Manganese uptake and accumulation in a woody hyperaccumulator, *Schima superba*

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ABSTRACT

A wide-spread subtropical tree species, *Schima superba* (*Theaceae*), occurring in a Mn mine wasteland, was found to contain unusually high Mn content in the leaf tissues. A pot growth experiment with different Mn treatments was conducted to further illustrate its Mn tolerance, accumulation and relocation capacity. *Schima* saplings grew well and showed no symptoms of Mn toxicity with Mn supply below 60 mmol/l. Total plant biomass decreased with the increase of Mn supply, but Mn contents in tissues increased significantly, and peaked (62 412.3 mg/kg) in stem at 150 mmol/l treatment. Under all treatments, Mn concentrations in the aboveground tissues were constantly greater than those in roots. When the external Mn supply was over 40 mmol/l, the Mn levels in the leaves and stems all exceeded 10 000 mg/kg, the suggested value for Mn hyperaccumulation. Most of the Mn taken from the substrates were transported to the aboveground tissues, e.g. over 86% accumulated in the aboveground parts at 150 mmol/l treatment. These findings confirmed that *Schima superba* is a Mn hyperaccumulator.

Keywords: hyperaccumulator; *Schima superba*; Mn accumulation; Mn toxicity; phytoremediation

Manganese is a common metal in the Earth's crust and its presence in soils mainly results from Mn in the parent material. Anthropogenic practices, especially the mining industry, have raised Mn content and availability in many soils. For instance, the extraction of Mn ore has led to elevated Mn and Cd levels in the minesoil (Yang et al. 2006, Li et al. 2007). Mn is an essential trace element for life tissues; however, exposure to excessive Mn can cause toxic effects on plants and people. As for plants, speckles presented on old leaves are the most typical symptoms of manganese toxicity, as well as lack of iron (El-Jaoual and Cox 1998). For people, exposure to excessive Mn can result in Parkinson-like symptoms (Gerber et al. 2002, Erikson and Aschner 2003) and abnormalities of the reproductive/immune system (Vartanian et al. 1999). In addition, one study conducted in Canada found that excessive manganese exposure was associated with decreased intelligence development among young children (Takser et al. 2003). The heavy metal contamination (e.g. Pb, Cd, Cu, Hg and Zn) attracted great interest of many scientists (Lasat 2002); the ecotoxicological effect of Mn was however rarely studied until recently. Some researches (Kuperman et al. 2004, Paschke et al. 2005) have been carried out in this area as Mn pollution becomes an increasing problem.

Phytoremediation of metal-contaminated soils is a cost-effective green technology mainly including phytoextraction and phytostabilization. Of the plants screened for remediation, woody species (such as willow and poplar) have proved as good candidates for phytoremediation purposes due to their rapid growth, high biomass, profuse root apparatus (Pulford and Watson 2003) and low impact on the food chain and human health. Currently 14 Mn hyperaccumulators have been reported including 9 species listed by Reeves and Baker (2000), an unidentified *Eugenia* species

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(Proctor et al. 1989) and the newly found tree species *Austromyrtus bidwillii* (*Myrtaceae*) from Australia (Bidwell et al. 2002) and three herbaceous plants *Phytolacca acinosa* Roxb. (Xue et al. 2003), *Phytolacca americana* (Yuan et al. 2007) and *Polygonum hydropiper* L. (Wang et al. 2007) from China.

Our previous studies on a Mn mineland indicated that *Schima superba* (*Theaceae*), a subtropical evergreen tree species commonly used for forest firebreak and gardening, contained unusually high Mn levels in the leaves (Yang et al. 2006). According to Baker and Brooks (1989) who defined Mn hyperaccumulation (concentration exceeding 10 000 mg/kg in the aboveground tissue), *S. superba* was a potential Mn hyperaccumulator. This tree species grows fast with wide ecological amplitude and has substantial biomass, giving a great potential for use in site remediation. The majority of studies about *S. superba* concerned its fire prevention and forestation, recently molluscidal effect of the methanol extract has also been reported (Luo et al. 2005). This study, based on the pot growth experiment under controlled conditions, aims to examine the tolerance of *S. superba* to Mn stress and identify its Mn-accumulation properties. The research findings may help to further illustrate its hyperaccumulation and provide more information on the possible use in the Mn-contaminated sites.

MATERIAL AND METHODS

Plant materials and experimental design

One-year old *Schima superba* saplings (about 30 cm in height) were prepared for the pot growth experiment. Saplings were transplanted in plastic pots, one individual per pot, and each pot was filled with 3.5 kg washed quartz sand. Hoagland's nutrient solution (pH = 4.79) was supplied every 5 days. After stabilization for two months, eighty-one strong, uniform saplings were selected and exposed to the following Mn treatments: 0 (control), 10, 20, 40, 60, 80, 100, 150 or 200 mmol/l, prepared with $MnCl_2 \cdot 4H_2O$. Each treatment was replicated three times. During the course of the experiment, 100 ml Hoagland's solution containing the relevant Mn amounts were added to the pots at a 7-day interval. Deionized water was supplied to leaves as necessary. The number of leaves was counted and plant height was measured every 15 days. Growth performance of saplings was carefully observed and recorded. Plants were harvested 90 days after the Mn treatment.

Sample preparation and chemical analysis

The whole plants were first washed with tap water completely and then rinsed three times with deionized water. Plant samples were separated into roots, stems and leaves, first dried at 105°C for 30 min, and then at 70°C to constant weight. Fresh weight (fw) and dry weight (dw) of plant materials were recorded. Dried plant materials were ground in a stainless steel mill to fine powder. Samples (about 0.1 g) were digested with concentrated HNO_3 + $HClO_4$ (5:1, v/v) and Mn concentration was determined by the atomic absorption spectrometry with flame atomizer (WFX-110, Beijing). Quality assurance of metal analysis was performed using certified Mn standard liquid added into the digested solution, and the average recovery rate was around 96%.

Statistical analysis

All statistical analyses were performed using the SPSS (Statistical Package for Social Science) for Windows. One-way ANOVA was carried out to assess the difference of means from various Mn treatments, followed by multiple comparisons using the least significant difference (LSD) test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Mn treatment on growth of *Schima superba*

Schima superba grew normally and showed no symptoms of Mn toxicity with Mn supply below 60 mmol/l during the period of treatment. When the external Mn concentration was above 80 mmol/l for 45 days, necrotic patches however appeared on the adult leaves and gradually grew bigger and continued to produce new leaves. At 150 mmol/l and 200 mmol/l Mn obvious symptoms of Mn toxicity appeared at 60-day treatment, such as the leaf senescence and withering; stems and roots still sustained growth. Basically, the number of leaves declined as the stress progressed when the Mn supply was over 60 mmol/l (Figure 1). Under all treatments, the stems remained growing but

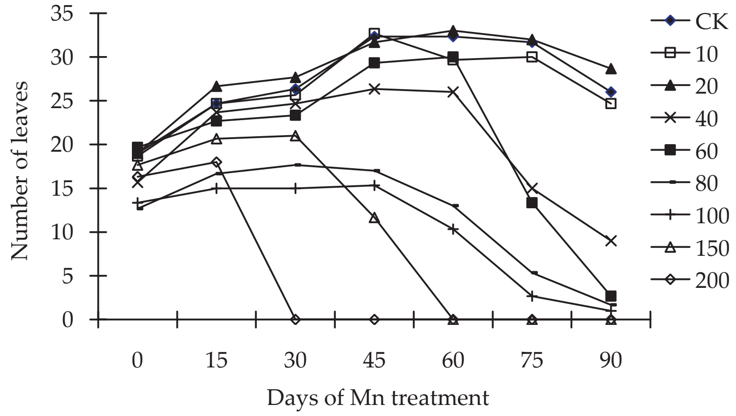

Figure 1. Number of *Schima superba* leaves under different Mn treatments in sand culture

no significant difference existed in height with the exception of the control (Figure 2). Treatments with higher Mn levels significantly decreased the biomass (dry weight) of plants, for instance at 200 mmol/l Mn treatment, 48.5% (leaf), 63.3% (stem) and 51.4% (root) of biomass reduced in comparison with the control (Table 1). It indicates that high external Mn levels may greatly affect the growth of leaf (> 60 mmol/l), stem (> 80 mmol/l) and root (> 150 mmol/l) of this plant.

Manganese uptake by *Schima superba*

Mn concentrations in roots, stems and leaves rose progressively with the increasing Mn supply (Figure 3). At Mn supply below 60 mmol/l, Mn concentrations in *Schima superba* tissues followed a trend of leaf > stem > root. Stem Mn concentrations increased quickly with Mn supply above 60 mmol/l, reaching the maximum of 62 412.3 mg/kg at 150 mmol/l. When the external Mn level was over 40 mmol/l, leaf Mn contents gradually increased and reached the peak (30 222.3 mg/kg) at 150 mmol/l. Root Mn concentrations remained increasing under all Mn treatments. Moreover, Mn concentrations in the above-ground tissues were constantly greater than those in roots. When Mn supply was over 40 mmol/l (7.91 g/l), Mn concentrations in the leaves and stems were all beyond 10 000 mg/kg, the suggested threshold value for Mn hyperaccumulation, further reflecting that this woody plant is a Mn hyperaccumulator.

Manganese accumulation and distribution in *Schima superba*

Mn accumulation in the plant tissues (Mn storage per plant) increased significantly with the elevation of external Mn levels (Table 1). The accumulation of Mn in leaves and stems first increased and then decreased with the maximum of 89.64 mg/plant

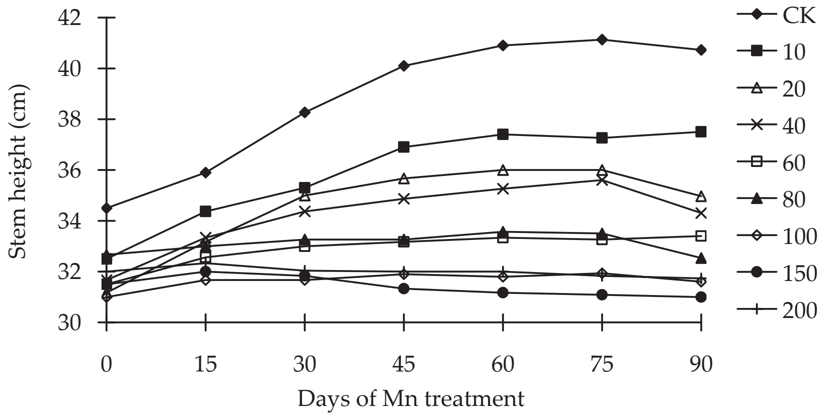

Figure 2. Stem height under different Mn treatments in sand culture

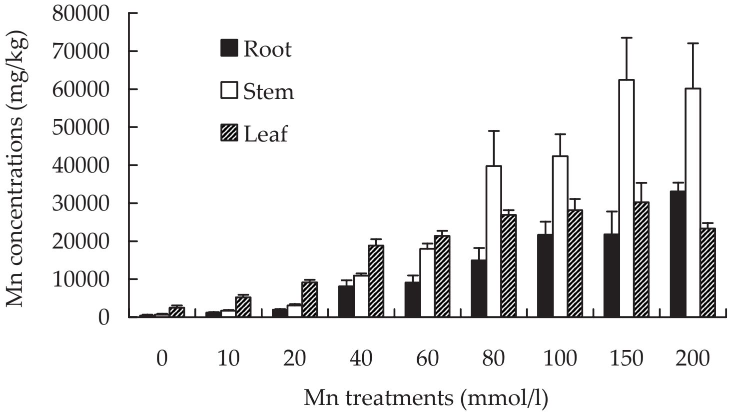

Figure 3. Mn concentrations in the root, stem and leaf of *Schima superba* under different treatments

for leaves and 110.06 mg/plant for stems occurring at 150 mmol/l Mn. Root Mn accumulation kept increasing under all Mn treatments, similar to the trend of Mn concentration as presented in Figure 3. When the Mn transportation within plant was concerned, the ratio of Mn accumulation in the aerial parts to those in the roots ranged from 3.5 to 13.0, indicating very high Mn transfer capacity from roots to aerial parts. Taking the 150 mmol/l treatment as an example, 38.9% of Mn taken stored in leaves, 47.7% in stems and 13.4% in roots, over 86% accumulated in aboveground parts and over 61% distributed in woody tissues.

Mn is involved in photosynthesis, respiration and as an activator of a number of enzymes in the tricarboxylic acid cycle. Mn treatment greatly altered the chlorophyll content and growth characteristics of a woody plant, *Populus cathayana* (Lei et al. 2007). Paschke et al. (2005) established Mn phytotoxicity thresholds for five grasses commonly used for restoration in North America. Baker and Brooks(1989) proposed two general strategies of metal tolerance, metal exclusion – plant taking heavy metals from substrate and excluding them somehow, or blocking their transportation within body, and metal detoxification – plant accumulating metals, but making them non-harmful in some way, such as being bound to cell wall, separated into vacuoles, or complexation to certain organic acids or proteins. A study on the woody Mn hyperaccumulator, *Austromyrtus bidwillii* found 40% of plant Mn existed as water-soluble form (Bidwell et al. 2002), and the total organic acids in leaf extractions were 123 × 10^3 mg/kg, suggesting

Table 1. Biomass and Mn accumulation (means ± SD, *n* = 3) of *Schima superba* under different Mn treatments in sand culture

Mn treatment (mmol/l)	Dry weight (g/plant)			Mn accumulation (mg/plant)			A/R
	leaf	stem	root	leaf	stem	root	
0	5.36 ± 0.41^{a}	2.40 ± 0.17^{a}	2.53 ± 0.40^{ab}	13.28 ± 1.02^{c}	1.67 ± 0.12^{b}	1.15 ± 0.18^{c}	13.0
10	4.69 ± 0.79^{ab}	2.27 ± 0.13^{ab}	2.27 ± 0.41^{a}	24.42 ± 4.12^{bc}	3.83 ± 0.21^{b}	2.80 ± 0.50^{c}	10.1
20	4.62 ± 0.39^{ab}	2.29 ± 0.08^{a}	2.55 ± 0.29^{ab}	42.42 ± 3.56^{bc}	7.08 ± 0.24^{b}	5.00 ± 0.57^{b}	9.9
40	4.03 ± 0.11^{bc}	2.05 ± 0.16^{abc}	1.75 ± 0.17^{ab}	75.97 ± 2.11^{ab}	22.50 ± 1.76^{abc}	14.25 ± 1.40^{ab}	6.9
60	3.80 ± 0.49^{bcd}	2.02 ± 0.06^{abc}	1.70 ± 0.47^{ab}	81.09 ± 10.47^{ab}	36.15 ± 1.11^{abc}	15.49 ± 4.27^{ab}	7.6
80	3.30 ± 0.10^{cd}	1.96 ± 0.10^{abcd}	1.68 ± 0.36^{ab}	88.83 ± 2.60^{a}	77.97 ± 4.07^{ab}	25.07 ± 5.39^{ab}	6.7
100	3.03 ± 0.40^{cd}	1.79 ± 0.28^{bcd}	1.59 ± 0.42^{ab}	85.33 ± 11.22^{a}	75.73 ± 11.66^{ac}	34.44 ± 9.09^{ab}	4.7
150	2.97 ± 0.06^{cd}	1.76 ± 0.26^{cd}	1.42 ± 0.12^{c}	89.64 ± 1.70^{a}	110.06 ± 16.20^{a}	30.93 ± 2.59^{a}	6.5
200	2.60 ± 0.33^{d}	1.52 ± 0.11^{d}	1.30 ± 0.16^{c}	60.63 ± 7.81^{a}	91.14 ± 6.85^{a}	42.89 ± 5.25^{a}	3.5

A/R: A – Mn accumulated in the aerial parts, R – Mn accumulated in the roots
Different letters in the same column indicate significant difference at $P < 0.05$ according to the least significant difference test

that Mn may probably be bound to water-soluble compounds like organic acids. In the present study, *Schima superba* grew normally and showed no symptom of Mn toxicity with Mn supply below 60 mmol/l during the treatment period. Although treatments with higher Mn levels significantly decreased the biomass of plants, Mn accumulation in the plant tissues increased significantly with the elevation of external Mn levels. These characteristics showed that *S. superba* had strong resistance to manganese and may have employed some metal detoxification mechanisms, which are subject of further studies.

Normal Mn concentrations in plant dry matter fall within a rather wide range of 20–500 mg/kg, and occasionally exceed 1 000 mg/kg in plants on normal soils (Reeves and Baker 2000). In comparison with normal plants, hyperaccumulators have the ability to accumulate heavy metals in their shoots far exceeding those observed in soil, without suffering from detrimental effects. Bidwell et al. (2002) found that the Mn concentration in dried leaves from *Austromyrtus bidwillii* was up to 19 200 mg/kg. Xue et al. (2003) found the first Chinese native Mn-hyperaccumulator, *Phytolacca acinosa*, containing 19 300 mg/kg Mn at highest in the leaf dry matter with a mean level of 14 480 mg/kg. In another newly reported Mn accumulator, *Phytolacca americana*, the maximum Mn concentration in leaf was 8 000 mg/kg. In Pingle Mn mine wasteland, Guangxi, two samplings of *Schima superba* showed its leaves containing Mn levels of 30 075.9 and 9 975.61 mg/kg, respectively (Yang et al. 2006), indicating that this species could be a prospective Mn-hyperaccumulator. Under sand culture conditions, all the leaf and stem Mn contents were above 10 000 mg/kg from 40 mmol/l treatment onwards, and the peak level in the stem reached 62 412.3 mg/kg. Furthermore, most of the Mn taken from the substrates was transported to the aboveground tissues, as seen from A/R value (Table 1). These characteristics confirmed *Schima superba* is a Mn hyperaccumulator. Moreover, the incomparable advantages in use for remediation include longer immobilization time of toxic metals in soil, larger extraction amount of metals from soil, wider range (area and depth) and less risk for the toxic metals to enter human food chain. This species can be used as a good woody plant for reclaiming metal-mined wastelands in south China at the middle-late restoration stage. Meanwhile, it provides a new resource for probing the biochemical mechanisms of Mn hyperaccumulation and detoxification.

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