Analysis on the Tolerance of Four Ecotype Plants Against Copper Stress in Soil

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Abstract

To screen copper tolerant species, Four ecotype plants, \textit{Catalpabungei C. A . Mey}, \textit{Catalpa ovata G. Don}, tall fescue (\textit{Festuca arundinacea}), and \textit{Sedum lineare Thunb}, were planted in soil contaminated by Cu in greenhouse to reveal their characteristics of survival, growth and morphology, as well as copper uptake, accumulation and translocation in plants. The survival rate(\(S\)) of \textit{Catalpabungei C. A . Mey} under Cu stress was high, while that of \textit{Catalpa ovata G. Don} was just the opposite. With the increasing of Cu concentrations in soil, tree height(\(H\)) increment percents of both \textit{Catalpabungei C. A . Mey} and \textit{Catalpa ovata G. Don} declined significantly(significant level, \(\alpha=0.05\)), but for stem bases(\(D\)) there were not obvious differences. The translocation factors (\(TF\)) and bioconcentration factors(\(BCF\)) of \textit{Catalpabungei C. A . Mey} and \textit{Catalpa ovata G. Don} were both lower than 0.5. The Cu content in roots of \textit{Catalpabungei C. A . Mey} was significantly positively correlated with that in soil(\(r=0.98; P<0.05\)). Tolerance index (\(TI\)) of tall fescue and \textit{Sedum lineare Thunb} were low. The concentrations of Cu in tall fescue and \textit{Sedum lineare Thunb} both had significantly positive correlation with those in soils. The results suggested that \textit{Catalpabungei C. A . Mey}, tall fescue, and \textit{Sedum lineare Thunb} were tolerant to Cu stress in soil to some extent, and were of application potential as pioneer species for ecological restoration in areas suffering Cu pollution.

Keywords: Catalpabungei C. A . Mey; Catalpa ovata G. Don; tall fescue; Sedum lineare Thunb; copper stress
1. Introduction

Cu is a kind of essential trace element for plants and plays an important role in some processes of physiological metabolism inside plant bodies[1]. Deficiency of Cu in soil can lead to plant deficiency disease, to the opposite, the excess of Cu in soil is also harmful to plant. In Cu enrichment environment, Cu is absorbed and accumulated by plant first in roots generally leading to inhibition or death of roots, then affecting the growth above the ground. According to published reports there is low concentration of Cu in natural environment, that is, Cu content of minimum and maximum in common soil are 2 and 100mg/kg and mean value is around 20--30mg/kg[2]. But in some areas polluted by Cu like copper mine tailings, the content of Cu in soil can reach up to 809.30--1395.54mg/kg[3]. Industrial and agricultural production such as mine exploitation, mining and smelting, manufacturing, cupric pesticides and fertilizers application, etc. dominates the copper pollution of soil[4-9]. At present, phytoremediation technology including phytoextration, phytostabilization, rhizofiltration, and phytovolatilization is widely used to deal with the soil contaminated by heavy metal. Phytoextration is a kind of in-situ soil remediation technology using hyperaccumulator to absorb and transport the heavy metal from soil and removing the heavy metal by mowing practice. Phytostabilization is another in-situ soil remediation technology using tolerant plants to make heavy metal in soil stabilization through uptake and retaining heavy metal in roots as well as precipitation around root zone, etc.. It is critical to screen and directly breed new species of heavy metal hyperaccumulation and tolerance. Until now, more than 400 species of heavy metal hyperaccumulators had been discovered all over the world, about 34 of which acting on Cu [10,11]. Elsholatzia haichowensis Sun, Commelina communis Linn., Rumex acetosa Linn., and Artemisia argyi were reported recently as Cu-enriched plants in China[12,13]. Nevertheless, hyperaccumulators have not been widely used in the practice of restoration in polluted soil for reasons of their slow growth, little phytomass, regionally different suitability, and so on. Therefore, it is still the objective for researchers to look for and breed new species with better effect and more utilization.

Catalpabungei C. A. Mey (Bignoniaceae; Catalpa), a species of deciduous tree, has the characters of strong germination, developed roots, quick canopy density, and long lifespan, et al. and ecological features of drought, cold and somewhat saline tolerance, SO2 and Cl2 resistance, soil and water conservation function and so on. Catalpabungei C. A. Mey is a kind of popular native tree which has a history of cultivation for more than 2000 years in China and distributes widely in temperate, warm temperate and subtropical zones. The main propagation ways are seeding, cutting and grafting. Now it is mainly used for wood furniture and ecological protection[14].

Catalpa ovata G. Don (Bignoniaceae; Catalpa), a species of deciduous tree, prefer to thick and moist soil rich in fertility, has the characters of fast growth, deep and thick roots and ecological features of SO2, HF, and smoke resistance, dust deposit, and somewhat saline tolerance. Its widespread areas locate in temperate, warm temperate and subtropical zones. It is reproduced mainly by seed propagation and applied for garden landscaping[15].

Sedum lineare Thunb (Crassulaceae; Sedum), a species of perennial herb, is a kind of succulent xerophyte whose stratum corneum of surface can prevent stems and leaves efficiently from evaporation. It adapts to soils easily and resists to most of the restrictive factors in environment such as cold, drought, salt, barrens, plant diseases and insect pests. It is widespread in China with the exception of such provinces as Xinjiang, Xizang, Qinghai, Neimenggu, and Gansu. It is propagated mainly by cutting and dividing and applied in roof greening for cooling and water saving.

tall fescue (Festuca arundinacea Schreb.; Poaceae; Festuca ), a species of perennial herb, is a kind of tufted grass having fibrous roots and wide adaptability. Its special characters, different from those mentioned above, are trampling and shade(50%) tolerance and sensitive to fertilizers. It is discovered in
most parts of China[16], propagated mainly with seeds, and used in garden landscaping and ecological rehabilitation.

Catalpabungei C. A. Mey, Catalpa ovata G. Don, Sedum lineare Thunb, and tall fescue all have wide and strong adaptability to environment and can be planted in most parts of temperate and Subtropical zones. Moreover, the four ecotype plants beyond the food chain of human. So, they can play important parts in garden landscaping and ecological rehabilitation. It had been reported that tall fescue had the potential of ecological rehabilitation on Cu contaminated land. But there were few research reports about the tolerance of Catalpabungei C. A. Mey, Catalpa ovata G. Don, and Sedum lineare Thunb against Cu stress [17-20]. In this paper, Catalpabungei C. A. Mey, Catalpa ovata G. Don, Sedum lineare Thunb, and tall fescue were studied with pot culture in greenhouse in order to reveal their characteristics of survival, growth and morphology, as well as copper uptake, accumulation and translocation in plants. What we did here were for the objective of screen of newer and more efficient materials for ecological recovery in the area suffering Cu pollution.

1. Materials and methods

1.1 Soil and plants. The red-yellow soil was collected at the foot of a hill in Fuyang city(30°03'41.72''N; 119°57'04.28''E) and was passed through a 5mm sieve to make it homogenized. The major properties of the soil were measured before pot experiment(Table 1).

Catalpabungei C. A. Mey and Catalpa ovata G. Don were one-year seedling from institute of subtropical forestry. Tall fescue using seed propagation and Sedum lineare Thunb using cutting propagation were bought from a commercial supplier named Xinhe flower and seedling company.

<table>
<thead>
<tr>
<th>Texture</th>
<th>Organic matter (g/kg)</th>
<th>Total N (g/kg)</th>
<th>Total P (g/kg)</th>
<th>Available P (mg/kg)</th>
<th>Available K (mg/kg)</th>
<th>Available Cu (mg/kg)</th>
<th>Total Cu (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy soil</td>
<td>4.89</td>
<td>0.37</td>
<td>0.27</td>
<td>0.71</td>
<td>6.06</td>
<td>37</td>
<td>0.96</td>
</tr>
</tbody>
</table>

1.2 Pot experiment. The pot experiment was performed in a greenhouse at Institute of Subtropical Forestry, Fuyang, China. The climate is subtropical, with a mean annual temperature of 16.2°C and rainfall of 1,452 mm.

Different quantity of CuSO₄·5H₂O(content > 99%) were directly homogenized into sieved soil to formulate mixtures containing Cu in gradient concentrations (mg/kg) of 50, 100, 200 and 500. At the same time, the control soil without extra CuSO₄·5H₂O were used as reference (marked 0). There were three replicates for each treatment and four pots were used for each replicate, totaling 240 pots in a randomized block design. Four kilograms of the substrate mixtures(dry weight) were loaded into cylindrical plastic pots (diameter 15 cm × height 20 cm). One seedling of Catalpabungei C. A. Mey and Catalpa ovata G. Don, 10 stem cuttings of Sedum lineare Thunb around 3cm in length and 50 seeds of tall fescue were planted into each pot, respectively.

1.3 Harvest and chemical analysis. Seven days after potting, tree height and diameter of stem base of Catalpabungei C. A. Mey and Catalpa ovata G. Don were measured. The background average values of Catalpabungei C. A. Mey in height and diameter were 44.07±6.96 cm and 6.68±0.78 mm, respectively and Catalpa ovata G. Don were 102.27±9.11 cm and 12.26±1.86 mm, respectively.
Four months after potting, survival rate($S$), height($H$) and stem base ($D$) increment percent of *Catalpabungei C. A. Mey* and *Catalpa ovata G. Don* were measured and phytomass, shoot and root morphology of *Sedum lineare Thunb* and tall fescue were analysed. After the plants were harvested, the shoots and roots of *Catalpabungei C. A. Mey* and *Catalpa ovata G. Don* were cut off and separated from each other in laboratory, while *Sedum lineare Thunb* and tall fescue were not separated into two parts for the little biomass of roots limited by Cu stress. All subsamples were washed firstly with tap water, then rinsed with deionized water three times, at last air dried, enzymes deactivated at 105℃ for half an hour, and oven-dried at 75℃ for 1 week to constant weight. The phytomass was measured by weighing method after drying. Total Cu in subsample was determined using flame atomic absorption spectroscopy (Solaar M6, Thermo Fisher Scientific Inc, USA) after digestion with 5 mL of a 4:1 mixture ($V/V$) of 80% HNO$_3$ and 20% HClO$_4$.

Soil samples were collected after potting trial, air dried, finely grounded, and passed sequentially through sieves of 2mm, 1mm, 0.25mm, and 0.149mm. Total and available Cu in samples were extracted with aqua regia and 0.1mol/L HCl, respectively, then analyzed by flame atomic absorption spectrometry (AAS).

### 1.4 Data analysis

Survival rate($S$) and growth rate consist of height($H$) and stem base ($D$) increment percent can indicate the adaptability of tree to Cu stress, which were calculated as:

$$S = \frac{n}{N} \times 100.$$  

Where $n$ means quantity of survival plants of a treatment and $N$ means quantity of total plants of the treatment, unit, %.

$$H = \frac{(H_2 - H_1)}{H_1} \times 100.$$  

Where $H_2$ means height after 4 moths cultivation and $H_1$ means height of background values, unit, %.

$$D = \frac{(D_2 - D_1)}{D_1} \times 100.$$  

Where $D_2$ means diameter of stem base after 4 moths cultivation and $D_1$ means diameter of stem base of background values, unit, %.

Tolerance index ($TI$) based on phytomass was used to assess the tolerance of plant to Cu stress. The tolerance index was calculated as:

$$TI = \frac{B_t}{B_c}.$$  

Where $B_t$ (g/pot) means treatment phytomass and $B_c$ (g/pot) means control phytomass.

Bioconcentration factors ($BCF$) used to estimate the plant’s ability of accumulating heavy metals from soils was calculated as the ratio of metal concentration in the aboveground plant tissues to that in the soil. Translocation factors ($TF$) used to estimate the plant’s ability of translocating heavy metals from roots to shoots was calculated as the ratio of total heavy metal in shoots to that in roots.

All statistical analyses (ANOVA and LSD test for mean comparisons) were conducted with SAS 8.0. Differences at $p < 0.05$ were considered significant.

### 2. Results

#### 2.1 Effect of Cu stress on growth of tested plants

*Catalpabungei C. A. Mey* survived and developed in soil with Cu concentration lower than 200 mg/kg, but died out all with Cu concentration at 500mg/kg. With the increasing of Cu level(minimum to maximum:0--200 mg/kg), the growth of *Catalpabungei C. A. Mey* gradually slowed down. For example, quantity and areas of leaves decreased, roots was inhibited and height increment percent declined significantly(significant level; $\alpha=0.05$). The stem base of *Catalpabungei C. A. Mey* had no differences among treatments(Fig. 1). Though *Catalpabungei C. A. Mey* was tolerant, it maybe became small aged tree under Cu stress.
The survival rates of *Catalpa ovata* G. Don planted in copper polluted soil with different concentrations (mg/kg) of 0, 50, 100, 200, 500 were 100%, 56%, 0, 0 and 0, respectively. Survival rates of *Catalpa ovata* G. Don declined sharply when Cu level in soil increased. It was discovered that the roots of *Catalpa ovata* G. Don was sensitive to Cu stress, that was, the roots stopped increasing when the concentration of Cu reached 50 mg/kg. The height increment percent of *Catalpa ovata* G. Don planted in copper polluted soil is significantly lower than that in control soil (significant level; $\alpha=0.05$). The stem base increment percent of *Catalpa ovata* G. Don had no differences among treatments (Fig. 2). Cu stress in soil had greatly negative effect on the growth of *Catalpa ovata* G. Don.

Tolerance index values of tall fescue (*Festuca arundinacea*) were 0.36 (Cu concentration at 50 mg/kg), 0.11 (100 mg/kg), 0.05 (200 mg/kg) and 0 (500 mg/kg), respectively. With the increasing of Cu in soil, phytomass of tall fescue decreased significantly (significant level; $\alpha=0.01$) (Fig. 3). Tolerance index values of *Sedum lineare* Thunb were 0.54 (Cu concentration at 50 mg/kg), 0.78 (100 mg/kg), 0.34 (200 mg/kg) and 0.31 (500 mg/kg), respectively. With the increasing of Cu in soil, phytomass of *Sedum lineare*
Thunb remained little and stable (Fig. 3). Sedum lineare Thunb could resist Cu pollution to some extent. High content of Cu in soil could inhibit the growth of roots of both tall fescue and Sedum lineare Thunb, for example leading to sparse roots.

2.2 Accumulation and translocation of Cu in tested plants. Cu content in shoots and roots of plants were measured separately. The results suggested that the range of Cu content in shoots of Catalpabungei C. A. Mey were 9.89 mg/kg to 41.58 mg/kg, and the mean value was 20.45 mg/kg. Cu content in roots of Catalpabungei C. A. Mey ranged from 21.88 mg/kg to 897.21 mg/kg, and the mean value was 335.78 mg/kg (Table 2). With the increasing of Cu in soil, Cu content both in shoots and roots of Catalpabungei C. A. Mey increased. The correlation coefficient of Cu content between roots and soil was 0.98 (P < 0.05). Increment of Cu content in roots was more than that in shoots. Bioconcentration factors and translocation factors of Catalpabungei C. A. Mey were both lower than 0.5, which showed the weak capability of enrichment and transportation of Cu in Catalpabungei C. A. Mey. The Cu absorbed by Catalpabungei C. A. Mey was mainly retained in the part of root and affected directly the growth of roots, leading to inhibition of aboveground. For the phenomena of high survival rate but low bioconcentration and translocation factors, Catalpabungei C. A. Mey might be considered as a species of Cu tolerant.

Since Catalpa ovata G. Don only survived in soil with Cu content lower than 50 mg/kg, Cu content in plant was measured only for the control and 50 mg/kg treatments. There was no significant difference of Cu content in shoots of Catalpa ovata G. Don between control and 50 mg/kg treatments. But Cu content in roots of 50 mg/kg treatment is significantly higher than that of control treatment (significant level; α = 0.01). Under Cu stress, the accumulation of Cu in Catalpa ovata G. Don was lower that in Catalpabungei C. A. Mey. Values of TF and BCF of Catalpa ovata G. Don were both lower than 0.5 (Table 2). It was week ability for Catalpa ovata G. Don to accumulate and transport Cu in plant.

Table 2 Accumulation (mg/kg) and translocation of Cu in Catalpabungei C. A. Mey and Catalpa ovata G. Don after 4 months

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Catalpabungei C. A. Mey</th>
<th>Catalpa ovata</th>
<th>Shoot</th>
<th>Root</th>
<th>T F</th>
<th>B CF</th>
<th>Shoot</th>
<th>Root</th>
<th>T F</th>
<th>B CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.89±1.55 B</td>
<td>21.88±0.54 C</td>
<td>0</td>
<td>0.14</td>
<td></td>
<td></td>
<td>6.78±0.14</td>
<td>25.20±6.79</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>14.33±3.86 B</td>
<td>120.88±2.69 B</td>
<td>0</td>
<td>0.82</td>
<td>0.45</td>
<td>44</td>
<td>7.81±0.82</td>
<td>99.08±5.58</td>
<td>0</td>
<td>0.89</td>
</tr>
<tr>
<td>100</td>
<td>15.98±1.77 B</td>
<td>303.16±3.84 B</td>
<td>0</td>
<td>0.03</td>
<td>.12</td>
<td>28</td>
<td>A</td>
<td>B</td>
<td>.08</td>
<td>13</td>
</tr>
<tr>
<td>200</td>
<td>41.58±27.5 A</td>
<td>897.21±241.25</td>
<td>0</td>
<td>0.02</td>
<td>.05</td>
<td>27</td>
<td>NA</td>
<td>NA</td>
<td>N</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data represent mean ± SD. NA No analysis. Different letters denote statistically significant differences among treatments. aSignificance level, α = 0.05. bSignificance level, α = 0.01.

Among different Cu stress treatments, Cu content of minimum to maximum in Festuca arundinacea were 33.99 to 1043.45 mg/kg, mean value was 416.17 mg/kg. And for Sedum lineare Thunb, Cu content ranged from 22.45 to 1788.43 mg/kg, mean value was 570 mg/kg. Under Cu stress, Cu content of Festuca arundinacea and Sedum lineare Thunb were much more than normal level (10 mg/kg) in plants. Moreover, with the increasing of Cu concentration in soil, the Cu content in Festuca arundinacea and Sedum lineare Thunb both increased quickly (Fig. 4). There were significantly positive correlation in Cu concentration between soil and Festuca arundinacea (r = 0.99; P < 0.05), and the same to Sedum lineare Thunb (r = 0.99; P < 0.01). Festuca arundinacea and Sedum lineare Thunb both had high capability of Cu accumulation.
3. Discussion

According to the criterion by Baker and Brooks (1983)[21], Cu concentration in shoots of hyperaccumulator is usually up to 1 000 mg/kg, and TF > 1. Copper-hyperaccumulating plant can endure high level of copper in soil. While Cu concentration in shoots of general plant is usually lower than 25 mg/kg, and TF ≤ 0.1[22]. For general plant, Copper usually accumulates in roots, leading to poisoning or even death of plant.

*Catalpabungei C. A . Mey* and *Catalpa ovata G. Don* were of much lower content of Cu in shoots compared with the criterion for hyperaccumulator. But the content of Cu in roots of these two plants increased sharply with the increasing of Cu in soil. For the low transportation of Cu in *Catalpabungei C. A . Mey* and *Catalpa ovata G. Don*, Cu absorbed was mainly accumulated in the parts of roots, only causing the inhibition of roots through the ways of shrinking nucleus of meristematic cell and preventing meristematic cell from dividing[23]. Moreover, the test showed that Cu level more than 100 mg/kg in soil could obviously restrain the development of roots of *Catalpabungei C. A . Mey* and even made *Catalpa ovata G. Don* to die. *Catalpabungei C. A . Mey* exhibited strong capability of Cu accumulation and endurance due to the high content(maximum value up to 897.21 mg/kg) of Cu in roots. *Catalpabungei C. A . Mey* might be a excluder species which kept the shoots off being poisoned by the way of preventing Cu from translocating from roots to stems and leaves[22]. For the high survival rate and obvious growth inhibition, *Catalpabungei C. A . Mey* might be considered just as a kind of Cu tolerant plant having application potential for ecological rehabilitation on Cu contaminated area. *Catalpa ovata G. Don* was susceptible to even low levels of copper. Compared to control treatment, the survival rate of *Catalpa ovata G. Don* in soil with copper content of 50 mg/kg declined around by 50%. *Catalpa ovata G. Don* could not live in the copper polluted soil.

Y.B. Wang(2006)[19] reported that in the condition of hydroponic culture, low level of Cu(<10mg/L) in nutrient solution had no obvious restriction for tall fescue, but as the increasing of Cu level, from 10 to 40 mg/L in nutrient solution, Cu stress showed some negative effects on the growth of tall fescue. In contrast with control treatment, For instance, tall fescue under Cu stress became dwarf shape, yellow leaves, short and sparse roots, as well as low biomass and plant pigment. In this paper, we tested the tolerance of tall fescue against different level of copper in solid substrate with pot experiment. The result suggested that the increment of tall fescue, on the basis of phytomass, was slowed down when Cu content in soil reached just low level of 50 mg/kg. In addition, with the increasing of Cu in soil, phytomass of tall fescue decreased obviously. Tall fescue, sensitive to the varied level of Cu, could bear Cu stress in soil to some extent. *Sedum lineare Thunb* showed a certain degree of tolerance against copper due to its constant value of phytomass in different treatments. The effect of abundant cytosol on the
dilution of copper ions was possibly the mechanism for *Sedum lineare Thunb* to avoid Cu toxicity. Moreover, with the increasing level of Cu in soil, the Cu content in tall fescue and *Sedum lineare Thunb* both increased sharply and the maximum content of Cu measured in tall fescue and *Sedum lineare Thunb* were 1043.45 mg/kg and 1788.43 mg/kg, respectively, which were the same to the results from Y. Wang(2007) and Y.B. Zhang(2010)[24,17]. Since the sensitivity and low phytomass of tall fescue observed in the trial process, it is still uncertain whether tall fescue could directly applied for ecological restoration on Cu contaminated land. While *Sedum lineare Thunb* was suggested to be applied for the surface cover of land polluted by Cu, retaining and promoting the content of organic matter of topsoil.

4. Conclusions

It was obvious that copper in soil was a key limiting factor restricting the growth of four ecotype plants discussed in this paper. With a certain degree of copper tolerant ability, *Catalpabungei C. A. Mey* and *Sedum lineare Thunb* had application potential as pioneer species for the ecological restoration in areas suffering Cu pollution. While *Catalpa ovata G. Don* did not. Tall fescue was suggested to be planted in soil with low level of Cu. The following is to screen and breed higher Cu tolerant cultivars of *Catalpabungei C. A. Mey*, Tall fescue and *Sedum lineare Thunb*, as well as to develop the reasonable equipment and integrated cultural practices to reduce Cu toxicity in planting area.

Acknowledgments

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