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Indexes of Radicle are Sensitive and Effective for Assessing Copper and Zinc Tolerance in Germinating Seeds of *Suaeda salsa*

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Abstract: Euhalophytes, such as *Suaeda salsa*, are ideal candidates to remediate heavy metal-polluted saline soils. However, the metal tolerance ability of dimorphic seeds and subsequent seedlings is largely unknown. This study investigated the tolerance of *S. salsa* seeds to different concentrations of Cu^{2+} (0–300 mM) and Zn^{2+} (0–300 mM) during germination and seedling growth stages. Results showed that dimorphic seeds of *S. salsa* had high metal tolerance during germination, and even germinated under 300 mM Cu and Zn treatments. However, seedling growth was more sensitive to metal solutions and radicle growth was almost completely inhibited by Cu at 10 mM, and by Zn at 50 mM. Germinating seeds and seedlings of *S. salsa* had a higher metal toxicity threshold of Zn than that of Cu. In all indexes, indexes of radicle were the most sensitive and effective indicator of metal tolerance. Seeds of *S. salsa* germinated successfully and seedlings survived under high Zn and Cu stress. The results suggest that *S. salsa* could be sown directly in heavy metal-contaminated soils for phytoremediation.

Keywords: dimorphic seed; germination index; halophyte; phytoremediation; seedling growth; *Suaeda salsa*

1. Introduction

Heavy metal pollution is a great environmental threat that reduces plant productivity [1]. With the rapid development of industry and agriculture worldwide, heavy metal-contaminated soil has become a serious environmental problem [2]. Polluted soils not only lead to deleterious effects on plants, but also pose human health risks via direct ingestion, contact with polluted soil, and the food chain [3,4]. In recent years, saline soils, including those in coastal areas and arid regions, are influenced by heavy metals [5–7]. Developing halophyte remediation technologies may help solve this problem [8,9]. The first critical step of phytoremediation is seedling emergence in the heavy metal-contaminated soils. Halophytes generally exhibit high salt tolerance during germination and seedling establishment. However, their ability to tolerate high levels of heavy metals is still largely unknown.

Seed germination is a critical transition because dry seeds are the most tolerant of and seedlings are the most sensitive to environmental stresses [10]. During the germination stage, seed germination percentage and velocity decrease with the increase in heavy metal concentrations [11,12]. Furthermore, seedling growth parameters are even more sensitive to heavy metal stress [13–15]. There are few studies on the inhibition of seed germination and seedling growth thereafter on wild species caused by heavy metals [6,16]. For example, seeds of several Australian native species, namely *Astrebla lappacea*, *Themeda australis*, *Austrostipa scabra* and *Acacia harpophylla*, were placed in different concentrations of arsenic (As), copper (Cu), zinc (Zn), manganese (Mn) and lead (Pb) to test their tolerance ability during germination and seedling growth stages [14].

Cu and Zn are essential micronutrients for plants at low levels of concentration, but they are toxic and retard growth or even cause death for most plant species at higher concentrations [11,17]. When in excess, Zn limits plant growth of root and expansion of leaves. Cu toxicity is characterized by chlorosis of leaves and the inhibition of root growth [18]. Cu and Zn toxicity have been reported for many plants, including many economically important crops. It has been reported that some plants have a high tolerance to Cu and Zn, and even accumulate large amounts of them in aboveground tissues [19,20].

Euhalophyte *Suaeda salsa* grows in inland saline soils and intertidal regions. This plant species has a high salt tolerance during its whole life cycle, from the germination phase to the fruiting phase [21]. In intertidal regions, *S. salsa* even can form a monospecific community. It can also absorb and accumulate different heavy metals, such as As, Pb, chromium (Cr) and Cu [7,17]. NaCl improved translocation and accumulation of Pb in *S. salsa* under Pb stress, thus enhancing the phytoextraction of Pb [22]. This species produces two types of seeds that differ in seed color, seed morph, dormancy type and germination characteristics. As Cu-tolerant halophytes, *S. salsa* plants grown from dimorphic seeds have different salt tolerances but exhibit similar responses to Cu [23]. Dimorphic seeds of *S. salsa* can germinate under a high NaCl concentration, and brown seeds have a higher germination percentage and velocity under salt stress during germination [21,24]. Although several studies have tested the tolerance ability of *S. salsa* seeds to different heavy metals [25,26], little information is known regarding the effect of seed type on heavy metal tolerance of *S. salsa*.

To evaluate the heavy metal tolerance of germinating seeds correctly, it is necessary to select an effective index. Though germination percentage and germination velocity are common indicators, several research studies suggest that indexes related to young seedlings are more suitable for metal tolerance analyses [6,14]. Thus, in this study, we selected two indexes for germinating seeds and four indexes for seedling establishment. The goal of this study was to determine the effect of Cu and Zn stress on the halophyte *S. salsa* at the germination and early seedling growth stages. Specifically, we asked the following questions: (1) Do dimorphic seeds of *S. salsa* have the same metal tolerance during germination? (2) Which index is more suitable for the proper analysis of heavy metal tolerance? (3) Does Cu exhibit stronger toxicity than Zn at the same concentrations?

2. Materials and Methods

2.1. Plant Materials

Mature fruits of *S. salsa* were collected from at least 50 plants growing in a salt desert in Karamay (North Xinjiang, China) in November 2019. Vegetation consists of plant species such as *Suaeda aralocaspica*, *Salsola subcrassa* and *Phragmites australis*. This site belongs to a temperate continental desert arid climate. The annual average temperature is 8.1 °C. The mean annual precipitation is 105 mm and mean annual potential evaporation is 3545 mm.

The fruits were allowed to dry naturally for 2 weeks in laboratory room. Seeds were separated from the dried plant material and sorted into brown seeds and black seeds. Each type of seed was pooled and stored at room temperature until used in experiments.

2.2. Chemicals

Water-soluble metal compounds were used throughout. $CuSO_4{\cdot}5H_2O$ and $ZnSO_4{\cdot}7H_2O$ were purchased from Fuchen Chemical Reagent Co., Ltd. (Tianjin, China).

2.3. Germination Experiment

Effects of different concentrations of Cu and Zn on the germination of dimorphic seeds of *S. salsa* were evaluated using single metal solutions prepared in deionized water. For each treatment, four replicates of 25 seeds were incubated in 50 mm diameter Petri dishes on two layers of No. 1 filter paper moistened with 2.5 mL of distilled water or with different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 150, 200, 250 and 300 mM) of Cu and Zn. To avoid evaporation, after being covered with lids, the edges of the Petri dishes were sealed with Parafilm. Then, the Petri dishes were transferred to light incubator chamber (GXZ-380, Jiangnan Instrument Factory, Zhejiang, China) and incubated at daily (12/12-h) temperature regimes of 10: 25 °C under a 12 h daylight photoperiod for 20 days. The dishes were opened daily to count the number of germinated seedlings. Because black seeds are smaller and the seed coat of black seeds is more rigid than that of brown seeds, the germination test standards for them are different. For black seeds, the radicle was ≥1mm. For brown seeds, the radicle was ≥2 mm. Germination percentage (%) = (the number of seeds germinated in different metal solutions/total number of seeds tested) × 100. The velocity of germination was estimated using a modified Timson's index of germination velocity [27].

2.4. Seedling Incubation

For the first week of the germination experiment, at each counting, germinated seeds were transferred into new Petri dishes (90 mm diameter) with the same solutions under the same temperature and light conditions. Incubation of the seedlings was terminated at the end of the germination experiment. Radicle length and shoot length were measured for 20 randomly selected seedlings under a microscope with Olympus CellSens software. Radicle tolerance index (%) = (mean length of the radicle in metal treatment/mean length of the radicle in distilled water control) × 100. Shoot tolerance index (%) = (mean length of the shoot in metal treatment/mean length of the shoot in distilled water control) × 100.

2.5. Data Analysis

Data for germination percentage, germination index, shoot and radicle tolerance index were analyzed by linear regression using the enter linear regression method (all independent variables were entered into the equation in a single step). The multiple linear regression model included seed type (brown and black seeds), heavy metal type (Cu and Zn) and metal concentration (0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 150, 200, 250 and 300 mM). All statistical analyses were conducted using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). All data were expressed as means ± SE. One-way ANOVA and Turkey's test were used to determine significant differences among different concentrations of Cu or Zn treatments for different indexes of each seed type of *S. salsa*. Independent samples T-Test was used to determine whether there was a difference between dimorphic seeds for different indexes of at the same Cu or Zn concentrations.

3. Results

3.1. Germination

Germination percentage and germination index were significantly affected by seed type ($p < 0.001$), metal type ($p < 0.001$) and metal concentration ($p < 0.001$) (Table 1).

Germination percentages of brown seeds in all Cu treatments ranged from 38% to 100%, whereas those of black seeds were 51–83% (Table 2). In other words, germination percentages of brown seeds were higher than that of black seeds at a low Cu concentration, similar to that of black seeds at a medium concentration, and lower at a high concentration. Germination percentages of dimorphic seeds in Zn solutions showed a similar trend. Germination indexes of brown seeds in all Cu treatments were 36.2–98.2%, whereas those of black seeds were 30.9–61.1% (Table 3). Germination indexes of brown seeds in all Zn treatments were 52.1–98%, whereas those of black seeds were 50.5–61%. At the same Cu or Zn concentration, the germination index of brown seeds was significantly higher than that of black seeds (Table 3).

Table 1. Multivariate analysis of correlates associated with germination percentage and index of dimorphic seeds of *Suaeda salsa*, radicle and shoot length of seedlings grown from both types of seeds.

Index	Factor	B (SE)	β	*p*-Value
Germination percentage	Metal type	8.607 (1.561)	0.259	<0.001
	Seed type	−10.036 (1.561)	−0.302	<0.001
	Solution concentration	−0.097 (0.008)	−0.598	<0.001
Germination index	Metal type	8.471 (1.41)	0.199	<0.001
	Seed type	−29.421 (1.41)	−0.692	<0.001
	Solution concentration	−0.102 (0.007)	−0.489	<0.001
Radicle length	Metal type	1.137 (0.209)	0.143	<0.001
	Seed type	−1.651 (0.209)	−0.208	<0.001
	Solution concentration	−0.016 (0.001)	−0.404	<0.001
Shoot length	Metal type	1.102 (0.14)	0.158	<0.001
	Seed type	−1.394 (0.14)	−0.201	<0.001
	Solution concentration	−0.024 (0.001)	−0.695	<0.001

Table 2. Effect of $CuSO_4$ and $ZnSO_4$ on germination percentage of dimorphic seeds of *S. salsa*.

Concentration (mM)	$CuSO_4$		$ZnSO_4$	
	Brown Seed	Black Seed	Brown Seed	Black Seed
0	100.0 ± 0.0 Aa	74.0 ± 2.6 Bab	100.0 ± 0.0 Aa	82.0 ± 2.6 Ba
0.01	100.0 ± 0.0 Aa	71.0 ± 1.9B Abc	99.0 ± 1.0 Aa	72.0 ± 5.4 Ba
0.05	96.0 ± 1.6 Aab	77.0 ± 4.4 Bab	100.0 ± 0.0 Aa	68.0 ± 2.3 Ba
0.1	99.0 ± 1.0 Aab	73.0 ± 3.4 Bab	98.0 ± 1.2 Aa	69.0 ± 5.7 Ba
0.5	100.0 ± 0.0 Aa	83.0 ± 2.5 Ba	99.0 ± 1.0 Aa	81.0 ± 1.9 Ba
1	98.0 ± 1.2 Aab	73.0 ± 6.6 Bab	99.0 ± 1.0 Aa	80.0 ± 5.2 Ba
5	81.0 ± 5.7 Ac	70.0 ± 4.8 Aabc	99.0 ± 1.0 Aa	76.0 ± 0.0 Ba
10	85.0 ± 3.8 Abc	71.0 ± 4.1 Babc	98.0 ± 1.2 Aa	81.0 ± 5.5 Aa
50	73.0 ± 4.4 Acd	66.0 ± 5.3 Aabc	78.0 ± 2.0 Ab	77.0 ± 3.0 Aa
100	61.0 ± 1.0 Ade	57.0 ± 5.7 Abc	70.0 ± 3.8 Abc	82.0 ± 5.0 Aa
150	55.0 ± 1.0 Be	63.0 ± 1.0 Aabc	77.0 ± 4.7 Ab	82.0 ± 2.6 Aa
200	55.0 ± 2.5 Ae	51.0 ± 3.4 Ac	69.0 ± 3.4 Abc	79.0 ± 5.3 Aa
250	63.0 ± 3.0 Ade	64.0 ± 3.7 Aabc	66.0 ± 4.2 Abc	77.0 ± 1.9 Aa
300	38.0 ± 4.8 Bf	52.0 ± 2.3 Ac	54.0 ± 10.5 Ac	78.0 ± 4.8 Aa

The standard error annotated by different lower-case letters among different concentration for the same seed type and metal type indicate significant differences at $p < 0.05$, Tukey's post hoc test. The standard errors annotated by different higher-case letters between dimorphic seeds at the same concentration of Cu or Zn indicate significant differences at $p < 0.05$, Tukey's post hoc test.

For each seed type, at the same concentration, seeds generally had a higher germination percentage and index in Zn solution than in Cu solution. For example, germination of brown seeds was 99% at 5 mM Zn solution, whereas only 81% germination was reached at 5 mM Cu solution (Table 2). Germination index of black seeds reached 53.9% at the highest level of Zn toxicity (300 mM), but the germination index of black seeds was only 30.9% at the same level of Cu toxicity (Table 3).

As Cu concentration increased from 0 to 300 mM, germination percentage and germination index of dimorphic seeds decreased (Tables 2 and 3). For brown seeds, germination percentage decreased from 100% at control to 38% at 300 mM. There was a different trend for the germination responses of dimorphic seeds in Zn solutions. The germination percentage and germination index of brown seeds also showed a decline trend as in Cu solutions. However, for black seeds, germination percentages and germination indexes did not show a significant difference among different Zn concentrations (Tables 2 and 3).

Table 3. Effect of $CuSO_4$ and $ZnSO_4$ on germination index of dimorphic seeds of *S. salsa*.

Concentration(mM)	$CuSO_4$		$ZnSO_4$	
	Brown Seed	Black Seed	Brown Seed	Black Seed
0	97.6 ± 0.4 Aa	49.0 ± 2.4B Abcd	97.4 ± 0.2 Aa	60.9 ± 2.2 Ba
0.01	97.8 ± 0.1 Aa	51.0 ± 1.0B Abc	96.5 ± 1.1 Aa	52.6 ± 4.3 Ba
0.05	93.5 ± 1.2 A Ab	56.2 ± 2.8 Bab	98.0 ± 0.4 Aa	50.5 ± 2.4 Ba
0.1	96.0 ± 0.7 Aa	50.3 ± 2.3 Babc	95.8 ± 1.0 Aa	51.1 ± 4.1 Ba
0.5	98.2 ± 0.1 Aa	61.1 ± 2.5 Ba	96.0 ± 1.1 Aa	58.7 ± 1.6 Ba
1	96.5 ± 1.1 Aa	51.9 ± 5.2B Ab	96.9 ± 1.0 Aa	58.2 ± 4.8 Ba
5	78.1 ± 5.2 Ac	49.8 ± 4.6B Abc	97.2 ± 1.1 Aa	56.9 ± 0.9 Ba
10	81.2 ± 3.9 Abc	47.5 ± 3.9B Abcd	94.9 ± 1.5 Aa	61.0 ± 3.9 Ba
50	70.5 ± 4.1 Acd	44.2 ± 3.7 Bbcde	76.4 ± 2.2 Ab	53.0 ± 3.0 Ba
100	56.7 ± 1 Ae	35.4 ± 5.5 Bbcde	68.2 ± 3.9 Abc	57.2 ± 3.9 Aa
150	52.0 ± 0.7 Ae	41.3 ± 0.5 Bde	74.5 ± 4.1 Ab	57.5 ± 2.0 Ba
200	52.6 ± 2.3 Ae	33.1 ± 1.8 Bde	66.9 ± 3.5 Abc	50.5 ± 2.9 Ba
250	59.0 ± 2.7 Ade	42.6 ± 2.2 Bbcde	63.9 ± 3.9 Abc	50.7 ± 1.3 Ba
300	36.2 ± 4.3 Af	30.9 ± 1.2 Ae	52.1 ± 10.0 Ac	53.9 ± 2.6 Aa

The standard errors annotated by different lower-case letters among different concentrations for the same seed type and metal type indicate significant differences at $p < 0.05$, Tukey's post hoc test. The standard errors annotated by different higher-case letters between dimorphic seeds at the same concentration of Cu or Zn indicate significant differences at $p < 0.05$, Tukey's post hoc test.

3.2. Seedling Growth

Radicle and shoot lengths were significantly affected by seed type ($p < 0.001$), heavy metal type ($p < 0.001$) and metal concentration ($p < 0.001$) (Table 1).

Brown seeds generally had higher shoot lengths than black seeds in the same Cu or Zn solutions (Figures 1 and 2). Radicle lengths of brown seeds in all Cu treatments were 2.2–14.3 mm, whereas those of black seeds were 1.2–8.3 mm (Figure 1). Radicle length of brown seeds was higher than that of black seeds at the same Cu concentrations. Similarly, the radicle of brown seeds had higher length growth than that of black seeds in the same Zn solutions (Figure 2).

For each seed type, at the same concentration, seeds generally had higher lengths and better growth of radicles and shoots in Zn solution than that in Cu solution. For example, the radicle length of brown seeds was 10.0 mm at 0.1 mM Zn solution, whereas it was only 4.4 mm at the same concentration of Cu. Radicle growth was almost completely inhibited by Cu at 10 mM, and by Zn at 50 mM (Figures 3 and 4).

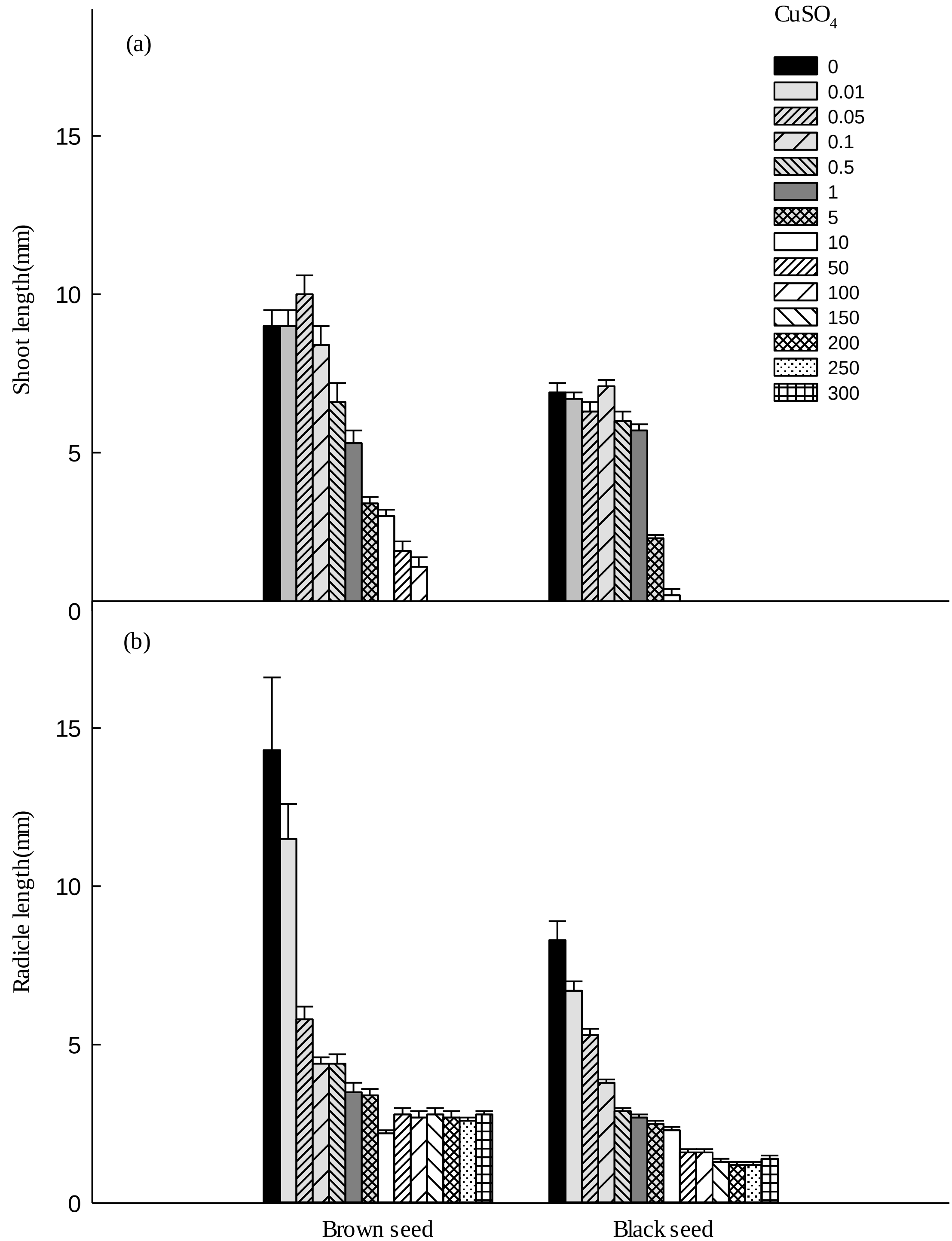

Figure 1. Effect of Cu on (**a**) shoot and (**b**) radicle length of seedlings grown from dimorphic seeds of *S. salsa*.

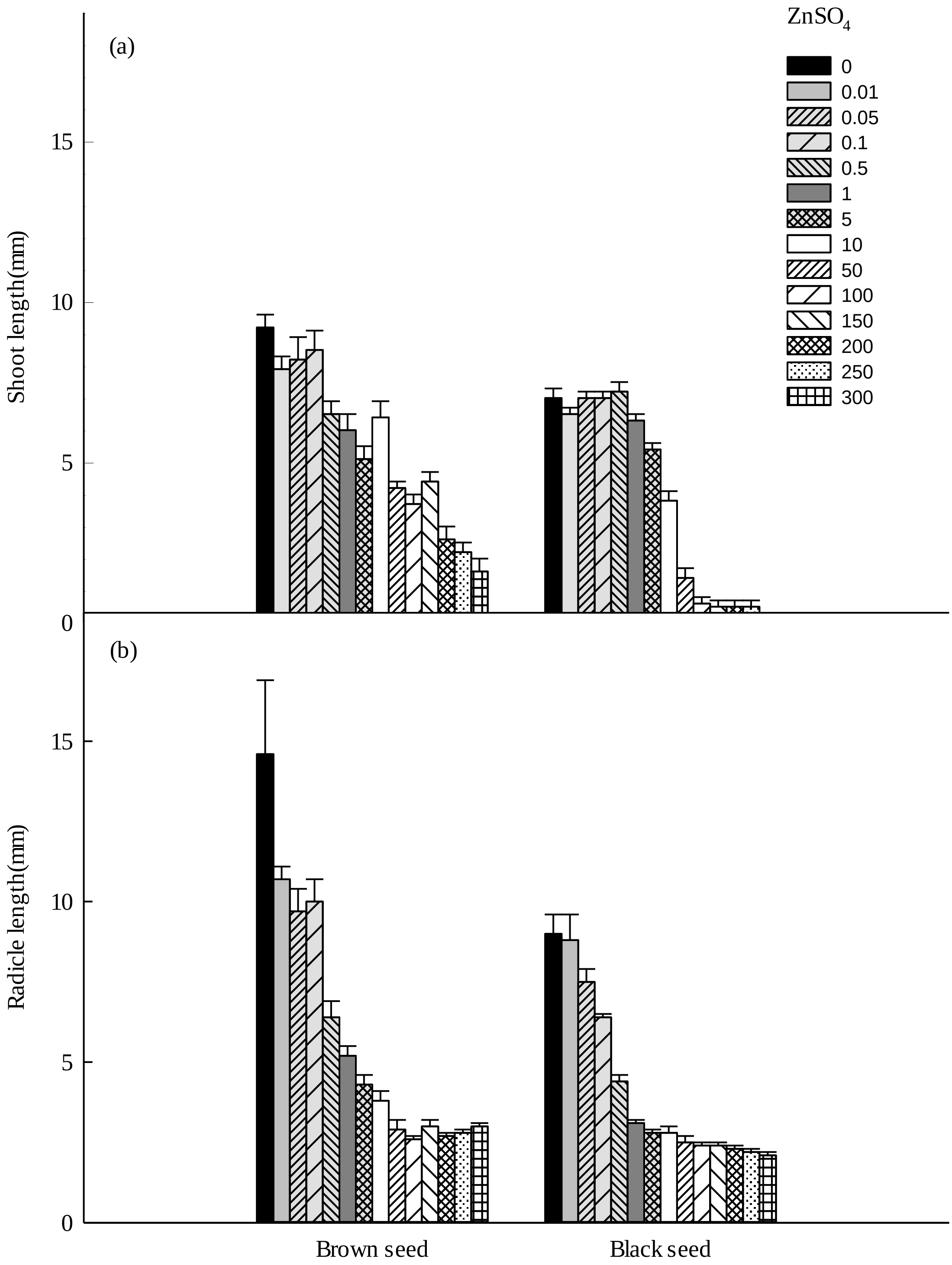

Figure 2. Effect of Zn on (**a**) shoot and (**b**) radicle length of seedlings grown from dimorphic seeds of *S. salsa*.

Figure 3. Radicle elongation of seedlings grown from dimorphic seeds of S. salsa after 20 days in various concentrations of Cu solutions.

Figure 4. Radicle elongation of seedlings grown from dimorphic seeds of *S. salsa* after 20 days in various concentrations of Zn solutions.

The radicle and shoot length of seedlings grown from dimorphic seeds decreased with the increase of metal concentrations. Radicle length from brown seeds was significantly reduced compared to the control at Cu concentrations of 0.05 mM and higher. Shoot length from brown seeds was not affected by Cu up to 0.1 mM but it was significantly reduced at 0.5 mM and progressively reduced further at higher concentrations compared to control (Figures 1 and 3). The decreasing trend of radicle and shoot lengths in Zn solutions was similarly observed in Cu solutions, but was less severe (Figure 2).

The radicle tolerance index and the shoot tolerance index showed similar changes in radicle and shoot lengths (Tables 4 and 5). Both indexes were usually higher in Zn solutions. The radicle tolerance index was more sensitive to metal toxicity than the shoot tolerance index. The radicle tolerance index reached limitation values in relatively lower metal concentrations. For example, the limitation value for the radicle tolerance index of seedlings from brown seeds is 10 mM Cu concentration. However, for shoot tolerance index, it was 150 mM Cu concentration (Table 4).

Table 4. Effect of $CuSO_4$ and $ZnSO_4$ on the radicle tolerance index of *S. salsa* seedlings grown from brown and black seeds.

Concentration (mM)	$CuSO_4$		$ZnSO_4$	
	Brown Seed	Black Seed	Brown Seed	Black Seed
0	100.00	100.00	100.00	100.00
0.01	80.33	81.08	73.40	97.68
0.05	40.53	63.80	66.88	83.18
0.1	30.65	45.70	68.61	70.8
0.5	30.66	34.71	43.82	48.47
1	24.40	32.26	35.95	34.83
5	23.77	29.59	29.53	30.62
10	15.35	27.62	26.05	30.73
50	19.25	18.93	19.68	27.82
100	18.73	18.79	17.79	26.07
150	19.52	16.23	20.62	26.60
200	18.56	14.04	18.79	26.06
250	17.91	14.95	18.91	23.94
300	19.21	16.32	20.76	23.03

Table 5. Effect of $CuSO_4$ and $ZnSO_4$ on shoot tolerance index of *S. salsa* seedlings grown from brown and black seeds.

Concentration (mM)	$CuSO_4$		$ZnSO_4$	
	Brown Seed	Black Seed	Brown Seed	Black Seed
0	100.00	100.00	100.00	100.00
0.01	110.38	96.15	86.03	95.08
0.05	95.48	91.51	88.74	99.15
0.1	93.58	102.76	92.82	100.06
0.5	72.58	87.00	71.07	102.76
1	58.40	81.77	65.50	90.03
5	37.51	33.47	54.97	76.59
10	33.28	7.61	69.47	53.57
50	21.58	0.00	45.73	19.57
100	15.96	0.00	40.69	8.16
150	0.00	0.00	47.44	7.73
200	0.00	0.00	28.13	7.55
250	0.00	0.00	23.56	7.42
300	0.00	0.00	17.39	0.00

4. Discussion

Although dormancy and germination of dimorphic seeds of *S. salsa* and eco-physiological responses of plants grown from dimorphic seeds to different environmental factors have been studied extensively [5,21,23,24], our study is the first to analyze germination of dimorphic seeds and seedling growth thereafter in different heavy metal solutions. In addition, our data indicate that both types of seeds of *S. salsa* have high Cu and Zn tolerance during the germination stage, and young seedling growth is a critical stage for metal tolerance. Cu is more toxic than Zn to germinating seeds, especially for radicle growth. The results suggest that seeds and seedlings of *S. salsa* are not only tolerant to high salinity but also to high levels of heavy metals, such as Cu and Zn.

Dimorphic seeds of *S. salsa* displayed differential germination responses to Cu and Zn toxicity. The germination percentage of brown seeds reached 38% and 54% at 300 mM Cu and Zn, respectively. However, germination of black seeds was 54% and 78%. The germination difference of dimorphic seeds in metal solutions might be related to their differential salt tolerances. Brown seeds of *S. salsa* are more salt tolerant than black seeds [21]. The contrasting abiotic tolerances of dimorphic seeds are also found in other plant species, such as *Bidens pilosa* [28], as well as *Arthrocnemum macrostachyum* [29] and *Torilis arvensis* [30]. This might be due to the difference in antioxidant ability between dimorphic seeds [29]. Furthermore, the tolerance difference of dimorphic seeds to abiotic stresses even has a carry-over effect. For example, the differential salinity tolerance of dimorphic seeds of *Suaeda splendens* carried over from seeds to seedlings [31]. Radicle and shoot lengths of brown seeds are generally longer than those of black seeds of *S. salsa* under the same metal treatment, however, this might be caused by the size difference between dimorphic seeds and the delayed germination of black seeds. Besides, the radicle and shoot tolerance indexes did not show a clear difference in pattern for dimorphic seeds. Moreover, adult plants grown from dimorphic seeds of *S. salsa* exhibited similar eco-physiological responses to Cu. Thus, heavy metal tolerance of seedlings between dimorphic seeds needs more indexes to evaluate.

Cu had more inhibitory effects on seed germination and, thereafter, seedlings of *S. salsa* compared to Zn. Cu and Zn are essential micronutrients and are required for different physiological processes. However, at high concentrations, both metals stimulate the production of ROS (Reactive oxygen species) and adversely affect plant growth and metabolism. Cu at a high concentration has higher toxicity than Zn [12]. This can also be reflected by the definition of Cu and Zn hyperaccumulator plants. Hyperaccumulation of Cu was defined as >300 ppm foliar Cu [32]. Zn hyperaccumulator can accumulate >10,000 ppm in aerial parts [20]. The germination index of brown and black seeds reached 81.2 and 47.5, respectively, at 10 mM Cu, but the germination index of brown and black seeds was 94.9 and 61, respectively, at the same concentration of Zn. Furthermore, at the seedling growth stage, the radicle tolerance indexes of brown and black seeds were 30.65 and 45.7, respectively, at 0.1 mM Cu, but that were 68.61 and 70.81 at 0.1 mM Zn. *S. salsa* seeds had high Cu and Zn tolerance during germination. Even at 100 mmol L^{-1} Cu and Zn, the germination percentages of both types of seeds were higher than 65%. Germination percentages of most plant species significantly decline under 0.5 mM Cu and Zn [14,33]. However, germination of *S. salsa* seeds did not show a significant difference until under 5 mM Cu and Zn. Seedlings of *S. salsa* did not show obvious toxic symptoms under 0.1 mM Cu and 1 mM Zn (Figure 3). Thus, *S. salsa* was tolerant to high concentrations of Cu and Zn at the germination and seedling stages.

Among all the indexes, radicle length and radicle tolerance index are more sensitive to Cu and Zn. Germination percentage and germination index are insensitive to metal stress, especially under Zn treatments. For black seeds, germination percentages and indexes did not significantly change under 0 to 300 mM Zn. Compared with the indexes of germinated seeds, the indexes of seedlings were more sensitive to metal stresses. With the increase in metal concentration, indexes of seedlings decrease gradually. For example, the radicle tolerance index of black seeds decreased from 100 to 48 when the Zn concentration changed from control to 0.5 mM Zn. It was also found that the indexes of radicle were more sensitive to metal stresses than those of shoot. For example, the radicle tolerance index of brown seeds was 24 under 1 mM Cu and the shoot tolerance index of brown seeds was 58 under the same Cu concentration. This high sensitivity of radicles can be explained by the fact that the radicle is the first part of a seedling to suffer from metal toxicity. Thus, when selecting high metal-tolerant halophytes, we should test not only the tolerance indexes of seeds and adult plants but also the indexes of seedlings.

5. Conclusions

In summary, this study shows that indexes of radicle are the most sensitive and effective indicator of metal tolerance for germinating seeds of *S. salsa*. These indexes may also serve as a useful index for future research on the tolerance of different metals for plants during germination and seedling growth stages. The present study demonstrates that dimorphic seeds of *S. salsa* are able to germinate under high Zn and Cu concentrations. However, seedling growth, especially the radicle growth, is more sensitive to the same level of metal stress. Overall, successful germination and survival of seedlings of *S. salsa* under medium Zn and Cu metal stress has been proved. Thus, this plant species might provide an important contribution to phytoremediation by sowing seeds directly into heavy metal contaminated soils.

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