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Copper uptake by four *Elsholtzia* ecotypes supplied with varying levels of copper in solution culture

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Abstract

The effects of copper (Cu) on the yield and Cu uptake of three ecotypes of *Elsholtzia splendens* and one of *Elsholtzia argyi* were studied using solution culture. Three Cu concentrations were compared: 0.31 (control), 50 and 100 $\mu\text{mol L}^{-1}$. Although *E. argyi* took up more Cu in the aboveground parts, typical visual symptoms of Cu toxicity appeared when the plants grew in 50 and 100 $\mu\text{mol Cu L}^{-1}$. In contrast, plants from all three populations of *E. splendens* showed high Cu tolerance and substantial shoot Cu accumulation of 58–144 mg kg^{-1} . Shoot Cu concentrations were about 16–27 times higher than root Cu concentrations. Root-to-shoot ratio of the *E. argyi* ecotype was halved when Cu was supplied at a level of 100 mg L^{-1} compared to the control (0.31 mg L^{-1}) but the ratio increased by 6–47% in the three *E. splendens* ecotypes. The increase in root-to-shoot ratio in *E. splendens* may be a mechanism by which the plants can tolerate high Cu concentrations. There were few differences in morphology among the three *E. splendens* ecotypes in response to added Cu. The results are discussed in relation to the possible use of *E. splendens* as a pioneer species in the phytostabilization of Cu-contaminated soils.

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Keywords: *Elsholtzia*; Cu tolerance; Copper uptake; Phytostabilization

1. Introduction

Copper (Cu) is an essential nutrient for plants. It is a component of several enzymes, including some that participate in electron flow and catalysis of redox reactions in plant cells (Ouzounidou et al., 1995). However, excessive Cu can lead to inhibition of root elongation and damage to root epidermal cells and root cell membranes (Lin et al., 2003). Excessive Cu may be toxic not only to plants but also to human beings via the food chain and may thus pose a potential threat to human health. Remediation measures are therefore needed for Cu contaminated soils.

In addition to the relatively harsh traditional engineering and chemical soil remediation methods there is a new developing phytoremediation technology in which metals

are removed or stabilized by hyperaccumulator plants. Over 500 plant species can hyperaccumulate heavy metals, most of which accumulate Ni. Only a few, including *Aeolanthus bifurcatus* and *Buchnera henriquesii* from central Africa, hyperaccumulate Cu. Very few studies have been carried out on these plants because of the difficulty in obtaining plant material or seed as a result of political unrest and instability in the area (Brooks, 1998). There has therefore been considerable interest in finding alternative Cu accumulators.

Elsholtzia splendens is a native Chinese plant in the family Labiata. It is an annual herb with an erect stem about 15 to 120 cm in height which flowers in the autumn (Tang et al., 1999). This species has been called the ‘copper flower’ in China and has been used in the past as a Cu indicator in metal prospecting. The potential use of *E. splendens* in phytoremediation of Cu-contaminated soils has therefore been studied by Tang et al. (2001) and Yang et al. (2002) who concluded that active adaptation and passive selection

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allow *E. splendens* to survive on Cu-contaminated soils. Contrary to Yang et al. (2002), Song et al. (2004) reported that *E. splendens* did not hyperaccumulate Cu in pot experiments, but the shoot biomass of *E. splendens* was almost five times greater than that of a typical Cu excluder, *Silene vulgaris*. A second *Elsholtzia* species, *E. argyi*, was originally found growing on copper ore, and both species are widely distributed in China. The aim of the present study was to investigate whether differences exist in the degree of Cu tolerance and accumulation among different *E. splendens* populations and *E. argyi*, and also to study the effects of Cu on the growth and yield of *Elsholtzia*.

2. Materials and methods

2.1. Sample collection

Seeds were collected from three populations of *E. splendens* growing on Cu tailings. One population was near the city of Tongling in Anhui Province and other two were near the cities of Jiande and Zhuji in Zhejiang Province. *E. argyi* seed was collected from Cu mine tailings in Sanmen county, Zhejiang Province. All four sites are located in subtropical east China.

2.2. Cultural conditions

The seeds were placed in a 1:1 (v/v) mixture of perlite and vermiculite within 1.5-L plastic containers in a growth chamber. The medium was kept moist with daily addition of distilled water. The growth chamber conditions were as follows: temperature of 25 °C, relative humidity of 65–70%, photoperiod of 14-h light/10-h dark, and photon flux density of 1.5×10^4 lux (lx). Ten days after seedling emergence, 25% Hoagland nutrient solution was supplied. One-month-old seedlings of uniform size were transferred into 2.5-L black polyethylene pots (3 plants per pot) containing 50% strength nutrient solution for a further two weeks pre-culture. The composition of the nutrient solution was as follows ($\mu\text{mol L}^{-1}$): $\text{Ca}(\text{NO}_3)_2$ 1000, MgSO_4 500, K_2HPO_4 50, KCl 100, H_3BO_3 10, MnSO_4 1.8, Na_2MoO_4 0.2, CuSO_4 0.31, NiSO_4 0.5, ZnSO_4 5 and Fe^{3+} –EDTA 60. The plants were then exposed to three concentrations of Cu: 0.31, 50 and 100 μmol

L^{-1} . Copper was supplied as CuSO_4 . The pots were arranged in a completely randomized design with three replicates per treatment. The solutions were continuously aerated and renewed every four days. The solution pH was adjusted to 5.3 ± 0.1 with 0.1 mol L^{-1} HCl or NaOH twice each day.

2.3. Experimental procedure and methodology

The plants were harvested after exposure to the Cu for 25 days. After measurement of shoot height, length of the longest root and root volume, the plants were separated into roots, stems and leaves. Roots were immersed in 20 mmol L^{-1} Na_2EDTA for 15 min to remove the putative absorbed Cu^{2+} (Long et al., 2002). All parts were then rinsed with distilled water and dried firstly at 105 °C for 30 min, oven-dried at 85 °C and weighed. The plant materials were milled with a stainless steel disintegrator. Sub-samples were digested with 85% HNO_3 and 15% HClO_4 and multi-element analysis conducted by inductively coupled plasma–atomic emission spectrometry (ICP–AES) using an IRIS Intrepid spectrometer.

2.4. Statistical analysis

The Data Processing System for Windows (DPS Version 3.11) was employed for analysis of variance analysis and multiple comparisons (Tang and Feng, 2002). Pairs of mean values were compared using least significant difference (LSD) at the 5% level.

3. Results and discussion

3.1. Effects of Cu on *Elsholtzia* plants

Plants from the three populations of *E. splendens* grew well in the presence of 50 and 100 $\mu\text{mol L}^{-1}$ Cu with a similar leaf colour to those grown under control conditions (0.31 $\mu\text{mol L}^{-1}$ Cu). In contrast, *E. argyi* appeared to exhibit Cu toxicity symptoms when grown with 50 and 100 $\mu\text{mol L}^{-1}$ Cu. Most of the leaves were purple in colour and chlorotic and some leaves fell from the plants in 100 $\mu\text{mol L}^{-1}$ Cu. All the roots of *E. splendens* appeared healthy and strong, while *E. argyi* roots were black and weak when Cu was supplied at 50 and 100 $\mu\text{mol L}^{-1}$. The differences in plant

Table 1
Effects of elevated Cu on yield of four *Elsholtzia* ecotypes in solution culture^a

<i>Elsholtzia</i> ecotype	Cu treatment ($\mu\text{mol L}^{-1}$)	Root length (cm)	Root volume ($\text{cm}^3 \text{ plant}^{-1}$)	Shoot height (cm)	Dry matter yield (g plant^{-1})		
					Leaves	Stem	Roots
<i>E. argyi</i> , Sanmen population	0.31	41.5±2.4 a	13.6±0.8 a	16.6±0.9 a	1.5±0.1 a	0.42±0 a	0.79±0.1 a
	50	14.8±1.2 b	0.61±0.1 b	6.50±0.3 b	0.05±0 b	0.02±0 b	0.02±0 b
	100	19.1±4.2 b	0.39±0.1 b	6.61±0.3 b	0.08±0 b	0.02±0 b	0.02±0 b
<i>E. splendens</i> , Jiande population	0.31	43.5±2.3 b	18.1±3.4	24.2±4.2	1.75±0.5	0.48±0.1	1.05±0.4
	50	48.1±1.1 ab	23.9±6.7	20.3±0.9	1.42±0.1	0.32±0	0.72±0
	100	49.8±0.6 a	16.7±0.4	20.5±1.5	0.98±0.2	0.20±0	0.60±0
<i>E. splendens</i> , Tongling population	0.31	50.6±0.3	13.2±0.4	25.2±0.8 ab	1.81±0.3 a	0.48±0 a	0.67±0.1 a
	50	44.1±9.2	13.3±3.5	26.4±0.3 a	1.34±0.1 a	0.38±0 a	0.55±0.1 ab
	100	48.6±4.8	8.3±2.2	21.0±2.2 b	0.43±0.1 b	0.12±0 b	0.30±0.1 b
<i>E. splendens</i> , Zhuji population	0.31	37.9±1.2	11.5±1.8	16.8±2.9	1.58±0.2 a	0.27±0.1 a	0.46±0.1
	50	40.1±5.1	12.7±3.5	16.5±1.3	1.15±0.1 b	0.16±0 ab	0.32±0.2
	100	42.7±1.8	10.1±2.1	14.8±2.4	0.77±0 b	0.09±0 b	0.35±0.2

^a Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

Fig. 1. Root-to-shoot ratios of *Elsholtzia* under Cu stress. TL, Tongling; SM, Sanmen; ZJ, Zhuji; JD, Jiande; all *E. splendens* except *E. argyi* at Sanmen.

morphology indicated that *E. splendens* tolerated Cu toxicity better than *E. argyi*.

The dry weight of *E. argyi* leaves, stems and roots showed a significant decline under 50 and 100 $\mu\text{mol L}^{-1}$ Cu compared with the control (Table 1), with total plant biomass declining by 97% and 95%, respectively. In contrast, the biomass of the three *E. splendens* ecotypes remained unchanged. The root-to-shoot ratio of *E. argyi* decreased as Cu concentration increased (Fig. 1), while the root-to-shoot ratio of all three *E. splendens* ecotypes tended to increase, although the increases were not significant. Compared to the control, the root-to-shoot ratios of *E. splendens* under 100 $\mu\text{mol Cu L}^{-1}$ increased by 6.0–39.0%.

Table 2
Tolerance index of *Elsholtzia* ecotypes subjected to different Cu treatments^a

<i>Elsholtzia</i> ecotype	Cu treatment (50 $\mu\text{mol L}^{-1}$)	Cu treatment (100 $\mu\text{mol L}^{-1}$)
<i>E. argyi</i> from Sanmen	35.5±2.30 c	34.3±0.40 c
<i>E. splendens</i> from Jiande	111±6.20 ab	115±8.80 a
<i>E. splendens</i> from Tongling	102±13.5 b	96.0±15.6 b
<i>E. splendens</i> from Zhuji	119±0.30 a	112±2.30 ab

^a Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

Table 3
Plant Cu concentrations (mg kg^{-1})^a

<i>Elsholtzia</i> ecotype	Cu treatment ($\mu\text{mol L}^{-1}$)	Leaves	Stem	Roots	Total aboveground	Whole plant
<i>E. argyi</i> , Sanmen population	0.31	9.78±0.1 c	8.36±0.2 b	19.2±1.6 c	9.50±0.1 c	12.3±0.4 b
	50	61.5±8.5 b	134±35 b	4391±134 b	84.0±9.2 b	1188±251 a
	100	139±17.6 a	392±79 a	6319±497 a	194±9.6 a	1293±217 a
<i>E. splendens</i> , Jiande population	0.31	5.95±0.7 b	7.91±0.6 c	13.7±1.4 c	6.40±0.6 c	8.70±1.1 b
	50	15.4±1.7 b	21.3±4.5 b	364±98.6 b	16.3±2.0 b	117±29.6 b
	100	58.3±4.4 a	57.3±3.9 a	1394±122 a	58.1±4.3 a	513±51.6 a
<i>E. splendens</i> , Tongling population	0.31	5.75±0.3 b	10.1±0.9 b	16.0±1.3 b	6.66±0.3 b	8.70±0.1 b
	50	19.7±1.5 b	23.7±1.0 b	371±28.6 b	20.5±1.2 b	105±14.9 b
	100	147±36.0 a	124±21.4 a	2294±264 a	144±34.0 a	845±64.0 a
<i>E. splendens</i> , Zhuji population	0.31	6.50±0.7 b	9.41±1.3 b	16.9±0.4 b	6.90±0.7 c	8.90±0.6 b
	50	26.8±5.5 b	57.7±14.5 b	432±57.4 b	30.1±5.5 b	91.7±19.2 ab
	100	65.1±10.9 a	143±24.6 a	1965±342 a	71.9±8.3 a	574±244 a

^a Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

This indicates that the plants developed more root biomass than shoot with an elevated Cu supply. Root lengths of *E. argyi* decreased significantly ($P<0.05$) with increasing Cu concentration in solution. Although the root length of *E. splendens* from Tongling decreased slightly, root length of the other two *E. splendens* ecotypes increased slightly at elevated Cu levels.

The tolerance indices of the three *E. splendens* ecotypes were calculated by the equation described by Shu et al. (2002) as follows:

Tolerance index (%)

$$= (\text{mean length of longest root in presence of added Cu} \div \text{mean length of longest root in unamended control}) \times 100.$$

The tolerance indices of *E. splendens* to the two elevated Cu treatments were significantly higher than of *E. argyi*. *E. splendens* from Jiande and Zhuji was less sensitive to copper than the ecotype from Tongling, while *E. argyi* was the most sensitive to elevated Cu. Root volume of the *Elsholtzia* plants showed similar trends to root length. However, the three *E. splendens* ecotypes exhibited maximum root volume and shoot height at 50 $\mu\text{mol L}^{-1}$ Cu. The results indicate a greater Cu tolerance in *E. splendens* than in *E. argyi* (Table 2).

3.2. Cu uptake by *Elsholtzia* plants

All four *Elsholtzia* ecotypes exhibited healthy growth with plant Cu concentrations within normal limits in the control nutrient solution containing 0.31 $\mu\text{mol Cu L}^{-1}$ (Table 3). Plant Cu concentrations increased with increasing Cu supply. *E. splendens* from Tongling and *E. argyi* had higher leaf Cu concentrations than *E. splendens* from Jiande or Zhuji at a solution Cu concentration of 100 $\mu\text{mol L}^{-1}$ ($P<0.05$). Leaf Cu concentrations in these two *E. splendens* ecotypes were below 100 $\mu\text{g g}^{-1}$ and were significantly ($P<0.05$) different from that of *E. splendens* from Tongling and *E. argyi*. *E. argyi* thus accumulated more Cu than *E. splendens* ($P<0.01$), and there was no significant difference in stem Cu concentration among the *E. splendens* ecotypes. In comparison with the control (0.31 $\mu\text{mol Cu L}^{-1}$), root Cu concentration increased sharply at 100 $\mu\text{mol L}^{-1}$ added Cu in all *Elsholtzia* plants; for example, root Cu in *E. argyi* increased from 19 to 6319

$\mu\text{g g}^{-1}$ DM. Root Cu concentrations were significantly different between *E. argyi* and *E. splendens*, but there were no differences among the *E. splendens* ecotypes. It is a commonly observed phenomenon that plants can restrict heavy metal translocation from roots to shoots. In the present work roots usually showed higher Cu concentrations than shoots and the role of the roots may be very important in acting as a site for metal deposition and inactivation (Ouzounidou et al., 1995). As for the increase in root-to-shoot ratio with Cu addition, it is possible that a more rapid increase in the biomass of roots than of shoots may be one strategy leading to increased tolerance to Cu in *E. splendens*. Increased sequestration of Cu in the roots may help to protect the shoots from Cu toxicity. With $100 \mu\text{mol Cu L}^{-1}$ in the nutrient solution, shoot Cu concentration reached $194 \mu\text{g g}^{-1}$ DM in *E. argyi* and $144 \mu\text{g g}^{-1}$ DM in *E. splendens* from Tongling. According to Brooks (1998), Cu hyperaccumulators are defined as plants that accumulate $>1000 \mu\text{g Cu g}^{-1}$ DM. Thus, *E. argyi* and *E. splendens* were not Cu hyperaccumulators under our experimental conditions. *E. argyi* was sensitive to Cu toxicity, while the three *E. splendens* ecotypes tolerated added Cu and behaved as Cu excluders (Song et al., 2004). Differences in Cu concentration were also found among the three *E. splendens* populations. Shoots of *E. splendens* from Tongling had a higher Cu concentrations than those of the other two *E. splendens* ecotypes.

Although the Cu concentrations in the plants were much lower than $1000 \mu\text{g g}^{-1}$, the plants may still have potential for phytostabilization since they can grow rapidly and grow in sites contaminated with mixtures of heavy metals. In our study, all the ecotypes showed their maximum Cu uptake under the highest Cu level. *E. splendens* from Tongling showed the highest Cu accumulation with up to $265 \mu\text{g pot}^{-1}$ Cu in the shoots (Table 4).

Copper uptake in leaves plus roots amounted to $>80\%$ of total plant uptake in control plants with the stems taking up $<20\%$ of total plant Cu (Fig. 2). Root absorption increased sharply with Cu addition. When Cu was supplied at the level of $100 \mu\text{mol L}^{-1}$, the proportion accumulated by the roots was similar in all four ecotypes and ranged from 87% to 93%. The large amounts partitioned in the roots resulted from both high tissue concentrations in the roots and the increase in root-to-shoot ratio. Under high Cu treatments, nearly 60% the total Cu in roots may be bound to the cell wall fraction and the cell wall–plasma

Fig. 2. Copper distribution in different plant parts. For legend of ecotypes, see Fig. 1.

membrane interface (Lin et al., 2003). Further studies are required to determine how the roots of *E. splendens* can tolerate and sequester Cu. The large differences between root and leaf concentrations may indicate an important restriction of the internal transport of metal from the roots towards stems and leaves.

Yang et al. (2002) considered *E. splendens* to be a Cu hyperaccumulator because plants can accumulate $>1000 \mu\text{g Cu g}^{-1}$ DM in their shoots. In the study by Yang et al. (2002), the highest Cu concentration in the nutrient solution was $1000 \mu\text{M}$, 10 times higher than we used in our experiments, and under these conditions nutrient absorption may have been inhibited by the high Cu concentrations in the growth medium. However, in our study all three populations of *E. splendens* accumulated more Cu in their roots than in the shoots and shoot Cu concentrations increased with increasing addition of Cu added, thus they acted as Cu excluders. Song et al. (2004) also found that the well-known Cu-tolerant species *Silene vulgaris* behaved as a typical Cu excluder. In common with other plant species, *E. splendens* accumulates most Cu in the roots. However, *E. splendens* can also grow naturally on Cu-contaminated soils as a dominant

Table 4
Plant Cu uptake ($\mu\text{g pot}^{-1}$)^a

<i>Elsholtzia</i> ecotype	Cu treatment ($\mu\text{mol L}^{-1}$)	Leaf	Stem	Root	Aboveground	Whole plant
<i>E. argyi</i> , Sanmen population	0.31	42.6±4.2 a	10.6±2.1 b	44.9±2.8 c	53.1±2.8 a	98.1±5.1 c
	50	6.9±4.8 b	8.5±2.3 b	258±24 a	15.4±5.2 b	273±23 b
	100	33.1±9.2 a	26.9±6.1 a	414±90 a	60.0±9.9 a	474±74 a
<i>E. splendens</i> , Jiande population	0.31	35.9±17.4 b	11.0±4.3 b	46.8±40.6 b	46.8±17.7 b	93.7±50.6 c
	50	66.5±18.9 b	19.9±5.8 b	785±384 b	86.3±20.1 b	872±329 b
	100	171±47 a	35.0±9.3 a	2521±512 a	206±44 a	2727±416 a
<i>E. splendens</i> , Tongling population	0.31	31.7±12.0 b	14.6±2.2 b	31.5±4.1 b	46.3±11.6 c	77.8±9.4 c
	50	79.1±13.1 b	26.8±3.1 ab	619±229 b	106±13 b	725±200 b
	100	224±60 a	40.8±17.4 a	1959±812 a	265±49 a	2224±640 a
<i>E. splendens</i> , Zhuji population	0.31	30.3±3.2 b	7.2±0.6 b	23.1±6.5 b	37.5±2.4 c	60.5±7.6 b
	50	94.1±40.5 ab	26.0±12.8 a	461±126 b	120±41 b	581±100 b
	100	150±46 a	34.1±6.6 a	2412±1587 a	184±36 a	2596±1303 a

^a Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

species, unlike many other species that cannot survive on Cu mine tailings. Lou et al. (2004) found that most of the Cu taken up by *E. splendens* was distributed in the cell walls and Cu was induced the synthesis of Cu-binding proteins together with low molecular substances such as organic acids, glutathione and amino acids in *E. splendens* roots. Our data indicate that *E. splendens* roots played an important role in Cu tolerance and detoxification in agreement with the results of Lou et al. (2004). The observed increases in of root-to-shoot ratio may enhance the effects of cell wall sequestration of Cu and may help to explain the ability of *E. splendens* to grow in soils highly contaminated with Cu.

Most metal-contaminated soils are anthropogenically polluted and may be unstable and are therefore potential sources of air and water pollution. Establishment of vegetation cover may be essential to stabilize the soil and minimize the pollution risk. The choice of appropriate plant species will be important (Wong, 2003). Plant species that are suitable for phytostabilization should have a healthy root system, tolerate high levels of the target contaminants and retain contaminants in the roots with minimal accumulation in the shoots (Tang et al., 2001). All three populations of *E. splendens* show similar characteristics and may be suitable for phytostabilization of some Cu-contaminated soils. In fact, this species is often a natural colonizer of Cu mine spoil that prevents erosion of the substrate into rivers or groundwater. There is therefore an urgent need for field studies on the stabilization of Cu-polluted sites by this plant species.

4. Conclusions

The roots of *E. splendens* may play an important role in Cu tolerance, as the root-to-shoot ratio and some indicators of root growth performance increased with increasing Cu level in the nutrient solution. There were some differences in Cu uptake capacity among the populations of *E. splendens* studied. Plants from the *E. splendens* Tongling population showed higher Cu accumulation than the other two *E. splendens* ecotypes. This plant species may have some potential for Cu phytoextraction, but is likely to be more suitable for Cu phytostabilization and further studies under field conditions are required.

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