



JOURNAL OF PLANT NUTRITION, 25(7), 1359–1375 (2002)

## GROWTH AND NUTRIENT COMPOSITION OF *ELSHOLTZIA SPLENDENS* NAKAI UNDER COPPER TOXICITY

Ming Jie Yang,<sup>1,2,\*</sup> Xiao E Yang,<sup>1</sup> and Volker Römheld<sup>3</sup>

<sup>1</sup>Department of Natural Resources, College of  
Environmental & Resources Science, Zhejiang University,  
Huajiachi Campus, Hangzhou 310029, China

<sup>2</sup>Institute of Modern Physics, Fudan University, Shanghai  
200433, China

<sup>3</sup>Institute of Plant Nutrition (330), University of  
Hohenheim, Stuttgart 70593, Germany

### ABSTRACT

Copper (Cu) tolerance and accumulation in *Elsholtzia splendens* Nakai were investigated by means of hydroponic culture. Enhanced growth was observed when plants were exposed to 50 and 100  $\mu\text{M}$  Cu. Copper uptake efficiency and translocation depended on Cu concentration in nutrient solution. Shoot Cu concentrations of 1133 and 3417  $\mu\text{g g}^{-1}$  dry wt were achieved by 500 and 1000  $\mu\text{M}$  Cu supply, respectively. Copper accumulation in *E. splendens* was accompanied by the ability to maintain the concentrations of other essential nutrients, except potassium, within the range considered sufficient for normal growth of

---

\*Corresponding author. E-mail: yangmj@fudan.edu.cn



higher plants. The results obtained suggest that *E. splendens* has a great potential for phytoremediation.

## INTRODUCTION

Soil contamination with heavy metals has become a worldwide challenge, leading to the losses in agriculture and hazardous health effects as they enter the food chain.<sup>[1]</sup> This problem may be partially solved by the emerging phytoremediation technology. The cost-effective plant-based approach to remediation takes the advantage of the remarkable ability of plants to concentrate heavy metals in their tissues from the environment.<sup>[2,3]</sup> However, the so-called phytoremediation has not achieved a lot partly due to the lack of basic information regarding the fundamental mechanisms employed by metal accumulators. The application of metal accumulators is also obstructed by some of their inherent defects, such as small biomass, slow growth rate, and poor adaptability.<sup>[4]</sup> Hence, before their practical application, the mechanisms responsible for the abnormal ability for metal tolerance and accumulation should be elucidated. Alternatively, efforts should be paid to explore new accumulators with ideal biological traits including high biomass and fast growth rate.

Different mechanisms have been proposed to explain the tolerance of plants to toxic heavy metals.<sup>[5-9]</sup> However, the mechanisms for metal accumulation are not fully understood, and this is particularly true in the case of Cu accumulators. Copper is an essential micronutrient but is toxic to plants when in excess.<sup>[10]</sup> It is a redox-active metal that functions as an enzyme activator and is an important part of prosthetic groups of many enzymes. Copper concentrations in healthy plant tissue range from 5 to 20  $\mu\text{g g}^{-1}$  dry wt.<sup>[11]</sup> In Cu-enriched environments, accumulation of Cu in plant tissues depends on the species or cultivars and ecotypes. In only a limited number of plant species a heritable tolerance or resistance occurs, which enables these plants to grow and to reproduce on metal-contaminated soils. There are very few examples of plants that hyperaccumulate copper though more than 400 species have been identified as metal-hyperaccumulators.<sup>[4,12]</sup> All of the copper-hyperaccumulators listed by Brooks et al.<sup>[13]</sup> are confined exclusively to the well-known Copper-Belt of Zaïre. *E. splendens* was first reported to be an endemic Cu-hyperaccumulator by Yang et al.<sup>[14]</sup> based on field survey around a copper-mining area in Zhejiang, southeast province of China. As yet, the documents on the mechanisms of tolerance and accumulation of copper in *E. splendens* are scarce.

The objectives of this study were to assess the ability of *E. splendens* in Cu tolerance and the effects of Cu accumulation on nutrients composition. The implication for *E. splendens* in phytoremediation was also discussed.



## MATERIALS AND METHODS

### Plant Culture

Seeds of *E. splendens* L. were collected from plants growing on a soil derived from copper-mining deposit in Zhuji County of Zhejiang, a southeast province of China. The seeds were surface sterilized by immersion for 25 min in 2.5% (w/v) bleach and rinsed four times in distilled water, and sown in quartz sands moistened with distilled water. One week after the emergence of seedlings, 0.1 strength of Hoagland nutrient solution was supplied. Three-week-old uniform seedlings were transferred into 3.6-L black polyethylene pot containing nutrient solution for another 1 week pre-culture. The composition of the nutrient solution was as follows: (in mM) 0.7 K<sub>2</sub>SO<sub>4</sub>, 0.1 KCl, 2.0 Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 MgSO<sub>4</sub>, and 0.1 KH<sub>2</sub>PO<sub>4</sub>; and (in μM) 10.0 H<sub>3</sub>BO<sub>3</sub>, 0.5 MnSO<sub>4</sub>, 0.5 ZnSO<sub>4</sub>, 0.25 CuSO<sub>4</sub>, 0.01 (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, and 60 Fe-EDTA.<sup>[15]</sup> Plants (7 per pot) then were exposed to various concentrations of Cu (0.25, 12.5, 25, 50, 100, 500, and 1000 in μM) added as sulphate. Pots were completely randomized, with three replicates. The solution was continuously aerated and renewed every 3 days. The solution pH was maintained at 5.3 ± 0.2 based on the pH of the soil where the plants were growing in the field. The plants were cultured in a growth chamber with following conditions: photoperiod–16 h light/8 h dark, photo flux density of 220 μmol · m<sup>-2</sup> s<sup>-1</sup>, day/night temperature 26/18°C, relative humidity 65–75%.<sup>[16]</sup>

### Plant Analysis

Plants were harvested at the start and at the end of the 24-day treatment. The length of the longest root and the heights of shoot were measured on 21 plants per treatment. Initial and final root length data were used to calculate relative root elongation rates, taking as a 100% reference the relative elongation rate of plants treated with 0.25 μM Cu. Roots of intact plants were immersed in aerated ice-cold 5 mM Pb(NO<sub>3</sub>)<sub>2</sub> solution for 30 min to remove the putative adsorbed Cu<sup>2+</sup>.<sup>[17]</sup> Plant were then rinsed with deionized water, blotted, separated into roots, stems, and leaves and subsequently oven-dried at 70°C. Sieved dry samples were digested with microwave digestion system (mLs-MEGA 1200) and the elements were determined with inductively coupled plasma-optical emission spectroscopy (ICP-OES, Model IRAS-AP, TJA Co.) using the procedure proposed by Yang et al.<sup>[16]</sup>

### Statistical Analysis

The SPSS statistical software package (Version 10.0) was employed for data processing and testing. One-way ANOVA, Non-linear Regression and



Correlation procedures were selected. LSD method was applied to test the difference among means.

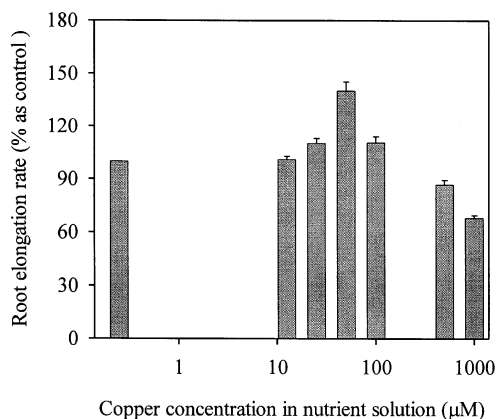
## RESULTS AND DISCUSSION

### Growth Performance

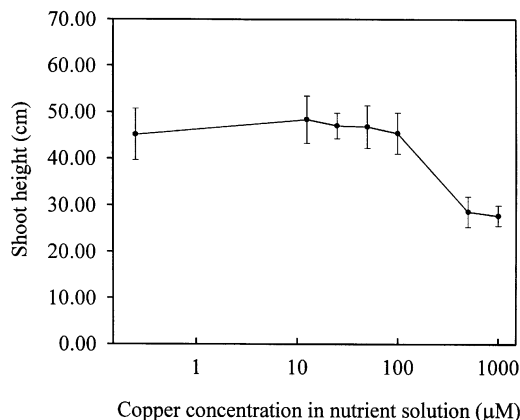
Root elongation rate is viewed as an important parameter characterizing the growth performance of plants.<sup>[18,19]</sup> Copper level of 0.25  $\mu\text{M}$  in nutrient solution can be regarded as control in the present study for it is generally adequate for optimum growth of the majority of higher plants.<sup>[10]</sup> Root length increased with increasing Cu supply from 2.5 to 100  $\mu\text{M}$  (Fig. 1). The highest root elongation rate was recorded at 50  $\mu\text{M}$  Cu treatment, which increased significantly ( $P < 0.01$ ) 41% in comparison with that of control. However, 500 and 1000  $\mu\text{M}$  Cu caused decline in root length. Nevertheless, the finding implicates that exposure to Cu enhances growth of *E. splendens*, which can be further confirmed by the observations that biomass production increased when plants grew with elevated Cu supply.

Shoot height changed slightly with increasing Cu concentrations from 0.25  $\mu\text{M}$  up to 100  $\mu\text{M}$  in nutrient solution (Fig. 2). However, significant ( $P < 0.05$ ) decreases in shoot height were found in treatments of 500 and 1000  $\mu\text{M}$  Cu supply.

Copper supply affected biomass production of *E. splendens* (Fig. 3). The dry weights of stems and leaves were significantly ( $P < 0.01$ ) higher in plants



**Figure 1.** Root elongation rate of *E. splendens* exposed to different levels of Cu. Vertical bars indicate  $\pm$  SE,  $n = 21$ .



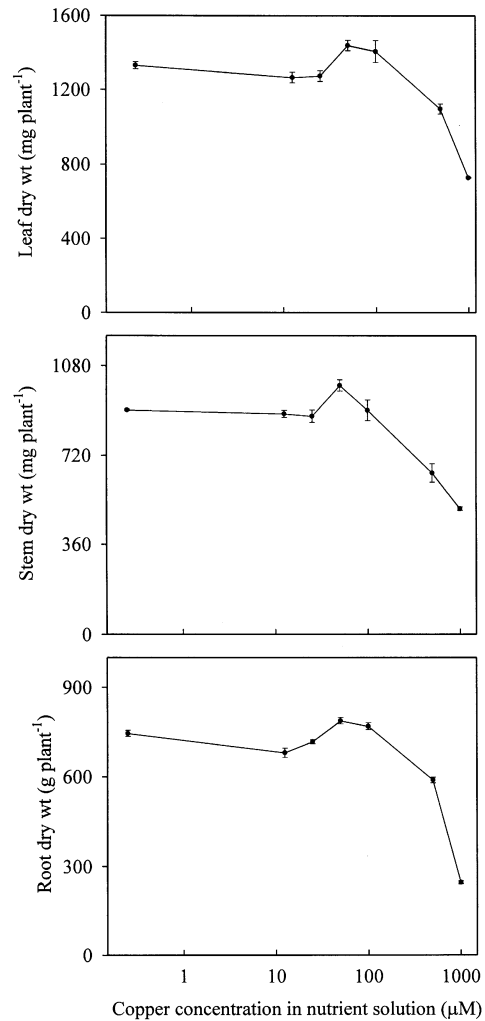
**Figure 2.** Shoot height of *E. splendens* exposed to different levels of Cu. Vertical bars indicate  $\pm$  SE,  $n = 21$ .

supplied with 50  $\mu\text{M}$  Cu than in plants receiving either lower or higher Cu concentrations. A pronounced increase in roots dry weights was noted in 50 and 100  $\mu\text{M}$  Cu supply in comparison with other Cu levels. The dry weights of stems and leaves remained constant with elevating Cu from 0.25 to 25  $\mu\text{M}$  in nutrient solution (Fig. 3). However, the fresh weights of stems and leaves in treatments of 12.5 and 25  $\mu\text{M}$  Cu were significantly higher than that of control (data not shown). These observations imply a change in water status of *E. splendens* caused by excessive Cu. The mechanisms underlying this change need further investigation. The biomass reduced significantly compared to control when *E. splendens* was exposed to 500 and 1000  $\mu\text{M}$  Cu. However, its biomass remained to be over one order of magnitude higher than in case of some well-known hyperaccumulators, such as *Thlaspi caerulescens* – a Zn hyperaccumulator,<sup>[20]</sup> *Thlaspi goesingense* – a Ni hyperaccumulator<sup>[21]</sup> and some Cu hyperaccumulators found in Zaïre<sup>[22]</sup>. Moreover, it is intriguing that *E. splendens* still survived and even completed the life cycle when toxicity occurred.

Good growth performance of *E. splendens* can be achieved by elevating copper supply up to 50 or 100  $\mu\text{M}$ , which are usually lethal to normal plants,<sup>[11]</sup> making it adaptable to metalliferous soils.

### Copper Uptake and Translocation

With increasing of Cu supply, root Cu concentrations increased and varied from 38 to 12,752  $\mu\text{g g}^{-1}$  dry wt (Fig. 4). Stem Cu concentrations remained

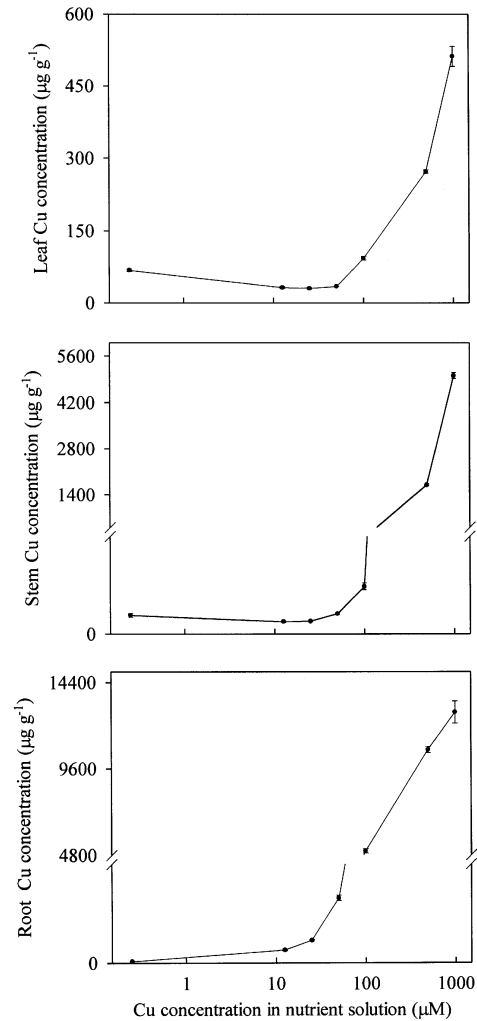


**Figure 3.** Biomass production of *E. splendens* exposed to different levels of Cu. Vertical bars indicate  $\pm$  SE,  $n = 3$ .

unaffected when plants exposed to Cu from 0.25 to 25  $\mu$ M. However, it dramatically increased at 500 and 1000  $\mu$ M Cu. In comparison with control, treatments of 12.5, 25, and 50  $\mu$ M Cu resulted in a significant decline in leaf Cu concentrations. Afterwards, leaf Cu concentrations sharply elevated at 500 and 1000  $\mu$ M Cu exposure.

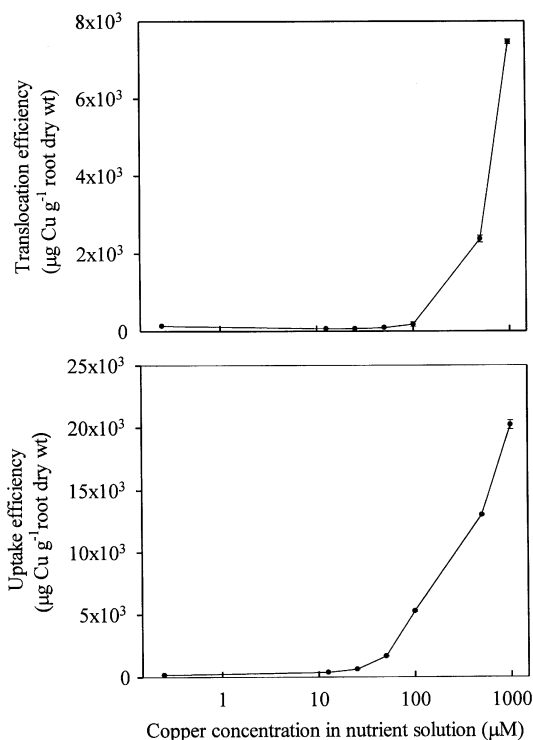
Cu AND *ELSHOLTZIA SPLENDENS* NAKAI

1365



**Figure 4.** Copper concentrations in organs of *E. splendens* exposed to different levels of Cu. Vertical bars indicate  $\pm$  SE,  $n = 21$ .

Copper uptake efficiency [total amount of Cu in the plant normalized by root dry wt,<sup>[23]</sup>] was dependent on Cu concentration in nutrient solution (Fig. 5). Below 50 µM Cu supply, Cu uptake efficiency increased exponentially with Cu increasing in nutrient solution and can be described by the function  $Y = -122 + 313 \times 1.04^X$  ( $r^2 = 0.997$ ), where  $Y$  and  $X$  represent Cu uptake



**Figure 5.** Copper uptake efficiency (total amount of Cu taken by plant normalized by root dry wt) and translocation efficiency (shoot Cu amount normalized by root dry wt). Vertical bars indicate  $\pm$  SE,  $n = 3$ .

efficiency and Cu concentration in nutrient solution, respectively. However, above 50  $\mu\text{M}$  Cu treatment, Cu uptake efficiency followed a different pattern described by the function of  $Y = -0.01X^2 + 29X + 1249$  ( $r^2 = 0.99$ ). Copper translocation efficiency (shoot Cu content normalized by root dry wt) remained low and similar between 0.25  $\mu\text{M}$  and 50  $\mu\text{M}$  Cu treatments whereas it soared above 100  $\mu\text{M}$  Cu supply (Fig. 5). A dramatic increase in Cu translocation may result from a breakdown in the tight restriction of translocation at the root/shoot interface.

Up to now, more than 400 species have been identified as hyperaccumulators, a term introduced by Brooks et al.<sup>[12]</sup> describing a plant which can accumulate metal(s) above  $1000 \mu\text{g g}^{-1}$  dry wt. However, there are very few examples of plant species not native to Africa that hyperaccumulate copper. All of the copper-hyperaccumulators listed by Brooks et al.<sup>[13]</sup> are confined exclusively to the well-known Copper-Belt of Zaïre. In the present study, we found that

**Cu AND *ELSHOLTZIA SPLENDENS* NAKAI**

1367

*E. splendens* could accumulate Cu up to 1133 and 3417  $\mu\text{g g}^{-1}$  dry wt in its shoots (stems plus leaves) when grew in solution containing 500 and 1000  $\mu\text{M}$  Cu, respectively. On a whole plant basis, Cu concentrations higher than 1000  $\mu\text{g g}^{-1}$  dry wt can be achieved by 100  $\mu\text{M}$  and higher Cu supply. Elevation of Cu in substrate can increase Cu uptake efficiency (Fig. 5) and thereby Cu concentration in plant organs (Fig. 4).

Phytoextraction, one of the various branches of phytoremediation, exploit pollutant-accumulating plants to remove metals or organics from soil by concentrating them in the harvested parts.<sup>[3]</sup> The ability of *E. splendens* in Cu tolerance and accumulation suggests it is suitable for removing Cu from contaminated soils.

A higher shoot/root ratio of heavy metal content in plants is important in practical phytoextraction for it can simplify the agricultural operation by only harvesting the aboveground parts of the plants. It could not be overlooked that Cu content in roots of *E. splendens* accounted for a large proportion of the total Cu taken by *E. splendens*, which might weaken the Cu-phytoextraction potential to some extent. However, the fact is that the mobility of Cu in soils and plants is very low.<sup>[24,25]</sup> In terms of phytostabilization-using plants to reduce the bioavailability and mobility of pollutant, and revegetation via root uptake and anchoring on contaminated soils that usually lack established vegetation cover due to toxic effects of pollutants or recent physical disturbance,<sup>[3]</sup> *E. splendens* has a great advantage. In fact, *E. splendens* is a pioneer plant in Cu-mining areas.<sup>[14]</sup> Our ongoing field experiments will further test its value in phytostabilization as well as in phytoextraction.

**Accumulation of Other Nutrients**

Copper hyperaccumulation in *E. splendens* suggests the existence of inner detoxification mechanisms. High amount of heavy metals in plant tissues may interfere with other essential nutrients and thereby disturb the mineral nutrition of plants.<sup>[26]</sup> Copper accumulation influenced uptake and distribution of other essential mineral nutrients (Tables 1–3).

With increasing Cu supply, plants exhibited significant potassium (K) loss from roots (Table 1). In stems, K concentration markedly decreased ( $P < 0.01$ ) in treatments of 500 and 1000  $\mu\text{M}$  Cu (Table 2). The lowest K concentration in leaves was observed in 1000  $\mu\text{M}$  Cu treatment. In general, Cu accumulation resulted in K depletion in *E. splendens*, and the negative correlation between K concentrations and Cu concentrations were statistically significant ( $r = -0.85$  in roots,  $r = -0.91$  in stems, and  $r = -0.76$  in leaves). Potassium loss in plant organs, especially in roots, during excessive Cu exposure was apparently the consequence of Cu toxicity. According to De Vos et al.,<sup>[27]</sup> Cu resulted in an

**Table 1.** Influence of Cu Supply on Nutrient Concentration in Roots of *E. splendens*

Element	Cu Concentration										LSD <sub>0.05</sub>
	0.25 $\mu\text{M}$	12.5 $\mu\text{M}$	25 $\mu\text{M}$	50 $\mu\text{M}$	100 $\mu\text{M}$	500 $\mu\text{M}$	1000 $\mu\text{M}$	10	4.0	0.9	
K $\text{mg g}^{-1}$	21	17	16	21	15	10	10	1.2			
Ca $\text{mg g}^{-1}$	4.3	3.9	3.3	3.4	3.1	4.5	4.0	0.16			
Mg $\text{mg g}^{-1}$	2.3	2.3	2.0	2.3	2.2	1.4	0.9	0.11			
P $\text{mg g}^{-1}$	8.9	10.1	11.3	8.8	9.3	11.2	12.0	0.70			
S $\text{mg g}^{-1}$	5.9	5.2	4.9	6.1	5.0	3.4	2.4	0.18			
Zn $\mu\text{g g}^{-1}$	77	77	58	45	39	41	49	3.4			
Fe $\mu\text{g g}^{-1}$	4,913	5,421	5,233	4,795	3,543	6,237	5,314	261.8			
Mn $\mu\text{g g}^{-1}$	812	553	452	178	95	368	335	28.5			

Cu AND *ELSHOLTZIA SPLENDENS* NAKAI

1369

**Table 2.** Influence of Cu Supply on Nutrient Concentration in Stems of *E. splendens*

Element	Cu Concentration										LSD <sub>0.05</sub>
	0.25 $\mu\text{M}$	12.5 $\mu\text{M}$	25 $\mu\text{M}$	50 $\mu\text{M}$	100 $\mu\text{M}$	500 $\mu\text{M}$	1,000 $\mu\text{M}$				
K $\text{mg g}^{-1}$	19	19	19	19	18	11	10			1.3	
Ca $\text{mg g}^{-1}$	3.1	3.1	3.1	3.1	3.4	4.1	6.5			0.19	
Mg $\text{mg g}^{-1}$	2.6	2.6	2.5	2.6	2.8	3.0	4.2			0.21	
P $\text{mg g}^{-1}$	6.0	5.3	5.6	5.4	4.7	3.1	5.2			0.16	
S $\text{mg g}^{-1}$	3.3	3.4	3.0	3.3	4.7	4.7	5.9			0.21	
Zn $\mu\text{g g}^{-1}$	69	42	56	71	45	40	62			5.3	
Fe $\mu\text{g g}^{-1}$	112	88	118	104	90	244	236			14.2	
Mn $\mu\text{g g}^{-1}$	25	29	33	79	57	19	25			3.9	

**Table 3.** Influence of Cu Supply on Nutrient Concentration in Leaves of *E. splendens*

Element	Cu Concentration									LSD <sub>0.05</sub>
	0.25 $\mu\text{M}$	12.5 $\mu\text{M}$	25 $\mu\text{M}$	50 $\mu\text{M}$	100 $\mu\text{M}$	500 $\mu\text{M}$	1000 $\mu\text{M}$	1000 $\mu\text{M}$	1000 $\mu\text{M}$	
K $\text{mg g}^{-1}$	20	22	20	19	19	17	17	17	17	1.2
Ca $\text{mg g}^{-1}$	3.2	3.4	3.2	3.2	3.3	3.2	3.2	3.2	3.2	0.17
Mg $\text{mg g}^{-1}$	2.9	3.4	2.9	3.0	3.2	2.6	2.6	2.6	2.6	0.20
P $\text{mg g}^{-1}$	6.5	6.9	6.1	5.3	5.7	4.7	4.7	4.2	4.2	0.20
S $\text{mg g}^{-1}$	4.1	3.8	2.9	3.3	4.3	4.6	4.6	5.1	5.1	0.18
Zn $\text{mg g}^{-1}$	72	136	170	335	235	105	105	119	119	15.3
Fe $\mu\text{g g}^{-1}$	817	830	718	834	754	606	606	759	759	39.1
Mn $\mu\text{g g}^{-1}$	78	101	103	183	143	53	53	66	66	4.1



immediate damage to the membrane especially by changing the activity of K channels in plant roots, thus causing a leakage across the membrane.<sup>[28]</sup> The observed  $K^+$  loss caused by excessive Cu supply is in agreement with studies in wheat,<sup>[29]</sup> *Mimulus guttatus*,<sup>[30]</sup> and *Silene compacta*.<sup>[31]</sup>

Calcium concentrations in *E. splendens* changed when plants received different levels of Cu. In roots, the lowest Ca concentration was detected in 100  $\mu$ M Cu treatment and the highest one was found in 500  $\mu$ M Cu treatment (Table 1). The concentrations of calcium in the stem rose with increasing copper concentration in nutrient solution and were positively correlated with Cu concentration in stems ( $r=0.994$ ). The highest Ca concentration in stems was observed in treatment of 1000  $\mu$ M Cu (Table 2) whereas no significant differences in leaf Ca concentrations was noted among the seven treatments. Changes in calcium concentrations have been found to be a general physiological response of plants against metal toxicity.<sup>[32,33]</sup> The increase of Ca following Cu increase in stems might alleviate Cu toxicity.<sup>[34]</sup>

Root magnesium (Mn) concentrations ranged from 0.9 to 2.3  $\text{mg g}^{-1}$  dry wt when plants were exposed to various levels of Cu, with the lowest one was recorded at 1000  $\mu$ M Cu. Stem Mg concentrations were positively correlated with stem Cu concentrations ( $r=0.982$ ). Leaf Mg concentrations fluctuated, being lowest in 500  $\mu$ M Cu treatment and highest in 12.5  $\mu$ M Cu treatment. Magnesium concentrations were within the range considered adequate for optimum growth for conventional plants.<sup>[10]</sup>

Phosphorous concentrations in roots varied from 8.8 to 12.0  $\text{mg g}^{-1}$  dry wt with different levels of Cu supplying, with root P concentration double those in stems and leaves. Leaf P concentrations were inversely correlated with leaf Cu concentrations ( $r=-0.83$ ), but still within the sufficient range.

Sulfur concentrations in roots, stems and leaves were within the range suitable for normal growth of conventional plants, varying from 2.4 to 6.1  $\text{mg g}^{-1}$  dry wt.

Extraordinarily high root iron concentrations were found across all Cu treatments, ranging from 3.5  $\text{mg} \cdot \text{g}^{-1}$  dry wt to 6.2  $\text{mg} \cdot \text{g}^{-1}$  dry wt (Table 1). The lowest one was detected at 100  $\mu$ M Cu supply and highest one resulted from the 500  $\mu$ M Cu treatment. Stem iron (Fe) concentrations varied from 90 to 236  $\mu\text{g g}^{-1}$  dry wt and were significantly correlated with Cu concentrations in stems ( $r=0.99$ ). Nevertheless, iron translocation from stems to leaves was pronounced in the present experiment (Tables 2 and 3), with leaf Fe concentrations being between 606 and 833  $\mu\text{g g}^{-1}$  dry wt. Chlorosis may be present when the total amount of Fe is sufficient because difficulty may exist in reducing iron from  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  in the cytosol.<sup>[10]</sup> The occurrence of chlorosis in treatments of 500 and 1000  $\mu$ M Cu might be induced by the deficiency of active Fe,<sup>[35]</sup> as the total iron concentrations in leaves in this study were adequate (Table 1).



Manganese partition in organs of *E. splendens* had a pattern similar to that of iron. The roots retained the majority of Mn taken by plant (Table 1), and Mn translocation from stems to leaves was also apparent (Tables 2 and 3). Zinc concentrations in roots (Table 1) were not markedly different from those in stems (Table 2). However, it was noticeable that zinc translocation from stems to leaves was the highest in treatments of 50 and 100  $\mu\text{M}$  Cu.

### CONCLUSIONS

*E. splendens* is highly tolerant to copper toxicity. *E. splendens* can accumulate copper in shoots to a concentration above  $1000 \mu\text{g g}^{-1}$  dry wt – the threshold defining a hyperaccumulator – with moderate reduction in growth. Copper accumulation in *E. splendens* is dependent on Cu status in substrate and accompanied by the ability to maintain the concentrations of essential nutrients within the sufficient range for normal growth of plants. Considering its high biomass and fast growth in addition to Cu accumulation, we suggest that *E. splendens* has great potential for phytoremediation of Cu-contaminated soils.

### ACKNOWLEDGMENTS

The present study was financed by National Natural Science Foundation of China (NSFC, No: 29977017) and Outstanding Young Scientist Grant from NSFC.

### REFERENCES

1. Dudka, S.; Miller, W.P. Accumulation of Potentially Toxic Elements in Plants and Their Transfer to Human Food Chain. *J. Environ. Sci. Health* **1999**, *B34* (4), 681–708.
2. Robinson, B.H.; Brooks, R.R.; Howes, A.W.; Kirkman, J.K.; Gregg, P.E.H. The Potential of High Biomass Nickel Hyperaccumulator *Berkheya coddii* for Phytoremediation and Phytomining. *J. Geochem. Explor.* **1997**, *60*, 115–126.
3. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 643–648.
4. Brooks, R.R. General Introduction. In *Plants that Hyperaccumulate Heavy Metals. Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining*; Brooks, R.R, Ed.; CAB International: Wallingford, England, 1998; 1–12.

Cu AND *ELSHOLTZIA SPLENDENS* NAKAI

1373

5. Baker, A.J.M.; Walker, P.L. Ecophysiology of Metal Uptake by Tolerant Plants. In *Heavy Metal Tolerance in Plants. Evolutionary Aspects*; Shaw, A.J., Ed.; CRC Press: Boca Raton, FL, 1990; 155–177.
6. Fernandes, J.C.; Henriques, F.S. Biochemical, Physiological and Structural Effects of Excess Copper in Plants. *Bot. Rev.* **1991**, *57*, 246–273.
7. Vázquez, M.D.; Poschenrieder, C.; Barceló, J.; Baker, A.J.M.; Hatton, P.; Cope, G.H. Compartment of Zinc in Roots and Leaves of the Zinc Hyperaccumulator *Thlaspi caerulescens* J&C Presl. *Bot. Acta* **1994**, *107*, 243–250.
8. Verkleij, J.A.C.; Shat, H. Mechanisms of Metal Tolerance in Higher Plants. In *Heavy Metal Tolerance in Plants.: Evolutionary Aspects*; Shaw, A.J., Ed.; CRC Press: Boca Raton, FL, 1990; 179–193.
9. Zhao, F.J.; Shen, Z.G.; McGrath, S.P. Solubility of Zinc and Interaction Between Zinc and Phosphorus in the Hyperaccumulator *Thlaspi caerulescens*. *Plant Cell Environ.* **1998**, *21*, 108–114.
10. Marschner, H. *Mineral Nutrition in Higher Plants*; Academic Press: London, 1995; 229 pp.
11. Robson, A.D.; Reuter, D.J. Diagnosis of Copper Deficiency and Toxicity. In *Copper in Soils and Plants*; Loneragan, J.F., Robson, A.D., Graham, R.D., Eds.; Academic Press: Sydney, 1981; 287–312.
12. Brooks, R.R.; Lee, J.; Reeves, R.D.; Jaffré, T. Detection of Nickeliferous Rocks by Analysis of Herbarium Specimens of Indicator Plants. *J. Geochem. Explor.* **1977**, *7*, 49–57.
13. Brooks, R.R.; Dunn, C.E.; Hall, G.E.M. *Biological Systems in Mineral Exploration and Processing*; Ellis Horwood: Hemel Hempstead, New Zealand, 1995; 539 pp.
14. Yang, X.; Shi, W.Y.; Fu, C.X.; Yang, M.J.; He, F. Copper Hyperaccumulators of Chinese Native Plants: Characteristics and Possible Use for Phytoremediation. In *Sustainable Agriculture for Food, Energy and Industry*; Bassam, N.E., Ed.; James & James Science Publishers Ltd.: London, 1998; 484–489.
15. Marschner, H.; Cakmak, I. High Light Intensity Enhances Chlorosis and Necrosis in Leaves of Zinc, Potassium, and Magnesium Deficient Bean (*Phaseolus vulgaris*) Plants. *J. Plant Physiol.* **1989**, *134*, 308–315.
16. Yang, X.; Baligar, V.C.; Martens, D.C.; Clark, R.B. Cadmium Effects on Influx, Transport of Mineral Nutrients in Plant Species. *J. Plant Nutr.* **1996**, *19*, 643–656.
17. Harrison, S.J.; Lepp, N.W.; Phipps, D.A. Uptake of Copper by Excised Roots II. Copper Desorption from the Free Space. *Z. Pflanzenernähr. Bodenkd* **1979**, *94*, 27–34.
18. Causton, D.R. Plant Growth Analysis: The Variability of Relative Growth Rate within a Sample. *Ann. Bot.* **1991**, *67*, 137–144.



19. Hagemeyer, J. Ecophysiology of Plant Growth under Heavy Metal Stress. In *Heavy Metal Stress in Plants-from Molecules to Ecosystems*; Prasad, Hagemeyer, J., Eds.; Springer-Verlag: Berlin, 1999; 157–182.
20. Brown, S.L.; Chaney, R.L.; Angle, J.S.; Baker, A.J.M. Zinc and Cadmium Uptake by Hyperaccumulator *Thlaspi caerulescens* Grown in Nutrient Solution. *Soil Sci. Soc. Am. J.* **1995**, *59*, 125–133.
21. Krämer, U.; Smith, R.D.; Wenzel, W.W.; Raskin, I.; Salt, D.E. The Role of Metal Transport and Tolerance in Nickel Hyperaccumulation by *Thlaspi goesingense* Hálácys. *Plant Physiol.* **1997**, *115*, 1641–1650.
22. Brooks, R.R.; Malaisse, F. *The Heavy Metal Tolerant Flora of Southcentral Africa*; Balkema: Rotterdam, The Netherlands, 1985; 199 pp.
23. Harper, F.A.; Smith, S.; Macnair, M. Can an Increased Copper Requirement in Copper-Tolerant *Mimulus guttatus* Explain the Cost of Tolerance? I. Vegetative Growth. *New Phytol.* **1997**, *136*, 455–467.
24. Shuman, L.M. Chemical Forms of Micronutrients in Soils. In *Micronutrients in Agriculture*, 2nd Ed.; Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M., Eds.; Soil Science Society of America, Inc.: Madison, WI, 1991; SSSA Book Series 4, 113–144.
25. Kochian, L.V. Mechanisms of Micronutrient Uptake and Translocation in Plants. In *Micronutrients in Agriculture*, 2nd Ed.; Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M., Eds.; Soil Science Society of America, Inc.: Madison, WI, 1991; SSSA Book Series 4, 229–296.
26. Welch, R.M. Micronutrient Nutrition of Plants. *Critic. Rev. Plant Sci.* **1995**, *14*, 49–82.
27. De Vos, C.H.R.; Schat, H.; De Waal, M.A.M.; Vooijs, R.; Ernst, W.H.O. Increased Resistance to Copper-Induced Damage of the Root Cell Plasmalemma in Copper-Tolerant *Silene cucubalus*. *Physiol. Plant.* **1991**, *82*, 523–528.
28. Murphy, A.S.; Eisinger, W.R.; Shaff, J.E.; Kochian, L.V.; Taiz, L. Early Copper-Induced Leakage of  $K^+$  from *Arabidopsis* Seedlings is Mediated by Ion Channels and Coupled to Citrate Efflux. *Plant Physiol.* **1999**, *121*, 1375–1382.
29. Jensen, P.; Adalsteinsson, S. Effects of Copper on Activity and Passive  $Rb^+$  Influx in Roots of Winter Wheat. *Physiol. Plant.* **1989**, *75*, 195–200.
30. Strange, J.; Macnair, M. Evidence for a Role for the Cell Membrane in Copper Tolerance of *Mimulus guttatus* Fisher ex DC. *New Phytol.* **1991**, *119*, 383–388.
31. Ouzounidou, G. Root Growth and Pigment Composition in Relationship to Element Uptake in *Silene compacta* Plants Treated with Copper. *J. Plant Nutr.* **1994**, *17*, 933–943.
32. Kinraide, T.B. Three Mechanisms for the Calcium Alleviation of Mineral Toxicities. *Plant Physiol.* **1998**, *118*, 513–520.



**Cu AND *ELSHOLTZIA SPLENDENS* NAKAI**

**1375**

33. Nieminen, T.; Helmisaari, H.-S. Nutrient Translocation in the Foliage of *Pinus sylvestris* L. Growing Along a Heavy Metal Pollution Gradient. *Tree Physiol.* **1996**, *16*, 825–831.
34. Maksymiec, W.; Baszysk, T. Are Calcium Ions and Calcium Channels Involved in the Mechanisms of  $\text{Cu}^{2+}$  Toxicity in Bean Plants? The Influence of Leaf Age. *Photosynthetica* **1999**, *36* (1–2), 267–278.
35. Römheld, V. The Chlorosis Paradox: Fe Inactivation in Leaves as a Second Event in Fe Deficiency Chlorosis. In *Iron Nutrition and Interaction in Plants*, Abstracts of 9th International Symposium; Römheld, V., Ed.; Hohenheim: Stuttgart, Germany, 1997; 10–10.

