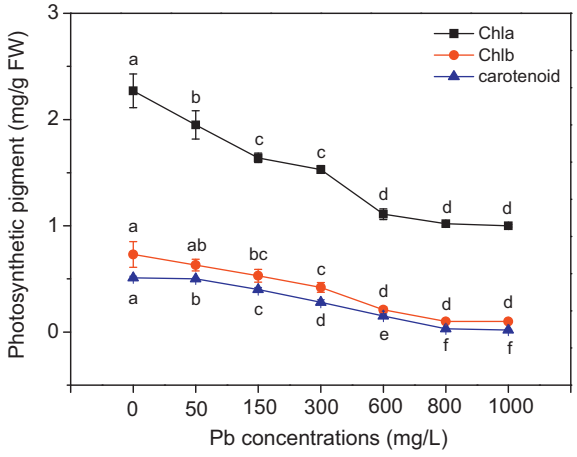

Fig. 4. Effects of Pb concentrations on photosynthetic pigment content of *Salsola passerina* Bunge. Results are means ± SE of three independent replicates. Different letters are significant at $p < 0.05$ according to Duncan test.

3.5. Pb distribution in stems and leaves of S. passerina

To further explore its tolerance mechanism of Pb, a higher stress (e.g., 1000 mg/L) was set up. The distribution of Pb in the *S. passerina* was rather uneven (Table 3). Subcellular localization in the plant's

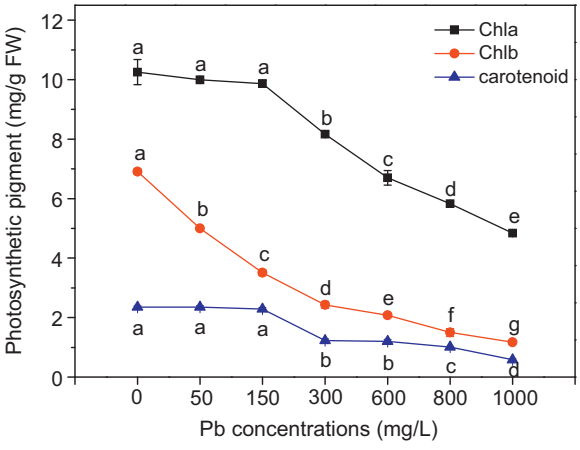

Fig. 5. Effects of Pb concentrations on photosynthetic pigment content of *Chenopodium album* L. Results are means ± SE of three independent replicates. Different letters are significant at $p < 0.05$ according to Duncan test.

Table 3
Pb distribution in subcellular parts of the stems and leaves in *Salsola passerina* Bunge under 1000 mg/L stress.

Components	Pb concentration (mg/L)	Distribution rates (%)
F1	71.41 ± 0.12[a]	75.22 ± 0.07
F2	6.2 ± 0.09	6.5 ± 0.06
F3	5.01 ± 1.22	5.28 ± 0.74
F4	12.31 ± 1.1	13 ± 0.67

[a] Means ± SE ($n = 3$). F1–F4 represent different subcellular components. F1: cell wall and residual, F2: karyon and chloroplast, F3: mitochondria, F4: cytosolic contained ribosomal protein.

stem and leaf cell to 1000 mg/L Pb decreased in the order: cell wall and residual > cytosolic contained ribosomal protein > karyon and chloroplast > mitochondria. A lot of Pb congregated at the extracellular, 75.22% of which assembled in cell wall (F1) of *S. passerina*, it was the highest Pb localization site, which could chelate the ions to prevent excessive Pb from seeping into the protoplasm, and to ensure the normal metabolism of organism. Furthermore, a little Pb entered into karyon and chloroplast (F2), combined with protein to reduce the toxicity. Thus, even at the highest concentration the *S. passerina* was still alive (Table 3).

3.6. Forms of Pb in root and stem of S. passerina

Different forms of extractable-Pb in root and stem of *S. passerina* are shown in Figs. 6 and 7. Acetic acid was the main extractable

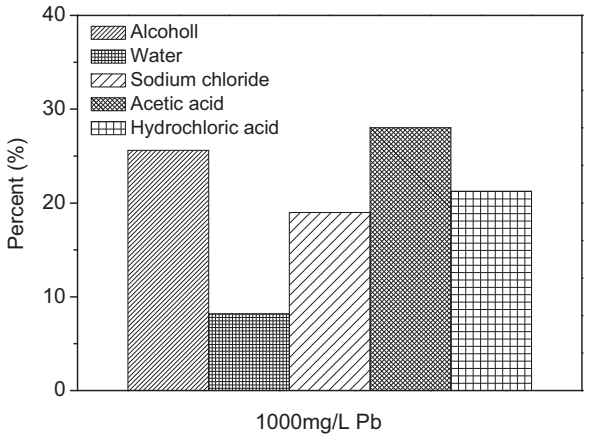

Fig. 6. Forms of extractable-Pb in root of *Salsola passerina* Bunge under 1000 mg/L of Pb treatment.

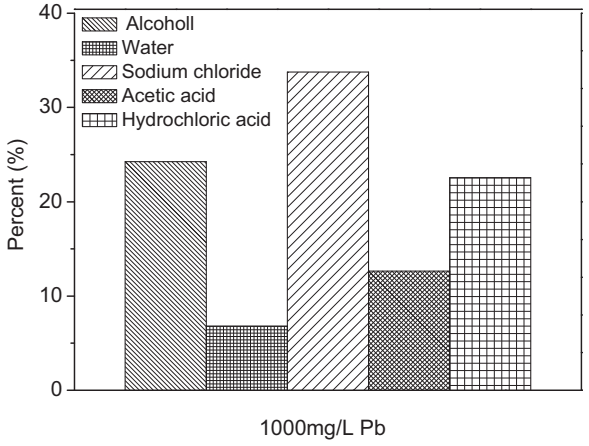

Fig. 7. Forms of extractable-Pb in stem of *Salsola passerina* Bunge under 1000 mg/L of Pb treatment.

forms, followed by ethanol extractable state, it was showed that insoluble salt which had little activity was the dominant form in the root. However, sodium chloride was the primary form in the stem, which made the Pb into the combinative form to reduce its toxic. Furthermore, the ethanol extractable state and hydrochloric acid extractable form were higher in the stem, indicating that the Pb was in the stable state. Either in root or stem, the water extractable form was the least one suggesting that there were little dissociative Pb in the *S. passerina*.

4. Discussion

When Pb accumulates in plant issue, it exerted negative effects on seed germination, seedling growth and plant photosynthetic processes and meanwhile inhibited enzyme activity [22,23]. The inter-specific variability of plant response to Pb differed among different species [24].

Seed germination, seed energy and seed index reflect the seed vigor and quality under stresses [14]. In the present study, the two tested species exhibited a different pattern in response to the addition of Pb in the seed germination stage, when the seeds of *S. passerina* and *Ch. album* were permeable to Pb solutions, water was intensely absorbed in the first stage of germination. At the end of this stage, water uptake decreased, and Pb penetrated into the embryo and inhibited germination [25,26]. Higher concentrations of Pb inhibited the seed germination for both experimental plants, whereas, *S. passerina* was more tolerant to Pb than *Ch. album* (Table 1). Under the lower Pb solution (e.g., 150 mg/L), the vigor of *Ch. album* seed was significantly inhibited. In contrast, *S. passerina* still has higher seed traits in seed germination, seed energy and seed index at 1000 mg/L of Pb solution, and the germination rate only declined by 27% compared to control. For comparison, the seed germination of wheat was significantly decreased by 27% just under 3 mmol Pb concentration [27]. The Pb tolerance of *S. passerina* seed might be contributed to its coat, which was an important defense against heavy metal stress. Moise et al. [28] reported that seed coat exhibited specific variation which would affect its permeability. Therefore, seed coat of *S. passerina* may restrain Pb from entering into the cell and thus protect the vigor of seed. This was evidenced by the seed germination even at a higher Pb stress (e.g., 1000 mg/L).

Our result showed that Pb inhibits plant growth, especially restrains the root growth of the two plants as Pb concentrations increased in the medium (Table 2), but *S. passerina* showed more tolerance than *Ch. album*. Root plays an important role in plant growth and development, and its modification will affect other parts of the plant [29]. Root of *Eruca sativa* was markedly inhibited at 7.62 mg/L of Pb solution [30], the root inhibition rate of lettuce, tomato and broccoli was 76%, 68% and 77%, respectively, under 339 mg/L of Pb treatment [31]. However, the root elongation of *S. passerina* was inhibited at 600 mg/L of Pb solution, even at the highest Pb solution (e.g., 1000 mg/L), it was still alive and the root inhibition rate was 69%. Results also showed that root growth was more sensitive than shoot growth, as root is the most important part of the complexation of heavy metals, and it is also the vulnerable contact point for factors of heavy metal toxic. When plant is under heavy metal stresses, root is the first point to contact with the toxins, and it has plentiful exchange sites on cell wall that heavy metal ions can fixed in, thereby preventing heavy metal ions from entering into issues [32,33]. The number of lateral roots decreased significantly with increment of Pb concentration. This agrees well with the findings of Faheed [34] and Kabir et al. [35]. In this experiment, a sharp decrease in the fresh weight of both plants was observed with increasing Pb concentration. This corroborates the previous findings [6,36]. One possible explanation is that plant uses more energy to deal with the stress than to produce biomass [37]. Likewise, Pb is perceived to prevent plant water absorption [38]. Our study indicated that *S. passerina* were more tolerant under higher Pb stress.

The ROS is the main production when plant is under the Pb stress [39,40], which can promptly cause the production of lipid peroxides, and membrane damage [41,42] thus inducing some key enzymes (SOD and POD) of the antioxidant defense system in the two xerophils. In this study, the activities of SOD and POD of the two plants increased with the increase of Pb concentrations (Figs. 1 and 2). When Pb concentration was 600 mg/L, the activities of SOD and POD of *Ch. album* were at their peak values, while the enzyme activity of *S. passerina* was enhanced even at the highest Pb concentration. This differs from the finding of Zhong's [43], implying that *S. passerina* has a better protection against oxidant damage.

MDA is the production of lipid peroxidation when plant is under Pb stress, and it is often served as an indicator to the extent of oxidative stress [44]. This study showed that the MDA content of *Ch. album* and *S. passerina* increased significantly at 150 and 600 mg/L of Pb, respectively (Fig. 3), implying that *Ch. album* was more sensitive to Pb toxicity. There was no substantial increase of MDA content at a higher Pb solution ($\geq$600 mg/L). This is attributed to the activity of antioxidative enzyme to reduce H_2O_2 levels and therefore minimize the damage on membrane [45].

Photosynthesis is highly sensitive to Pb toxicity, the physiological characteristics of the two plants were investigated under different soil Pb concentrations. In this study, chlorophyll content of the two plants decreased as Pb concentration increased. There was a lesser decrease in *S. passerina* compared to that in *Ch. album*, especially under higher Pb treatment (Figs. 4 and 5). Chlorosis of *Ch. album* was observed at 300 mg/L of Pb solution, but it was not observed in *S. passerina* at the same solution. Reduction of the chlorophyll contents was attributed to Pb stress by reducing chlorophyll synthesis, and preventing plants from taking up essential elements such as Mg and Fe [46]. As a result, it damaged the photosynthetic apparatus or degraded chlorophyll in response to increased chlorophyllase activity [47]. The study also revealed the decline of Chla was more significant than Chlb, indicating that Chla was more sensitive to Pb stress compared to Chlb. Our results corroborate the previous studies [48,49], but contradict with those of Gajewska et al. [50]. This may be due to that diverse plants have different response mechanism to the stress of heavy metal. Compared to *Ch. album*, the *S. passerina* exhibited a higher Pb tolerance, and the tolerance of plant to heavy metals was related to the distribution of the metal in various parts of plant. In this study, with sub-components of *S. passerina*, Pb mainly precipitated in the cell wall of the apoplast (F1) which were the 'dead' tissues in the plants with lower physiological metabolism activity. The cell wall usually includes protein and polyoses, which have a lot of potential ligands such as hydroxyl, carboxyl, amino group, aldehyde group, phosphate, and thiol [51] and these ligands can participate in a variety of reactions containing ion exchange, adsorption, complexation, precipitation and crystallization, leading to metal sequestration under metal toxicity [52]. When the *S. passerina* exposed to the higher Pb stress, the cell wall chelated the ions to the apoplast parts, it is the chief site for *S. passerina* for the detoxification of Pb [51]. Thus, *S. passerina* can tolerate higher Pb stress and grew well even at the 1000 mg/L Pb solution, exhibited higher tolerance compared to *Avicennia marina* which is the Pb tolerant species and can bear 800 mg/L of Pb stress [8].

Heavy metal in different parts of plant has different existing forms. The quality and activity of the dominant extractable form have a significant impact on the migration, accumulation and toxicity of heavy metal in plant [53]. In the present study, Pb in the root of *S. passerina* was in an acetic acid form (Fig. 6), followed by ethanol extractable fractions. These extracted heavy metals were insoluble

salt. They transferred the free heavy metal into the immovable combination state to reduce the Pb toxicity significantly. In the stem, the primary extractable state was in sodium chloride extractable form. It extracted pectic acid salt and caused the protein to evolve into an absorbed state, to lessen the content of free heavy metal (Fig. 7). Our results corroborate the findings that the Pb tolerant mechanism of *S. passerina* is to transform the free Pb into anchorage state [54].

5. Conclusion

Our results revealed that the seed germination and seedling growth of *S. passerina* Bunge and *Ch. album* L. were both inhibited when under the Pb stress, while *S. passerina* showed more tolerance than *Ch. album* and that such a tolerance was associated with the stronger activity of the antioxidant enzymes and less MDA accumulated. Furthermore, *S. passerina* reduces the toxicity of Pb through cell wall precipitation, and transfer of the free Pb into an anchorage state. In conclusion, *S. passerina* is a higher Pb tolerant species and can be therefore considered as a promising species for phytoremediation of Pb contaminated arid lands.

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