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# Accumulation and Resistance to Copper of Two Biotypes of *Cynodon dactylon*

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**Abstract** The effects of copper accumulation and resistance in two biotypes of *Cynodon dactylon* were studied. Results showed that at a low concentration of copper (<100 mg/kg), the growth of *Cynodon dactylon* was generally unaffected. As copper concentration increased, negative effects on the growth of *Cynodon dactylon* became apparent. The critical concentration at which the plant exhibited poisoning symptoms was different for the two biotypes of *Cynodon dactylon*. At 500 mg/kg copper concentration in soil, the biotype from the polluted area showed significantly higher tolerance of copper than the biotype from the unpolluted area.

**Keywords** *Cynodon dactylon* · Copper · Biotype · Resistance

In recent years, much scientific research has been done concerning the recovery and restoration of heavy metal tailings (Baker and Brooks 1989; Ewais 1997; Roser et al. 2006). These studies have emphasized creating screening tests for hyperaccumulator or accumulator plants and examining mechanisms of tolerance in plants, particularly cultivated crop plants (Archer and Caldwell 2004; Wang et al. 2004a, b; Roser et al. 2006). On the one hand, the current limited variety and number of hyperaccumulators hardly allow extensive applications of heavy metal hyperaccumulators in various conditions (Wei et al. 2004); on the other hand, there are quite a few deserted areas of land polluted with heavy metal tailings in mountain and river valleys (Wang et al. 2004a, b). There is no urgent need to cultivate this land at present. The urgent need is to ameliorate the soils of these uncovered and unsteady wastelands and restore vegetation that can eventually reduce the harmfulness of the soils. Because cultivation of crop plants on this polluted land would introduce heavy metals into the food chain, wild plants that can resist heavy metals and survive on deserted land, such as lawn grass or mound grass, may be the best alternative for restoring the land. This strategy was not only practical, but also economical.

Various species of weed and turfgrass have been studied for their response to heavy metals (Ewais 1997; Archer and Caldwell 2004), and *Cynodon dactylon* (L.) Pars is an important species among them because of its fast growth rate and good reproductive capacity. It is not only a common weed in farmland, but also a variety of fine grass for lawns. It can resist drought, trampling, alkali, saline, and other environmental stresses, and the resistance of the wild variety is stronger. It has been extensively used for athletic fields, parks, graveyards, and soil and slope protection (Archer and Caldwell 2004). Wang et al. (2004a, b) indicated that *C. dactylon* grew normally on a wasteland of tailings with high Cu concentration and that it might be able to restore copper mine tailings. However, few other reports have been published concerning the copper tolerance of *C. dactylon*. The objective of this study is to understand the potential of two biotypes of *C. dactylon* as soil remediation plants for copper mine tailings using cultivation experiments.

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## Materials and Methods

*Cynodon dactylon* plants were collected from the Shizishan copper tailings yard in Tongling City, Anhui

Province, China (polluted area biotype) and from an area of farmland 10 km away from the copper tailings yard with no evident heavy metal contamination (unpolluted area biotype). During sampling, *C. dactylon* seedlings of similar height and biomass were collected, and they were cultivated for 2 days before experimental treatments began. The soil used for cultivation was taken from the ecological plantation of Anhui Normal University: it is yellow-brown earth containing 13.35 g/kg of organic matter and has N, P, K, and Cu content levels of 1.25, 0.15, 10.89 g/kg, and 26.3 mg/kg respectively. The Cu treatments consisted of 100, 200, 500, 1,000, 2,000, or 3,000 mg Cu (in the form of $CuSO_4 \cdot 5H_2O$) per kilogram of dry soil. A soil sample without Cu served as the control. For the treated soil samples, 2 kg of each soil was placed in a plastic pot (diameter: 20 cm), and the pot experiment was conducted in the ecological plantation of Anhui Normal University. The reaction time between Cu and soil was 30 days. The seedlings of *C. dactylon* were about 5 cm in height, and their roots were cut off to 5 mm. Each pot contained 10 plants, and 10 replications (A replication is one pot with 10 plants) were used in this study. The group with no cultivated plants served as the control group. The cultivation experiment took place between April 5, 2005 and June 4, 2005, and the plant samples were collected and measured on May 5 and June 4 of the same year. Fresh weight, dry weight, root length, and height of the aboveground part were measured for each *C. dactylon* plant. The aboveground part and underground part of each plant were separated at sample collection. Samples were washed 3 times in distilled water, sanitized for 0.5 h under 105°C, and then dried under 70°C for 72 h. The dried samples were digested by $HNO_3$-$H_2SO_4$-$HClO_4$ (8:1:1) after being triturated, and the concentrations of Cu were determined by atomic adsorption spectrophotometry (AA6800, Shimadzu, Japan); the Cu standard sample of the state environmental protection administration of China was used for correction (Environmental Monitoring of China 1992). Soil samples were extracted by 0.1 mol/L HCl (liquid:soil = 5:1) for 90 min, to determinate the concentration of available Cu in soils. The mixture was then centrifuged and the supernatant was filtered. The concentration of Cu in the supernatant was determined by atomic adsorption spectrophotometer (AA6800, Shimadzu, Japan) (Environmental Monitoring of China 1992). To avoid cross-contamination of Cu or other metals, all receptacles had been soaked in 2% $HNO_3$ for more than 24 h before used. Data were analyzed using SPSS 11.0 software, and regression, correlation analysis and *t*-test were used for determining the significance of difference among various groups of plant and soil samples.

## Results and Discussion

In regard to external Cu toxicity symptoms in *C. dactylon*, poisoning symptoms and critical Cu concentrations were obviously different in the two biotypes of *C. dactylon*. For the unpolluted area biotype of *C. dactylon*, both the control and treated plants were doing well at low Cu concentration (<200 mg/kg) for 15 days after cultivation. However, when the Cu concentration became higher than 200 mg/kg, the plants began to express external symptoms, including discoloration of leaves. As time went on, the plants treated with the low concentration of Cu showed no obvious change, but the external symptoms of the plants treated with high Cu concentrations were much more notable: the leaves were small, thin, and feeble, and the discolored areas spread. When the Cu concentration in the soil reached 2,000 mg/kg, the leaves became thin, yellow and wilting. In contrast, the plants of the polluted area biotype of *C. dactylon* in both control and low-Cu-concentration groups grew slowly. These plants were short and small, and their leaves were long and narrow. Interestingly, at 500 mg/kg Cu in the soil – the point when the unpolluted area biotype of *C. dactylon* started to show toxic symptoms – the polluted area biotype of *C. dactylon* grew extremely well, with the best results among all the treatments.

The Cu stress had obvious effects on the growth of *C. dactylon*. For the unpolluted area biotype at low Cu concentrations, slight increases were observed in dry weights of both aboveground and underground parts, average root length, and height of aboveground parts. However, when the Cu concentration was increased to 500 mg/kg, the biomass, root length, and height of the aboveground part decreased sharply. These changes were significantly negatively related to Cu concentration, while the polluted area biotype of *C. dactylon* demonstrated a greater tolerance than the unpolluted area biotype (Table 1). At the level of 500 mg/kg Cu, the biomass, root length, and height of the aboveground part of the polluted area biotype reached their maximum. Further increases in Cu concentration enhanced the value of each growth index of the polluted area biotype of *C. dactylon* compared to those of the unpolluted area biotype ($p < 0.05$). This indicated that the resistance of the polluted area biotype of *C. dactylon* was much higher than that of the unpolluted area biotype, and that it could grow well in soils contaminated by Cu. However, at Cu concentrations lower than 200 mg/kg, the dry weights, average root lengths, and heights of the aboveground parts of the polluted area biotype plants were generally lower than those of the unpolluted area biotype, probably due to genetic difference between these two biotypes of *C. dactylon*. This existing standpoint can be regarded as a kind of resistance cost, because the polluted area biotype needs to consume a

**Table 1** Effects of Cu concentrations on the growth of *C. dactylon*

| Biotype | Concentration (mg/kg) | Dry weight of aboveground part (mg) | | Dry weight of underground part (mg) | | Average length of root (cm) | | Height of aboveground part (cm) | |
|---|---|---|---|---|---|---|---|---|---|
| | | I[a] | II[b] | I | II | I | II | I | II |
| Unpolluted area biotype | Control | 71 ± 6.5[c] | 75 ± 7.6 | 52 ± 5.3 | 57 ± 6.6 | 3.5 ± 0.3 | 3.9 ± 0.4 | 10.7 ± 1.2 | 11.8 ± 1.3 |
| | 100 | 85 ± 8.6 | 92 ± 10.8 | 67 ± 6.6 | 78 ± 8.9 | 4.0 ±0.4 | 4.6 ± 0.5 | 11.2 ± 1.6 | 12.1 ± 1.2 |
| | 200 | 82 ± 8.4 | 84 ± 6.9 | 60 ± 6.2 | 71 ± 8.8 | 3.6 ± 0.4 | 4.2 ± 0.4 | 10.9 ± 0.9 | 11.9 ± 1.1 |
| | 500 | 65 ± 5.9 | 79 ± 8.4 | 55 ± 5.1 | 62 ± 6.3 | 3.2 ± 0.3 | 3.6 ± 0.4 | 10.3 ± 1.1 | 11.2 ± 0.8 |
| | 1,000 | 48 ± 5.1 | 58 ± 5.3 | 37 ± 3.8 | 44 ± 4.5 | 2.9 ± 0.3 | 3.2 ± 0.4 | 9.6 ± 0.9 | 10.4 ± 0.9 |
| | 2,000 | 40 ± 5.3 | 49 ± 4.2 | 34 ± 3.6 | 38 ± 4.2 | 2.7 ± 0.2 | 2.9 ± 0.3 | 7.5 ± 0.8 | 9.6 ± 0.9 |
| | 3,000 | 39 ± 4.6 | 45 ± 4.3 | 28 ± 3.1 | 35 ± 4.1 | 2.4 ± 0.2 | 2.5 ± 0.3 | 6.2 ± 0.7 | 7.5 ± 0.8 |
| Polluted area biotype | Control | 66 ± 7.8 | 74 ± 8.1 | 51 ± 5.5 | 58 ± 6.8 | 3.0 ± 0.2 | 3.8 ± 0.5 | 8.6 ± 0.8 | 10.4 ± 0.8 |
| | 100 | 72 ± 6.9 | 89 ± 8.5 | 56 ± 5.9 | 66 ± 6.7 | 3.4 ± 0.3 | 3.9 ± 0.5 | 9.9 ± 0.9 | 11.6 ± 1.3 |
| | 200 | 83 ± 9.4 | 95 ± 8.8 | 66 ± 6.2 | 72 ± 8.1 | 3.8 ± 0.4 | 4.3 ± 0.4 | 11.5 ± 1.5 | 12.5 ± 1.3 |
| | 500 | 112 ± 10.1 | 129 ± 13.6 | 78 ± 8.1 | 89 ± 9.6 | 5.6 ± 0.6 | 6.7 ± 0.8 | 15.3 ± 1.6 | 22.3 ± 1.9 |
| | 1,000 | 73 ± 7.4 | 78 ± 7.6 | 68 ± 7.4 | 78 ± 8.3 | 5.2 ± 0.5 | 6.1 ± 0.6 | 12.5 ± 1.3 | 20.6 ± 2.0 |
| | 2,000 | 58 ± 5.5 | 66 ± 7.1 | 43 ± 4.6 | 49 ± 5.5 | 4.2 ± 0.5 | 4.5 ± 0.5 | 12.3 ± 1.3 | 18.2 ± 1.8 |
| | 3,000 | 48 ± 4.1 | 56 ± 5.3 | 31 ± 4.2 | 40 ± 4.2 | 2.5 ± 0.3 | 3.1 ± 0.3 | 8.0 ± 0.9 | 10.2 ± 1.1 |

[a] May 5th, 2005; [b] June 4th, 2005; [c] Stdevp, n = 10

certain amount of energy for combating heavy metal stress, and the cost of this situation is the decrease in biomass (Kidd et al. 2004; Koivunen et al. 2004). Cu appeared to have a greater effect on the growth of the underground part than on the growth of the aboveground part, reflected in the root/shoot ratios. As Cu concentration increased, especially at the level of 500 mg/kg, the root/shoot ratio decreased extremely markedly and the regression coefficient $r$ was $-0.924$ ($p < 0.01$). This raised concern because the underground part of the plant was in fact the direct functional part of Cu. Therefore, a low level of Cu might have a good effect on the growth of *C. dactylon*. However, when Cu concentration was raised to a certain level, it certainly had an impact on the growth of *C. dactylon*. The results were consistent with reports that heavy metals influence root tip cell mitosis, decrease the velocity of cell division, and cause biomass to decrease (Yang et al. 2002; Seregin and Kozhevnikova 2006).

Table 2 shows that Cu content in the aboveground and underground parts of these two biotypes of *C. dactylon*

**Table 2** Cu concentrations in above-ground parts and underground parts of *C. dactylon*

| Biotype | Concentration (mg/kg) | Aboveground part (mg/kg DW) | | Underground part (mg/kg DW) | |
|---|---|---|---|---|---|
| | | I[a] | II[b] | I | II |
| Unpolluted area biotype | Control | 11.93 ± 1.31[c] | 18.04 ± 1.64 | 23.25 ± 3.98 | 28.45 ± 3.45 |
| | 100 | 20.24 ± 2.22 | 25.67 ± 3.95 | 71.68 ± 10.64 | 83.22 ± 10.24 |
| | 200 | 31.35 ± 3.53 | 41.54 ± 5.62 | 123.07 ± 26.78 | 140.54 ± 21.11 |
| | 500 | 72.10 ± 6.84 | 80.75 ± 10.11 | 203.43 ± 30.12 | 224.54 ± 31.64 |
| | 1,000 | 143.10 ± 16.48 | 158.19 ± 24.65 | 367.50 ± 38.65 | 395.17 ± 42.45 |
| | 2,000 | 217.68 ± 22.43 | 224.58 ± 33.87 | 488.10 ± 60.26 | 504.08 ± 68.93 |
| | 3,000 | 399.01 ± 41.51 | 478.08 ± 56.46 | 903.15 ± 120.67 | 979.45 ± 136.43 |
| Polluted area biotype | Control | 21.68 ± 2.66 | 30.68 ± 3.55 | 32.42 ± 3.95 | 40.45 ± 4.85 |
| | 100 | 30.05 ± 2.89 | 41.42 ± 5.92 | 89.60 ± 10.45 | 128.78 ± 15.67 |
| | 200 | 41.93 ± 5.01 | 55.58 ± 7.88 | 134.70 ± 18.69 | 184.53 ± 25.44 |
| | 500 | 79.98 ± 8.67 | 91.53 ± 13.21 | 275.38 ± 40.16 | 305.18 ± 46.17 |
| | 1,000 | 180.93 ± 20.33 | 207.48 ± 36.98 | 431.75 ± 55.98 | 458.54 ± 59.82 |
| | 2,000 | 249.95 ± 25.63 | 310.54 ± 51.29 | 705.83 ± 80.71 | 848.38 ± 91.56 |
| | 3,000 | 421.58 ± 51.69 | 496.54 ± 60.48 | 1,188.48 ± 122.64 | 1,248.15 ± 143.55 |

[a] May 5th, 2005; [b] June 4th, 2005; [c] Stdevp, n = 10

increased continuously with the increment of Cu processing density. Along with the extension of growth time, Cu concentration was increased markedly ($p < 0.05$). In addition, Cu content in the underground parts of these two biotypes of *C. dactylon* was much higher than in the aboveground parts, and with the increment of Cu processing density, the increased value of Cu concentration was greater than that of the aboveground parts. It was also observed that Cu content in the aboveground and underground parts of the polluted area biotype of *C. dactylon* were consistently higher than those in the unpolluted area biotype ($p < 0.01$) regardless of the time of exposure. On a relative scale, however, the Cu content levels in the aboveground and underground parts of the polluted area biotype of *C. dactylon* were only 7.13 and 10.53 times greater than those in its companion control, compared to 10.38 and 12.39 times greater than the control levels for the unpolluted area biotype ($p < 0.05$). This phenomenon was more obvious at the high Cu concentrations. When the Cu treatment concentration rose to 3,000 mg/kg, the concentrations in the aboveground and underground parts of the polluted area biotype of *C. dactylon* were 17.82 and 26.42 times those in the control, while the concentrations in the unpolluted area biotype reached 29.97 and 32.67 times those in its companion control. Meanwhile, when the Cu concentration was higher than 500 mg/kg, the migration ratio of Cu from the underground part to the aboveground part for the polluted area biotype of *C. dactylon* was significantly lower than that in the unpolluted area biotype ($p < 0.05$) (Table 3). Thus, the underground part of the polluted area biotype of *C. dactylon* showed strong retention of Cu, which could obstruct the transportation of $Cu^{2+}$ from the underground part to the aboveground part. Table 1 shows that as the Cu concentration increased to 500 mg/kg, the growth reduction for the polluted area biotype of *C. dactylon* was smaller than that for the unpolluted area biotype, suggesting that the retention of Cu in the underground part of the polluted area biotype of *C. dactylon* had somehow reduced the harmful effect of Cu on the aboveground part. When plants can grow in an area contaminated with a high content of Cu over a long period of time, the mechanism by which they resist Cu toxicity must be considered. The mechanism we propose here matches with the exclusion mechanism provided by Baker (1981) and blocking was one of the important reasons in strengthening the anti-Cu ability in the polluted area biotype of *C. dactylon* (Morrison et al. 1979; Kidd et al. 2004).

Metal accumulation, defined as the product of the metal concentration and the biomass of the aboveground part of the plant, were the most important factors in evaluating phytoremediation efficiency. By examining the relative accumulations in the aboveground part and the underground part of *C. dactylon*, it could be found that, except for the control samples and the treatment with 100 mg/kg Cu, Cu accumulation in the aboveground parts of the two biotypes of *C. dactylon* increased remarkably with the increment of Cu concentrations in this study. Though the biomass of the underground part of *C. dactylon* was obviously smaller than that of the aboveground part (Table 1), the accumulation of Cu in the underground part was still greater than that of the aboveground part (Table 3). However, the ratio of Cu accumulation in the

**Table 3** Cu accumulation in *C. dactylon*

| Biotype | Concentration (mg/kg) | Total accumulation (mg) | | Relative accumulation of above-ground part (%) | |
|---|---|---|---|---|---|
| | | I[a] | II[b] | I | II |
| Unpolluted area biotype | Control | 2.06 ± 0.43[c] | 2.98 ± 0.47 | 41.20 ± 12.39 | 45.48 ± 8.53 |
| | 100 | 6.52 ± 1.26 | 8.85 ± 1.96 | 26.37 ± 5.64 | 26.68 ± 6.75 |
| | 200 | 9.96 ± 2.11 | 13.47 ± 2.98 | 25.82 ± 6.97 | 25.91 ± 6.12 |
| | 500 | 15.88 ± 3.44 | 20.30 ± 4.18 | 29.52 ± 5.46 | 31.42 ± 8.94 |
| | 1,000 | 20.47 ± 3.69 | 26.56 ± 5.04 | 33.56 ± 7.62 | 34.54 ± 9.73 |
| | 2,000 | 25.30 ± 4.75 | 30.16 ± 6.56 | 34.41 ± 8.16 | 36.49 ± 11.47 |
| | 3,000 | 40.85 ± 9.17 | 55.79 ± 12.79 | 38.09 ± 11.07 | 38.56 ± 11.58 |
| Polluted area biotype | Control | 3.08 ± 0.58 | 4.62 ± 0.91 | 46.39 ± 14.81 | 49.18 ± 16.54 |
| | 100 | 7.18 ± 1.22 | 12.17 ± 2.25 | 30.13 ± 5.80 | 30.25 ± 7.69 |
| | 200 | 12.37 ± 2.42 | 18.57 ± 4.14 | 28.13 ± 6.94 | 28.44 ± 7.51 |
| | 500 | 30.44 ± 6.25 | 38.97 ± 8.82 | 29.43 ± 7.08 | 30.30 ± 8.52 |
| | 1,000 | 42.57 ± 9.37 | 51.95 ± 11.58 | 31.03 ± 9.85 | 31.15 ± 10.32 |
| | 2,000 | 44.85 ± 8.14 | 62.07 ± 13.40 | 32.33 ± 6.45 | 33.02 ± 10.40 |
| | 3,000 | 57.08 ± 12.48 | 77.73 ± 15.55 | 35.45 ± 11.13 | 35.77 ± 9.68 |

[a] May 5th, 2005; [b] June 4th, 2005; [c] Stdevp, n = 10

**Table 4** Effects of *C. dactylon* growth on concentrations of available Cu in soils

| Concentration (mg/kg) | Without plant (mg/kg) | | Unpolluted area biotype *C. dactylon* (mg/kg) | | Polluted area biotype *C. dactylon* (mg/kg) | |
|---|---|---|---|---|---|---|
| | I[a] | II[b] | I | II | I | II |
| Control | 3.82 ± 0.33[c] | 4.02 ± 0.42 | 4.46 ± 0.43 | 4.92 ± 0.52 | 4.64 ± 0.45 | 4.75 ± 0.55 |
| 100 | 15.33 ± 1.86 | 16.59 ± 1.55 | 18.69 ± 1.76 | 20.21 ± 3.01 | 16.84 ± 1.83 | 18.86 ± 2.05 |
| 200 | 28.36 ± 3.60 | 29.63 ± 3.87 | 31.48 ± 3.98 | 32.68 ± 4.11 | 39.76 ± 4.24 | 41.25 ± 4.65 |
| 500 | 43.84 ± 5.66 | 45.55 ± 6.01 | 48.71 ± 6.55 | 51.75 ± 6.43 | 72.18 ± 9.46 | 81.52 ± 10.58 |
| 1,000 | 78.72 ± 9.16 | 85.13 ± 11.23 | 90.75 ± 9.98 | 105.45 ± 11.15 | 145.48 ± 18.47 | 133.62 ± 12.49 |
| 2,000 | 185.34 ± 22.42 | 196.74 ± 23.62 | 224.81 ± 24.46 | 235.86 ± 24.32 | 285.13 ± 30.33 | 329.25 ± 41.58 |
| 3,000 | 322.89 ± 35.46 | 335.38 ± 45.28 | 456.14 ± 61.49 | 489.68 ± 62.01 | 506.24 ± 61.45 | 513.17 ± 69.84 |

[a] May 5th, 2005; [b] June 4th, 2005; [c] Stdevp, n = 10

aboveground part of *C. dactylon* was still more than 30% on average with Cu pollution. This demonstrated that although the Cu accumulation of the underground part was higher than that of the aboveground part in *C. dactylon*, the quantity of Cu migrated from the underground part to the aboveground part was also elevated. Therefore, this plant possessed a stronger ability to remediate Cu contamination in the soil environment. However, because the biomass and Cu concentration were both smaller (Tables 1 and 2), the total Cu accumulation in the aboveground part of the unpolluted area biotype was remarkably lower than that in the polluted area biotype. This indicates that the polluted area biotype of *C. dactylon* has a great potential for phytoremediation in soils polluted by Cu.

The available concentration of a heavy metal is bound up with soil physicochemical properties, plant growth condition, etc. (Zhou and Sun 2002; Sun et al. 2006). It directly affects plant growth and influences transformation of the heavy metal among fractions. The content of available Cu in soils was linearly correlated to the treated concentrations of Cu in soils, and the correlation coefficient was 0.985 (Table 4). Compared to the controls, although cultivated plants had assimilated Cu to a certain degree and the absorption increased sharply with the increment of plant biomass, the available Cu in soils was increased (rather than reduced) significantly ($p < 0.05$) (Table 4). Furthermore, this phenomenon was even more evident in the polluted area biotype than in the unpolluted area biotype. This suggests that the growth and uptake of Cu by *C. dactylon* accelerates the transformation of Cu among fractions in the soil. The available Cu fraction can be utilized by organisms, and it is more mobile in soil than any other fractions. Factors such as eluviation (Zhou and Sun 2002) can advance the migration of the available Cu in soil. Thus, it can be concluded that plant uptake should not be the only factor studied in Cu phytoremediation. A comprehensive investigation should include growth, uptake, root secretion, etc. of *C. dactylon* activated Cu in soil as well as the accelerated transformation of Cu from various fractions to the available fraction. The greater the proportion of available Cu in the soil, the greater the transformation of Cu to other fractions, because the available Cu is apt to be taken away by other exotic factors such as rain and the effect of activation and uptake propels the restoration of Cu-polluted soil. The absorption capability of the polluted area biotype of *C. dactylon* is obviously stronger than that of the unpolluted area biotype. Therefore, it can be concluded that *C. dactylon* is a Cu-tolerant plant that can be used to restore Cu-polluted soil, and of the two biotypes examined in this study, the polluted area biotype is more appropriate for this purpose.

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