***Pak. J. Bot.*, 50(3): 871-877, 2018.**

COPPER-INDUCED SIMILAR CHANGES IN GROWTH AND PHYSIOLOGICAL RESPONSES OF PLANTS GROWN FROM DIMORPHIC SEEDS OF *SUAEDA SALSA*

LI JIANG[1,2], LEIWANG[3*], KE ZHANG[3] AND CHANGYAN TIAN[3]

[1]Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography,
Chinese Academy of Sciences, Urumqi 830011, China
[2]Turpan Eremophytes Botanical Garden, Chinese Academy of Sciences, Turpan 838008, China
[3]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography,
Chinese Academy of Sciences, Urumqi 830011, China
[*]Corresponding author's E-mail: egiwang@ms.xjb.ac.cn

Abstract

There are few studies related to the responses of plants grown from heteromorphic seeds to heavy metals. Therefore we tested the hypothesis that plants grown from dimorphic seeds of the halophyte *Suaeda salsa* have high copper (Cu) tolerance but differ in physiological and growth responses. We examined the effect of Cu on plant growth, copper accumulation, chlorophyll content and other mineral elements of both types of plants via a pot experiment. The results demonstrated that, although dry weight, chlorophyll content and most of the mineral elements were significantly influenced by Cu, there were no significant differences in tolerance responses and copper accumulation between plants from dimorphic seeds. Dry weight and tolerance index did not significantly decrease until ≥ 800 mg kg^{-1} Cu. With increasing the content of Cu, chlorophyll content increased and then decreased. Roots accumulated higher levels of Cu than shoots. The bioconcentration and translocation factors were <1.0 under all Cu addition treatments. Our study is the first report of ecophysiological responses of plants grown from heteromorphic seeds to a heavy metal and suggests that both types of *S. salsa* plants are considered as Cu-tolerant halophyte and exhibit similar response to Cu.

Key words: Copper; Dimorphic seed; Halophyte; Phytoremediation; *Suaeda salsa*.

Introduction

Mineral mining and smelting, application of sewage sludge, use of fungicides, etc., may lead to copper (Cu) enrichment and even soil pollution (Kabata-Pendias, 2010). The accumulation of Cu in soils and plants poses risks for animals and humans because Cu can be ingested via the consumption of contaminated water and plants. In consideration of toxicity of Cu and its impact on health and the environment, many methods have been proposed for remediating Cu contaminated soil (Koptsik, 2014). However, most of these methods are expensive and/or cause degradation of soil quality (Pilon-Smits, 2005). To date, the best way to clean Cu polluted soils is by phytoremediation, including the two most common techniques: phytoextraction and phytostabilization (Sarwar *et al.*, 2017). Selecting suitable plants to extract or stabilize Cu from contaminated soils receives more attention than using traditional remediation methods, mostly because this alternative ecological technology is inexpensive and has the advantage of preserving the biological and physical properties of the soils (Mateos-Naranjo *et al.*, 2013; Goswami &Das, 2016).

Screening accumulators and hyperaccumulators as well as the stabilizer plant species is a crucial step towards feasible phytoremediation of Cu contaminated soils (Zhang *et al.*, 2010; Goswami & Das 2016). Up to now, 34 species of Cu hyperaccumulators have been found (Reeves, 2003). However, few of them can grow well in saline soils (Wang *et al.*, 2014). When faced with the combined pollution caused by salt and Cu, it is impossible to remediate Cu contaminated saline soils by using glycophytic accumulators or stabilizers. Halophytes potentially have high tolerance to Cu, partly due to their physiological basis for salt tolerance (Manousaki & Kalogerakis, 2011; Cambrollé *et al.*, 2013). Therefore, halophytes could be excellent candidates for phytoremediation of Cu polluted saline soils. Recent studies show that several halophytes have an inherent tolerance to Cu. For example, the halophytic species *Atriplex halimus* can tolerate and accumulate high contents of Cu (Mateos-Naranjo *et al.*, 2013).

As an essential trace element for plant growth and metabolism, Cu is involved in carbohydrate, protein, photosynthesis, cell wall metabolism, etc (Peña *et al.*, 1999; Quartacci *et al.*, 2003; Kabata-Pendias, 2010; Adrees *et al.*, 2015). However, excessive amounts of Cu lead to growth inhibition by inhibiting enzyme activity, membrane integrity, photosynthesis, and other physiological processes (Quartacci *et al.*, 2003; Zhang *et al.*, 2010; Mateos-Naranjo *et al.*, 2013). For example, chlorophyll and carotenoid pigment contents of Calendula officinalis plants decreased significantly at all Cu contents (150-400 mg kg^{-1}) (Goswami & Das, 2016). However, in several studies halophytes have been reported to be able to grow in Cu-polluted soils without a marked impact on their growth (Mateos-Naranjo *et al.*, 2013; Chai *et al.*, 2014). It also has been shown that*Suaeda salsa* can tolerate high contents of Cd and Zn (Liu *et al.*, 2011; Wu *et al.*, 2013; Liu *et al.*, 2016), which indicates that this plant is likely to tolerant high Cu content.

Suaeda salsa (L.) Pall. (Amaranthaceae) is an annual halophytic herb with succulent leaves that is particularly abundant in saline and alkaline soils of beaches and lake shores in China and appears to grow optimally under moderately saline conditions (Song & Wang, 2015). *Suaeda salsa* is suggested to be a heavy-metal bioindicator and potential accumulator to remediate heavy metal polluted soil. It produces non-dormant brown seeds and black seeds with non deep physiological dormancy (Song & Sun, 2014). Plants derived from dimorphic *S. salsa* seeds exhibit different salt tolerance, but they have similar growth and physiological responses to nitrate nitrogen (Jiang *et al.*, 2012). However, information is lacking about the responses of plants grown from dimorphic seeds of *S. salsa* to heavy metals.

Based on the high and differential salt tolerance of plants grown from dimorphic seeds of *S. Salsa* (Jiang *et al.*, 2012), we hypothesized that the two types of plants have high Cu tolerance but differ in growth and physiological responses to Cu. To test this hypothesis, we measured growth parameters, chlorophyll content, ion content, and Cu accumulation of plants from both types of *S. salsa* seeds under a series of Cu treatments.

Materials and Methods

Seeds: Mature seeds were collected from plants of *S. salsa* growing in a natural population located on an inland saline area (37°20′ N, 118°36′ E) on the Yellow River Delta in Shandong Province, China, in October 2013. Seeds were stored dry at 4°C until transferred to Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, on 29 March 2014. Then, the seeds were stored dry at room temperature (20-23°C) for 15 days before they were used in pot experiments.

Plant culture and treatments: The pots were placed in a greenhouse at Fukang Field Research Station of the Chinese Academy of Sciences (44°17′ N, 87°56′ E; 460 m a.s.l.), located in the southern part of the Junggar Basin of Xinjiang, China. Non polluted gray desert topsoil (0-20 cm) was collected from the field and used as the growth substrate and control. Soil pH was 8.47, organic matter 0.92%, total N 0.96 g kg^{-1}, available N 89.54 mg kg^{-1}, available P 9.24 mg kg^{-1}, available K 341.16 mg kg^{-1}, and Cu content 34.95 mg kg^{-1}. The soil was air-dried and homogenized, after which it was sieved through 4-mm mesh.

Each plastic pot (20 cm height, 20 cm diameter) was filled with 3 kg of air-dry soil mixed with different contents of Cu (solution prepared by dissolving analytical grade $CuSO_4·5H_2O$): 0 (CK without external Cu), 50, 200, 400, 800, and 1500 mg kg^{-1}. After the pot soil was homogenized for four weeks, dimorphic seeds of *S. salsa* were sown into each pot on 14 April, 2014. There were five replicates for each treatment, and they were arranged in a randomized block design. Each pot received 6 g granular lawn fertilizer (Osmocote 301, Scotts, Marysville, OH, USA) once at the beginning as the basic fertilizer. Each pot was irrigated daily with 100 mL tap water. A plastic pallet was placed under each pot to collect potential leachate. Plants were harvested after 90 days.

Plant growth parameters: After harvest, plants were separated intoleaves, stems and roots. The leaves and stems were rinsed with distilled water three times and then blotted with filter paper. The roots were dipped in a $CaCl_2$ solution for 5 minutes to get rid of trace elements adsorbed at the root surface and then rinsed three times with distilled water. The dry weight was measured after the fresh material was oven-dried at 80°C for 48 h.

Tolerance index: Tolerance index, a measure of the tolerance of plants to biotic and abiotic stresses (in our study Cu stress) was determined by comparing the dry biomass of plants subjected to metal treatment with the control using the equation: Tolerance index = (Biomass of treated plants / Biomass of control plants) × 100 (Wilkins, 1978).

Chlorophyll contents: Fresh mature whole leaves (0.2 g) were extracted in dark at 4ºC with a 5 ml mixture of acetone and ethanol (v/v = 1:1) until the color of the tissue disappeared completely. After centrifugation, light absorbance at 663 and 645 nm was measured. The contents of chlorophyll a, chlorophyll b, and chlorophyll (a + b) were calculated using adjusted extinction coefficients (Lichtenthaler, 1987) and expressed as μmol g^{-1} FW.

IonContents: Cu^{2+}content of dried plant shoots were determined using inductively coupled plasma spectroscopy (ICP-OES) after digestion with HNO_3:$HClO_4$ (3:1, v/v).Water-soluble Na^+, K^+, Mg^{2+}, and Ca^{2+}contents of dried shoots were determined with inductively coupled plasma spectroscopy (ICP-OES) after extraction with distilled water. Water-soluble Cl^- and SO_4^{2-} contents of dried shoots were determined by ion chromatography (ICS-5000) after extraction with distilled water.

Bioconcentration factor and translocation factor: The bioconcentration factor (BCF), which indicates the ability of plants to translocate heavy metals from soil to roots, was calculated by dividing the metal contents in roots by the metal content in soils, on a dry weight basis. Translocation factor (TF), which indicates the ability to translocate heavy metals from roots to shoots, was calculated by dividing the metal content in shoots over the metal content in roots, on a dry weight basis.

Statistical analysis

All analyses were performed with SPSS Version 16.0 (SPSS Inc.) and data were log transformed when necessary to meet the requirements for normality and homogeneity of variances. Two-way ANOVA was used to determine the effects of plant type and Cu content, as well as their interaction on dry weight, tolerance index, chlorophyllcontent, ion content, BCF, and TF. Duncan's test was performed to determine significant ($p<0.05$) differences between individual treatments.

Results

Plant growth: There was no significant difference between plants grown from the two seed morphs of *S. salsa* in terms of leaf, stem and root dry weight at the same Cu treatments (Table 1). Dry weight did not decrease significantly until the added Cu treatment exceeded 800 mg kg^{-1} (Fig. 1A, B, C). Stems and roots even had significantly higher dry weights in the 50 mg kg^{-1}Cu treatment than in the control (Fig. 1B, C).

Tolerance index in the series of Cu treatments was a downward parabola, reached the maximum value at 50 mg kg^{-1}Cu treatment, and significantly decreased at 1500 mg kg^{-1}Cu treatment (Fig. 1D).

Table 1. F values from ANOVA for the effects of seed type, copper and their interactions on leaf dry weight, stem dry weight, root dry weight, Chl a content, Chl b content, Chl (a+b) content, Na^+ content, K^+ content, Mg^{2+} content, Ca^{2+} content, Cl^- content, SO_4^{2-} content, shoot Cu^{2+}content, and root Cu^{2+}content of plants grown from dimorphic seeds of *S. salsa*.

Source	Copper	Seed type	Copper ×Seed type
Leaf dry weight	7.11***	0.07	0.11
Stem dry weight	13.52***	1.16	0.41
Root dry weight	15.80***	0.01	0.59
Chl a content	29.45***	0.00	0.72
Chl b content	23.80***	0.46	1.20
Chl (a+b) content	25.74***	0.06	1.02
Na^+ content	6.00***	1.91	1.24
K^+ content	11.83***	0.15	1.35
Mg^{2+} content	13.04***	0.01	0.52
Ca^{2+} content	3.18**	0.90	0.46
Cl^- content	40.16***	0.29	1.16
SO_4^{2-} content	3.99**	0.03	0.03
Shoot Cu^{2+}content	189.36***	1.32	0.27
Root Cu^{2+}content	396.66***	1.52	0.20

Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Five replicates for leaf dry weight, stem dry weight, root dry weight, Chl a content, Chl b content, Chl (a+b) content, Na^+ content, K^+ content, Mg^{2+} content, Ca^{2+} content, Cl^- content, SO_4^{2-} content, shoot Cu^{2+}content, and root Cu^{2+}content of plants grown from dimorphic seeds of *S. salsa*

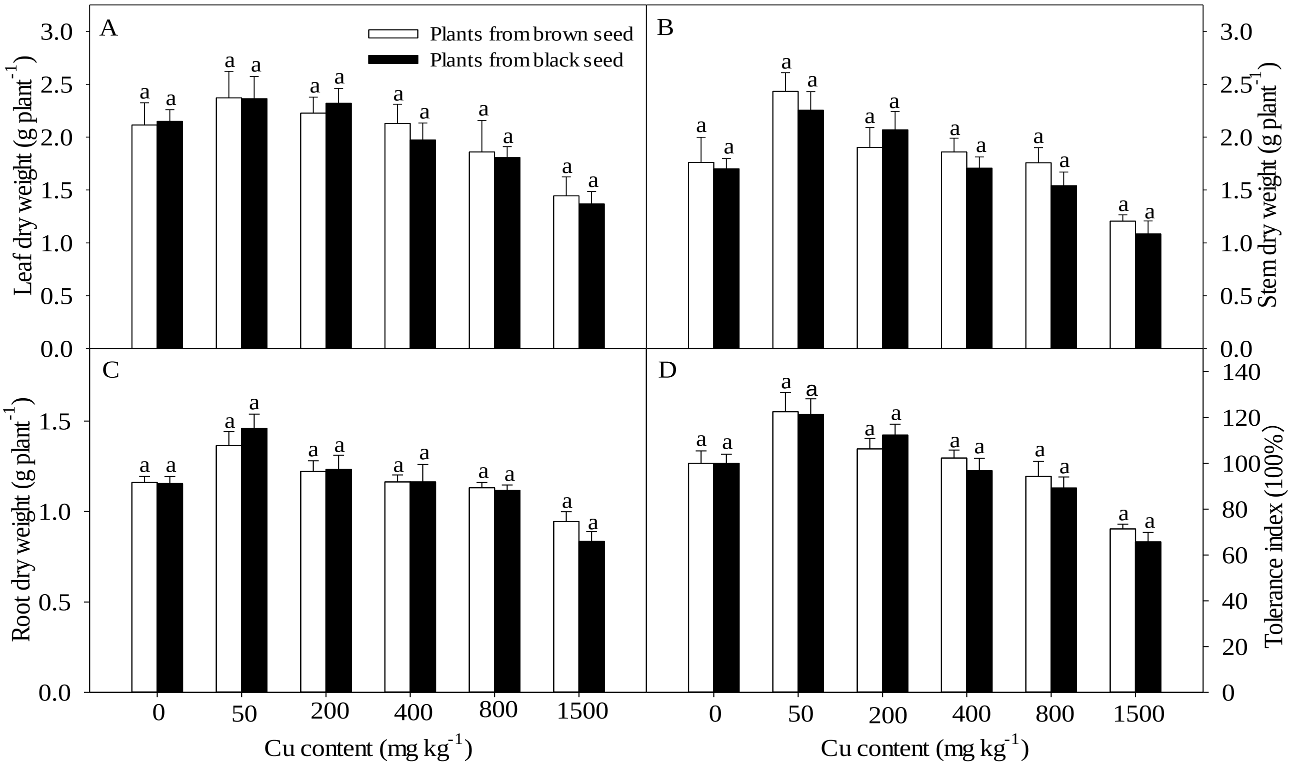

Fig. 1. Effect of Cu on leaf dry weight (A), stem dry weight (B), root dry weight (C), and tolerance index (D) of plants grown from dimorphic seeds of *S. salsa*. Values with different lowercase letters are significantly different ($p<0.05$) for treatment effect within the same copper level. Values represent the means ± S.E. of five replicates.

Chlorophyll contents: There was no significant difference between the two plant types of *S. salsa* in chlorophyll a, chlorophyll b, and chlorophyll (a + b) contents in the same Cu treatment. Chlorophyll content was significantly affected by soil-added Cu (Table 1). Except for chlorophyll a content of plants grown from brown seeds, chlorophyll a content of plants grown from black seeds, chlorophyll b and chlorophyll (a + b) content of both plant types reached the highest value at 200 mg kg^{-1} Cu. Chlorophyll a, b, and (a + b) contents had the lowest values at 1500 mg kg^{-1}Cu (Fig. 2).

Ion contents: Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} contents in shoots showed significant responses to Cu treatments, although there was no significant difference between the two plant types in ion contents at the same Cu treatment (Table 1).

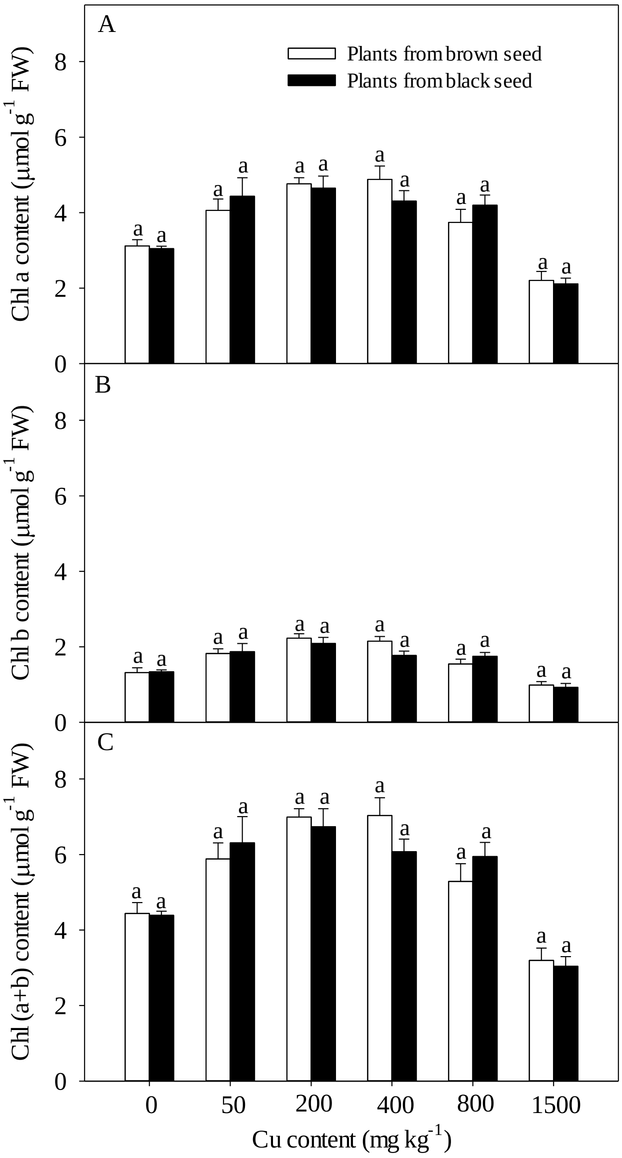

Fig. 2. Effect of Cu on Chl a (A), Chl b (B), and Chl (a+b) (C) contents of plants grown from dimorphic seeds of *S. salsa*. Values with different lowercase letters are significantly different ($p<0.05$) for treatment effect within the same copper level. Values represent the means ± S.E. of five replicates.

Na^+ and K^+ contents in shoots increased under Cu treatments from 0 to 800 mg kg^{-1} and then decreased from 800 to 1500 mg kg^{-1}(Fig. 3A, B). The trajectories of Mg^{2+} and Ca^{2+} contents showed an increase from 0 to 400 mg kg^{-1} Cubut a decrease from 400 to 1500 mg kg^{-1}Cuwas recorded (Fig. 3C and D).

Cl^- and SO_4^{2-} contents in shoots differed. Cl^- contents were increased under Cu treatments from 0 to 1500 mg kg^{-1} (Fig. 3E), whereas SO_4^{2-} contents were decreased (Fig. 3F).

Cu accumulation and translocation: There was no significant difference between plants from the two seed types in Cu^{2+}content. Cu^{2+}contents in shoots and roots of both plant types significantly increased as soil-added Cu increased from 0 to 1500 mg kg^{-1}. Cu^{2+} contents were higher in roots than in shoots in the same Cu treatment (Fig. 4A and B).

Plants from dimorphic seeds did not differ significantly in BCF and TF. Under Cu treatments from 0 to 1500 mg kg^{-1}, BCF of plants from brown seeds ranged from 1.27 to 0.58 and those from black seeds from 1.31 to 0.62 (Fig. 4C). TF values of plants from brown and black seeds were from 1.01 to 0.13 and from 0.95 to 0.11, respectively.

Discussion

Although dormancy and germination of hetermorphic seeds and ecophysiological responses of heteromorphic plants to various environmental factors have been studied extensively (Mandak, 1997; Imbert, 2002; Redondo-Gomez *et al.*, 2008; Lu *et al.*, 2013; Cao *et al.*, 2015), our study is the first one on responses to a heavy metal. Our data indicate that the two plant types of *S. salsa* do not differ significantly in growth and physiological responses to Cu treatments. BCF and TF indicate that *S. salsa* is not a Cu-accumulator, just a Cu-tolerant excluder halophyte.

Unlike some Cu accumulator plants, which can have >1000 mg kg^{-1} Cu in shoots (Koptsik, 2014), the critical toxicity level of Cu in shoots of *S. salsa* was about 100 mg kg^{-1} dry matter (Fig 4A). Cu content in tissues increased dramatically with soil Cu addition (from 50 to 1500 mg kg^{-1}), reaching values between 63 and 111 mg kg^{-1} and 96 and 955 mg kg^{-1} for shoots and roots, respectively. BCFs and TFs did not exceed the critical value (1.0) under any of the soil Cu addition treatments. The low content of Cu in shoots and the values of BCFs and TFs indicate that *S. salsa* is not a Cu accumulator. Cu toxicity thresholds of both plant types were determined by the analysis of its growth, ion content and Cu content in shoots and roots. Cu content causing a significant decrease in these indexes was> 800 mg kg^{-1}. This threshold content indicates that this species is a Cu-tolerant halophyte and has great potential to be grown in Cu contaminated saline soil.

The effect of Cu on plants is not only in growth performance but also on physiological responses (Cambrollé *et al.*, 2013; Chai, 2014; Adrees *et al.*, 2015). As an essential trace element, Cu increased plant growth of *S. salsa* when incubated at 0-200 mg kg^{-1}. However, Cu content at 1500 mg kg^{-1} significantly inhibited plant growth. These changing dynamics may be ascribed to the variation in chlorophyll content. Cu is a component of the plastocyanin and other chloroplast Cu proteins, and thus it has a marked effect on photosystems I and II (Aguirre & Pilon, 2015). Proper Cu content in shoots is essential for photosynthesis to perform normally. Therefore, the high tolerance of *S. salsa* was related to its capacity to avoid excessive absorption of Cu in the leaves. Cu also influences the absorption of essential mineral elements, K, Mg, Ca, S, Cl, and of Na^+, which is beneficial, but not essential, for plant growth. Ion homeostasis is critical for plants to sustain physiological-biochemical functions. As a euhalophyte, *S. salsa* plants accumulate a large number of Na^+ and Cl^- ions that function in osmotic adjustment and balance the osmotic pressure in the cytoplasm (Maeda *et al.*, 2010). *S. salsa* needs to absorb a great deal of Cl^- to balance the Cu ions entering plant tissues when treated with high content of Cu. Meanwhile, the content of other cations (K^+, Ca^{2+}, and Mg^{2+}) and the anion (SO_4^{2-}) also need to change for ion balance.

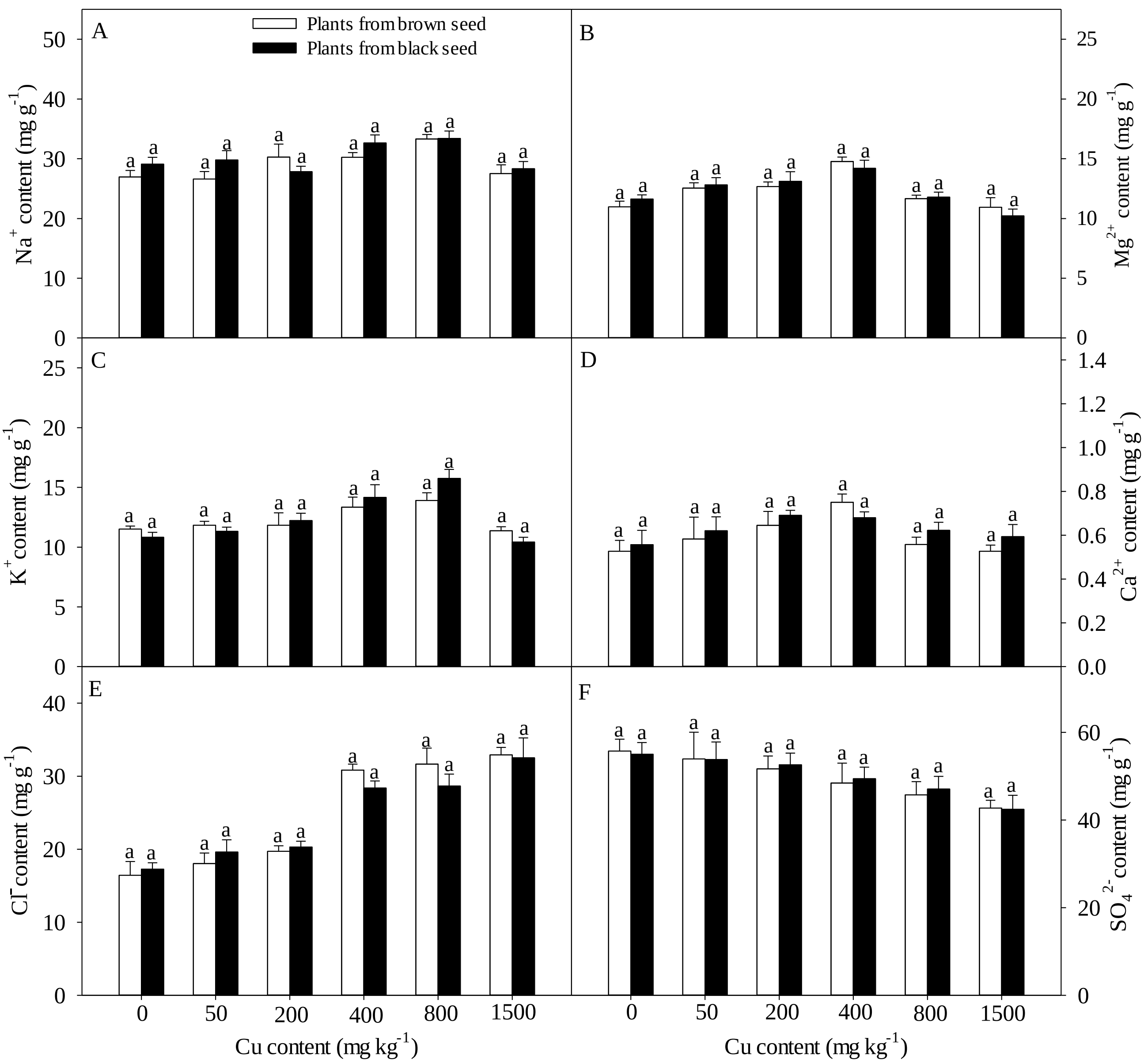

Fig. 3. Effect of Cu on Na^+ (A), K^+ (B), Mg^{2+} (C), Ca^{2+} (D), Cl^- (E), and SO_4^{2-} (F) contents in shoot of plants grown from dimorphic seeds of *S. salsa*. Values with different lowercase letters are significantly different ($p<0.05$) for treatment effect within the same copper level. Values represent the means ± S.E. of five replicates.

Contrary to our expectation, plants of *S. salsa* grown from dimorphic seeds did not differ significantly in their tolerance to Cu. The most remarkable trait of *S. salsa* is the apparent carry-over of differential salinity tolerance from seed type to plants (Jiang *et al.*, 2012). Using this trait, *S. salsa* can adjust to the deal with temporal and spatial variation of salinity in its habitat, apart from benefiting from applying differences in seed dormancy and germination (Li *et al.*, 2015, Wang *et al.*, 2015). Nitric Oxide (NO) contributes to the differential salt tolerance of seedlings grown from dimorphic seeds of *Atriplex centralasiatica* by modulating antioxidative enzyme activity, hydrogen peroxide accumulation and the ion equilibrium (Xu *et al.*, 2011). So next step is to analyze the possible underlying mechanism of NO on the differential salt tolerance of both plant types of *S. salsa*. As for the similar Cu tolerance for both plant types, we speculate that Cu is not an important ecological factor for *S. salsa*. However, it is necessary for osmotic adjustment and for activating fundamental physiological pathways in this euhalophyte.

In conclusion, both plant types of *S. Salsa* have similar high tolerance to Cu stress and accumulate a small amount of Cu in shoots and a relatively large amount of Cu in roots. This indicates that *S. salsa* has the potential to be used as a Cu phytostabilizor in saline soils. However, since *S. salsa* is an annual plant it would die and decay after completing its life cycle and thus Cu accumulated in the roots would be released back into soil. Thus, *S. salsa* can only be used as phytoextractor for Cu-contaminated saline soils. Meanwhile, *S. salsa* absorbs a small amount of Cu in aboveground tissues, reducing health risks when served as wild vegetable or forage. Further research is needed to explore the physiological and molecular basis of similarity in Cu tolerance responses of plants from the two types of *S. Salsa* seeds.

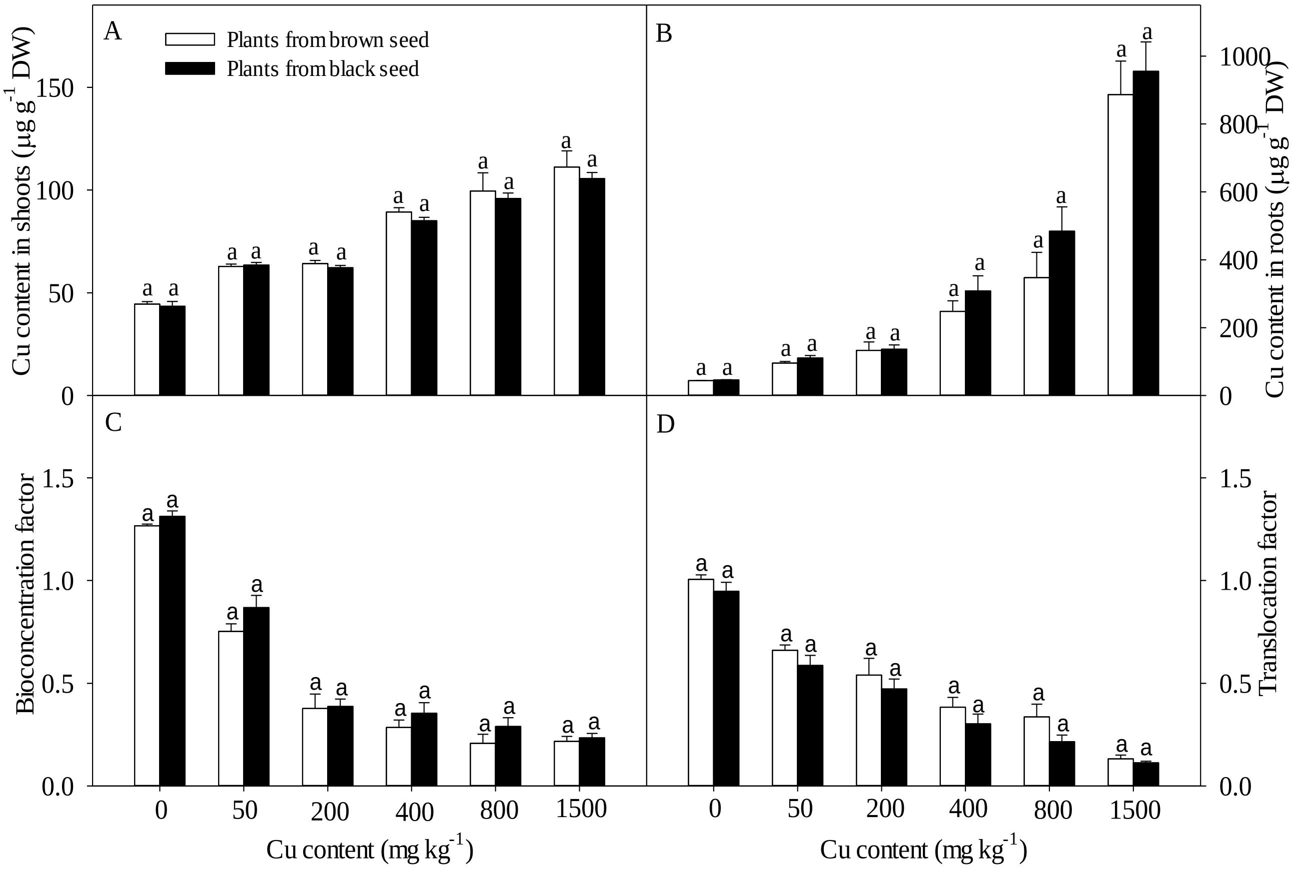

Fig. 4. Effect of Cu on copper content in shoot (A) and root (B), bioconcentration factor (C) and translocation factor (D) of plants grown from dimorphic seeds of *S. salsa*. Values with different lowercase letters are significant different ($p<0.05$) for treatment effect within the same copper level. Values represent the means ± S.E. of five replicates.

Acknowledgements

We thank Prof. Carol Baskin and Prof. Jerry Baskin (University of Kentucky) for assistance with the manuscript revision. This research was supported by the National Key Research and Development Program (2016YFC0501400), the National Natural Science Foundation of China (31770451), and the China Postdoctoral Science Foundation (2014M562489).

References

Adrees, M., S. Ali, M. Rizwan, M. Ibrahim, F. Abbas, M. Farid, M. Zia-ur-Rehman, M.K, Irshad and S.A. Bharwana. 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environ. *Sci. Pollut. R.,* 22: 8148-8162.

Aguirre, G. and M. Pilon. 2015. Copper delivery to chloroplast proteins and its regulation. *Front. Plant Sci.,* 6: 1250-1260.

Cambrollé, J., J.M. Mancilla-Leytón, S. Muñoz-vallés, E. Figueroa-Luque, T. Luque and M.E. Figueroa. 2013. Effects of copper sulfate on growth and physiological responses of *Limoniastrum monopetalum*. *Environ. Sci. Pollut. R.,* 20: 8839-8847.

Cambrollé, J., J.M. Mancilla-Leytón, S. Muñoz-vallés, T. Luque and M.E. Figueroa. 2012. Tolerance and accumulation of copper in the salt-marsh shrub *Halimione portulacoides*. *Mar. Pollut. Bull.,* 64: 721-728.

Cao, J., X.Y. Lv, L. Chen, J.J. Xing and H.Y. Lan. 2015. Effects of salinity on the growth, physiology and relevant gene expression of an annual halophyte grown from heteromorphic seeds. *AoB Plants,* 7: 1-20.

Chai, M.W., F.C. Shi, R.L. Li, G.Y. Qiu, F.C. Liu and L.M. Liu. 2014. Growth and physiological responses to copper stress in a halophyte *Spartina alterniflora* (Poaceae). *Acta Physiol. Plant.,* 36: 745-754.

Goswami, S. and S. Das. 2016. Copper phytoremediation potential of *Calandula officinalis* L. and the role of antioxidant enzymes in metal tolerance. *Ecotox. Environ. Safe,* 126: 211-218.

Imbert, E. 2002. Ecological consequences and ontogeny of seed heteromorphism. *Persp. Plant Ecol. Evol. Syst.,* 5: 13-36.

Jiang, L., L. Wang, C.H. Yin and C.Y. Tian. 2012. Differential salt tolerance and similar responses to nitrogen availability in plants grown from dimorphic seeds of *Suaeda salsa*. *Flora,* 207: 565-571.

Kabata-Pendias, A. 2010. Trace Elements in Soils and Plants. (4th Ed) CRC Press, Boca Raton.

Koptsik, G.N. 2014. Problems and prospects concerning the phytoremediation of heavy metal polluted soils: A review. *Eurasian Soil Sci.,* 47: 923-939.

Li, W.Q., S. Yamaguchi, M.A. Khan, P. An, X.J. Liu and L-S.P. Tran. 2015. Roles of gibberellins and abscisic acid in regulating germination of *Suaeda salsa* dimorphic seeds under salt stress. *Front. Plant Sci.,* 6: 1235-1245.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.,* 148: 350-382.

Liu, X.L., C.Y. Yang, L.B. Zhang, L.Z. Li, S.J. Liu, J.B. Yu, L.P. You, D. Zhou, C.H.Xia and J.M. Zhao. 2011. Metabolic profiling of cadmium-induced effects in one pioneer intertidal halophyte *Suaeda salsa* by NMR-based metabolomics. *Ecotoxicology,* 20: 1422-1431.

Liu, X.L., X.J. Shen, Y.K. Lai, K. Ji, H.S. Sun, Y.Y. Wang, C.Z. Hou, N.N. Zou, J.L. Wan and J.B. Yu. 2016. Toxicological proteomic responses of halophyte *Suaeda salsa* to lead and zinc. Ecotox. *Environ. Safe,* 134: 163-171.

Lu, J.J., D.Y. Tan, J.M. Baskin and C.C. Baskin. 2013. Trade-offs between seed dispersal and dormancy in an amphi-basicarpic cold desert annual. *Ann. Bot.,* 112: 1815-1827.

Maeda, Y., M. Yoshiba and T. Tadano. 2010. Physiological role of sodium in the growth of the halophyte *Suaeda salsa* (L.) Pall. under high-sodium conditions. *Crop Sci.,* 50: 2492-2498.

Mandák, B. 1997. Seed heteromorphism and the life cycle of plants: a literature review. *Preslia*, 69: 129-159.

Manousaki, E. and N. Kalogerakis. 2011. Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. *Ind. Eng. Chem. R.,* 50: 656-660.

Mateos-Naranjo, E., L. Andrades-Moreno, J. Cambrollé and A. Perez-Martin, 2013. Assessing the effect of copper on growth, copper accumulation and physiological responses of grazing species Atriplex halimus: ecotoxicological implications. *Ecotox. Environ. Safe.,* 90: 136-142.

Peña, M.M.O., J. Lee and D.J. Thiele. 1999. A delicate balance: Homeostatic control of copper uptake and distribution. *J. Nutr.*, 129: 1251-1260.

Pilon-Smits, E. 2005. Phytoremediation. *Annu. Rev. Plant Biol.,* 56: 15-39.

Quartacci, M.F., C. Pinzino, C.L.M. Sgherri, F.D. Vecchia and F. Navari-Izzo. 2003. Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. *Physiol. Plant.*, 108: 87-93.

Redondo-Gomez, S., E. Mateos-Naranjo, J. Cambrollé, T. Luque, M.E. Figueroa and A.J. Davy. 2008. Carry-over of differential salt tolerance in plants grown from dimorphic seeds of Suaeda splendens. *Ann. Bot.,* 102: 103-112.

Reeves, R.D. 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant Soil,* 249: 57-65.

Sarwar, N., M. Imran, M.R. Shaheen, W. Ishaque, M.A. Kamran, A. Matloob, A. Rehim and S. Hussain. 2017. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere,* 171: 710-721.

Song, H.L. and Z.G. Sun. 2014. Temporal variations and bioaccumulation of heavy metals in different *Suaeda salsa* marshes of the Yellow River estuary, China. Environ. *Sci. Pollut.* R. 21: 14174-14187.

Song, J. and B.S. Wang. 2015. Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. *Ann. Bot.,* 115: 541-553.

Wang, F.X., Y.G. Xu, S. Wang, W.W. Shi, R.R. Liu, G. Feng and J. Song. 2015. Salinity affects production and salt tolerance of dimorphic seeds of *Suaeda salsa*. *Plant Physiol. Bioch.,* 95: 41-48.

Wang, H.L., C.Y. Tian, L. Jiang and L.Wang. 2014. Remediation of heavy metals contaminated saline soils: A halophyte choice? Environ. *Sci. Technol.,* 48: 21-22.

Wilkins, D.A. 1978. Measurement of tolerance to edaphic factors by means of roo-growth. *New Phytol.,* 80: 623-633.

Wu, H.F., X.L. Liu, J.M. Zhao and J.B. Yu. 2013. Regulation of metabolites, gene expression, and antioxidant enzymes to environmentally relevant lead and zinc in the halophyte *Suaeda salsa*. *J. Plant Growth Regu.*, 32: 353-361.

Xu, J., H.X. Yin, L.L. Yang, Z.X. Xie and X.J. Liu. 2011. Differential salt tolerance in seedlings derived from dimorphic seeds of Atriplex centralasiatica: from physiology to molecular analysis. *Planta,* 233: 859-871.

Zhang, H.X., F.Q. Zhang, Y. Xia, G.P. Wang and Z.G. Shen. 2010. Excess copper induces production of hydrogen peroxide in the leaf of *Elsholtzia haichowensis* through apoplastic and symplastic CuZn-superoxide dismutase. *J. Hazard. Mater.,* 178: 834-843.

Zhang, S.R., M.Y. Chen, T. Li, X.X. Xu and L.J. Deng, 2010. A newly found cadmium accumulator-*Malva sinensis* Cavan. *J. Hazard. Mater.,* 173: 705-709.

(Received for publication 22 August 2017)