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Effects of Chelating Agents on Mn Absorption by *Polygonum pubescens* Blume from Mn Ore-containing Soils

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Pot experiment was carried out to evaluate the effects of incorporation of various doses of ascorbic acid, tartaric acid and EDTA on the Mn absorption by hyperaccumulator *Polygonum pubescens* Blume from soil. The results showed that the incorporation of chelating agents could effectively raise the exchangeable Mn content in Mn ore soil, activate Mn in soil and enhance plant Mn uptake. For Mn-activation ability, the order was EDTA > ascorbic acid > tartaric acid. 10 mmol/kg EDTA displayed the maximum effect of 1 135 % on accumulation capacity of the shoots of *Polygonum pubescens* Blume in mine tailing soils. EDTA is recommended to be coupled with *Polygonum pubescens* Blume for remediation of Mn ore soils. 5 mmol/kg EDTA was the optimal regulating dose for the Mn transport ability and Mn remediation efficiency of *Polygonum pubescens* Blume in Mn mine tailing area.

Keywords: Chelating agent, Mn, hyperaccumulator, Pot culture, Mn mine tailing.

INTRODUCTION

Hyperaccumulators are highly heavy metal-tolerant and phytoremediation technology which uses hyperaccumulators to remediate heavy metal-contaminated soils has become an international hot spot in administering soil heavy metal contamination. However, most heavy metals in soil are usually compounded with organic matters or Fe and Mn oxides. Their low solubility leads to their low bioavailability for plant uptake. Chelate-induced phytoextraction is a technology of increasing the bioavailability of heavy metals to enhance plant uptake by applying chelating agents to soils to activate the heavy metals[1-3]. Currently, research coupling hyperaccumulator and chelate-induced phytoextraction mainly focuses on the four heavy metals of Pb, Cd, Se and Cu[4-8]. There are no reports on the effects of chelating agent application on Mn uptake by hyperaccumulators. Manganese toxicity has been confirmed and too much Mn will damage the membrane, proteins and DNA of plant cells, causing lipid peroxidation and consequently oxidative stress[9-11]. *Polygonum pubescens* Blume is a Mn hyperaccumulator newly discovered in a Mn mining area in North Guangxi, China[12]. It grows quickly with a high biomass. More importantly, it can be grown using cuttings. Therefore, it is a fairy good plant for remediation. In this study, pot experiment was performed to investigate the growth and Mn accumulation characteristics of Mn hyperaccumulator *Polygonum pubescens* Blume by applying different doses of EDTA, tartaric acid and ascorbic acid to Mn-containing soils. This study aimed to find a method to enhance the Mn accumulation capacity of hyperaccumulators by applying chelating agents of certain dose to soils so as to effectively administer and remediate Mn-polluted soils.

EXPERIMENTAL

Soils and plant used in this study: The mine tailing soil was collected from a Mn mining area in Lipu County in north Guangxi, China. Centering at the mine, soil samples were taken from the surface 20 cm in an S-pattern. Soil sample from each sampling site was a composite of multiple points. The soil from a recovery zone was collected in a forested area 1 km away from the mining area. The soil free of Mn pollution and used for artificial contamination was collected from the biological park of Guangxi Normal University. *Polygonum pubescens* Blume was collected from the mining area and cultured indoors with cuttings. Uniform plants were selected for study.

The basic physico-chemical properties of these three soils are shown in Table-1.

Experimental method: Pot culture was conducted in this test. The soils were air-dried. After pebbles and debris were removed, the soils were pounded to pieces to pass a 2 mm sieve. The soils were weighed and put into plastic pots at 2 kg/

TABLE-1
PHYSICO-CHEMICAL PROPERTIES OF THE THREE SOILS USED IN THIS STUDY

Soil samples	pH	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Total manganese (mg kg^{-1})
Mine tailing soil	5.02	2.324 ± 0.05	0.98 ± 0.08	35312
From the recovery zone	5.23	3.322 ± 0.25	1.48 ± 0.13	555
Used for artificial contamination	4.74	1.975 ± 0.03	0.64 ± 0.02	154

pot. For artificial contamination, manganese was added in the form of $MnCl_2 \cdot 4H_2O$ and set up two adding value for simulation background: 1 000 mg/kg (I) and 2 000 mg/kg (II), adding the mine tailing soil and the soil from the recovery zone, there were now totally four soils in terms of Mn content. For each soil, 10 treatments were set up to do an experiment and for each treatment, there were 5 replications. CK were those with only plants but no chelating agent added. In other treatments, chelating agents were added at levels of 2, 5, or 10 mmol/kg and the corresponding treatments were denoted as A2, A5, or A10, respectively for ascorbic acid, T2, T5, or T10, respectively for tartaric acid and E2, E5, or E10, respectively for EDTA. There were totally 200 pots. The soils in the pots were sprayed with deionized water, mixed thoroughly and left for balance for 2 months. *Polygonum pubescens* Blume about 15 cm high grown from cuttings was transplanted into the pots for 2 plant/pot. After transplanting, plant growth and management was carried out in a greenhouse at the biological park of Guangxi Normal University to lessen the interference of external conditions on the test, such as raining. The plants were watered regularly and harvested after 120 days of growth.

Sample processing and analytical methods

Biomass: Plant samples were divided into shoots and roots by cutting from the soil surface with a scissor. After harvested, the plants were first rinsed with tap water, soaked in 20 mmol/L EDTA-Na solution for a 15 min to remove surface-adsorbed metal ions and then rinsed with deionized water for 3 times. After blotted dried, the plants were first oven-dried at 105 °C for 0.5 h and then at 70 °C for 48 h. The dried plant parts were weighed, ground with a stainless steel pulverizer and passed through a 60-mesh nylon screen for Mn content determination.

Extraction and determination of enzymes: Weigh 0.100 g leaf of *Polygonum pubescens* Blume, add 8 mL of 0.1 mmol/L phosphate buffer (pH 7.0, containing 0.1 ìmol/L EDTA and 1 % polyvinyl pyrrolidone (PVP)) and a small amount of quartz sand, grind to homogenate with a glass mortar and centrifuge at 10 000 g for 15 min (4 °C). Take the supernatant for superoxide dismutase (SOD) activity determination[13].

Determination of Mn: Plant samples were microwave-digested with HNO_3:H_2O_2 (9:2, v:v) and soil samples were microwave-digested with HNO_3:HF:$HClO_4$ (9:4:2, v:v:v). Manganese contents were measured using flame atomic absorption spectrometry (WFX-110).

Weigh 2 g (dry weight) of oven-dried soil that had been passed through a 100-mesh net and sequentially extract the various Mn species as follows:

Exchangeable: Mix the soil sample with 16 mL 1 mol/L $MgCl_2$ (pH = 7.0), shake the suspension at 25 ± 1 °C for 1 h and centrifuge the suspension at 3500 rpm for 30 min. Take the supernatant and dilute as needed for exchangeable Mn determination. Wash the precipitate with deionized water, centrifuge the suspension at 3500 rpm for 15 min, discard the supernatant and keep the precipitate for the next extraction.

Carbonate-bound (weak acid-soluble): Add 16 mL of 1 mol/L NaOAc to the precipitate obtained from last extraction, adjust the pH to 5.0 using HAc, shake the suspension at 25 ± 1 °C for 5 h and centrifuge the suspension at 3500 rpm for 30 min. Take the supernatant and dilute as needed for carbonate-bound Mn determination. Wash the precipitate with deionized water, centrifuge the suspension at 3500 rpm for 15 min, discard the supernatant and keep the precipitate for the next extraction.

Fe-Mn oxide-bound (oxidizable): Add 20 mL of 0.04 mol/L $NH_2OH \cdot HCl$ (in 25 % (v/v) CH_3COOH solution) to the precipitate obtained from last extraction, shake the suspension at 96 ± 3 °C for 6 h with occasional stirring and centrifuge the suspension at 3500 rpm for 30 min. Take the supernatant and dilute as needed for Fe-Mn oxide-bound Mn determination. Wash the precipitate with deionized water, centrifuge the suspension at 3500 rpm for 15 min, discard the supernatant and keep the precipitate for the next extraction.

Organic matter-bound (reductable): Add 6 mL of 0.02 mol/L HNO_3 and 10 mL of H_2O_2 (30 %) to the precipitate obtained from last extraction, adjust the pH to 2 and shake the suspension at 85 ± 2 °C for 2 h. Add another 6 mL of H_2O_2 (30 %) and shake the suspension at 85 ± 2 °C for 2 h. Cool the suspension to 25 ± 1 °C, add 5 mL of 3.2 mol/L NH_4OAc [in 20 % (v/v) HNO_3], dilute to 20 mL, shake for 30 min, centrifuge the suspension at 3500 rpm for 30 min. Take the supernatant and dilute as needed for organic matter-bound Mn determination. Wash the precipitate with deionized water, centrifuge the suspension at 3500 rpm for 15 min, discard the supernatant and keep the precipitate for the next extraction.

Residual: Oven-dry the precipitate obtained from the last extraction at 75 °C to constant weight. Weigh 0.2500 g of the dried precipitate to a Teflon digest vessel, add 9 mL HNO_3 and 4 mL HF and microwave-digest the precipitate. Let the digest to cool, transfer it to a Teflon crucible, add 3 mL $HClO_4$, heat until smoke goes out and let evaporation to continue to remove the excessive acid. Transfer the digest to a volumetric flask, add 5 mL lanthanum nitrate, add deionized water to volume and measure Mn in residual form.

Statistical analysis

Transport factor (TF) = (shoot Mn mass proportion × shoot biomass) / (root Mn mass proportion × root biomass)

The effects of chelator incorporation on the accumulation capacity of shoots, En = (shoot Mn content after chelator incorporation – shoot Mn content in CK) / shoot Mn content in CK × 100 %

All determinations were carried out for three times. Data were analyzed with One-Way ANOVA using the SPSS software package. Differences ($p < 0.05$) between treatments were tested

with the LSD procedure when variances are equal and with the Games-Howell procedure when variances are unequal.

RESULTS AND DISCUSSION

Effects of chelating agents on Mn species in Mn mine tailing soils: As shown in Table-2, for CK, the proportion of exchangeable Mn was low, being only 0.56 % and most Mn was bound to Fe-Mn oxide and organic matter. After the addition of chelating agents, the proportion of exchangeable Mn increased significantly ($p < 0.05$) with increasing dose of chelating agents. E10 displayed the largest increase of 2.57 % which was 5.6 times of that in CK. Table-2 also showed that with the incorporation of chelating agents, carbonate- and organic matter-bound Mn increased and Fe-Mn oxide-bound and residual Mn tended to decrease, indicating that chelating agent incorporation can drive the transformation of Mn from Fe-Mn oxide-bound to exchangeable and from residual form to carbonate-bound and organic matter-bound and that chelating agents activated Mn in soil and enhanced Mn uptake by plant roots. For Mn activation ability, the three chelating agents were in the order of: EDTA > ascorbic acid > tartaric acid. Mao[14] believed that heavy metal bioavailability is related to its species. Exchangeable heavy metals can be easily used by organisms. Carbonate-bound, Fe-Mn oxide-bound and organic matter-bound heavy metals can be taken up by organisms, while heavy metals in residual form are inert and are not bioavailable. Table-3 shows Mn species in root zone soil after *Polygonum pubescens* Blume was planted. Compared with that before *Polygonum pubescens* Blume was planted (Table-2), exchangeable and carbonate-bound Mn decreased significantly, demonstrating that these two Mn species can be utilized by *Polygonum pubescens* Blume.

Effects of chelating agents on the accumulation and distribution of Mn in *Polygonum pubescens* Blume: As shown in Table-4, for the control groups, the transport factor (TF), was in the order of II > I > mine tailing zone > recovery zone. Such results indicated that Mn uptake and transport by plants were closely related to the content and species of Mn in soil. In treatments I and II, as $MnCl_2 \cdot 4H_2O$ was added, Mn existed as Mn ion which was favourable for plant uptake and transport. Higher concentration of Mn^{2+} in II than in I resulted in a larger transport factor for II. Yet in mine tailing area and recovery zone, most Mn existed as insoluble minerals whose bioavailability for plant uptake is low.

With the addition of chelating agents, Mn content in *Polygonum pubescens* Blume tended to increase with increasing dose of added chelating agents in the soils of mine tailing area and recovery zone. It is apparent that chelating agents application drives the transformation of Mn to exchangeable species, leading to enhanced plant uptake. Table-4 showed that EDTA had a greater effect than ascorbic acid and tartaric acid. The E10 treatment showed the most pronounced effect with shoot Mn content in *Polygonum pubescens* Blume being 24 925 mg/kg and 5 177 mg/kg in mine tailing area and recovery zone, respectively. This was related to the higher Mn activation ability of EDTA compared with that of ascorbic acid and tartaric acid. Most Mn in treatments I and II was in its ionic form and Mn content in *Polygonum pubescens* Blume showed a decreasing trend with increasing EDTA concentration. Based on the finding by Vassil *et al.*[15] that at high concentrations, Pb and EDTA of free state are more biotoxic than their chelating state (Pb-EDTA). It is believed that the biotoxicity of free Mn^{2+} and EDTA will be enhanced with their increasing concentrations and the vitality of plants will be lowered.

The changes of transport factor and En varied with soil type and which chelating agent was applied. In mine ailing zone and mine recovery zone, both transport factor and En went up after EDTA addition. For treatment E10, En was up to 1 135 % and 1 471 %, indicating that the complex of EDTA and Mn is favourable for Mn uptake and transport by *Polygonum pubescens* Blume. Transport factor did not change much or even dropped after the application of ascorbic acid or tartaric acid, which was not in line with the changing trend of exchangeable Mn as shown in Table-2. Therefore, it can be concluded that chelating agents increase the solubility of heavy metal in soil solution by compounding with heavy metal but it is not confirmed whether compounded heavy metal can be taken up by plants. Therefore, it is important to select an effective chelating agent to activate heavy metals in soil. The transport factor in treatments I and II decreased after chelating agents application, which was related to the high free Mn concentration as too much free Mn can damage the membrane, proteins

TABLE-2
MASS PROPORTION (%) OF MANGANESE SPECIES IN MINE TAILING SOILS AFTER CHELATING AGENTS APPLICATION

Species	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Exchangeable	0.56	0.96	1.81	2.90	0.78	1.42	2.56	1.41	2.87	4.32
Carbonate-bound	0.46	0.81	1.31	1.56	0.60	1.13	1.60	0.90	1.62	2.57
Fe-Mn oxide-bound	64.22	62.62	60.02	58.05	62.47	59.63	58.04	62.45	58.69	56.75
Organic matter-bound	30.98	32.00	33.42	34.25	32.51	34.32	34.57	31.70	33.45	33.22
Residual	3.78	3.61	3.45	3.24	3.64	3.50	3.23	3.54	3.38	3.14

TABLE-3
MASS PROPORTIONS (%) OF VARIOUS MANGANESE SPECIES IN THE MINE TAILING SOILS AFTER *Polygonum pubescens* Blume WAS PLANTED

Mn species	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Exchangeable	0.38	0.41	0.42	0.48	0.38	0.38	0.39	0.41	0.88	0.92
Carbonate-bound	0.19	0.35	0.36	0.37	0.30	0.35	0.38	0.35	0.61	0.61
Fe-Mn oxide-bound	64.51	62.22	62.33	59.69	62.32	61.76	61.06	63.72	61.15	59.73
Organic matter-bound	31.05	33.15	33.37	36.05	33.20	33.89	34.66	31.85	33.87	35.33
Residual	3.88	3.87	3.53	3.41	3.79	3.62	3.50	3.67	3.50	3.42

TABLE-4
IMPACTS OF CHELATING AGENTS ON MANGANESE CONTENT (mg/kg DW) IN *Polygonum pubescens* Blume

Soil	Item	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Mine tailing area	Shoot Mn content	2019 ± 43	2650 ± 84^a	3743 ± 37^b	7592 ± 35^c	2592 ± 42^a	3002 ± 21^b	3643 ± 91^c	7086 ± 52^a	16236 ± 339^b	24925 ± 389^c
	Root Mn content	575 ± 37	911 ± 27^a	1365 ± 61^b	2137 ± 52^c	1153 ± 64^a	1734 ± 43^b	1603 ± 55^b	1836 ± 60^a	2377 ± 47^b	5153 ± 91^c
	TF	3.51	2.91	2.74	3.55	2.25	1.73	2.27	3.86	6.83	4.84
	En (%)	–	31	85	276	28	49	80	251	704	1135
Recovery zone	Shoot Mn content	330 ± 13	318 ± 14^a	634 ± 33^b	668 ± 36^b	321 ± 15^a	357 ± 13^{ab}	387 ± 23^b	614 ± 11^a	2485 ± 25^b	5177 ± 43^c
	Root Mn content	148 ± 13	135 ± 7^a	188 ± 9^b	247 ± 13^c	129 ± 7^a	144 ± 8^a	173 ± 10^b	207 ± 11^a	214 ± 18^a	295 ± 11^b
	TF	2.23	2.36	3.38	2.71	2.48	2.48	2.24	2.97	11.61	17.55
	En (%)	–	-3	92	103	-3	8	17	86	654	1471
I	Shoot Mn content	11446 ± 222	9441 ± 203^a	11498 ± 173^b	13512 ± 346^c	8948 ± 124^a	9831 ± 212^b	12417 ± 305^c	8723 ± 49^a	7165 ± 143^b	6665 ± 100^c
	Root Mn content	624 ± 37	574 ± 60^a	560 ± 18^a	924 ± 37^b	410 ± 20^a	524 ± 23^b	755 ± 29^c	499 ± 25^a	531 ± 28^{ab}	604 ± 23^b
	TF	18.34	16.46	20.53	14.62	21.82	18.76	16.46	17.48	13.51	11.03
	En (%)	–	-18	0	18	-22	-14	8	-24	-37	-42
II	Shoot Mn content	15207 ± 293	14109 ± 143^a	14860 ± 142^b	16429 ± 226^c	15801 ± 389^a	16210 ± 234^a	16559 ± 119^a	13980 ± 168^a	12078 ± 165^b	10090 ± 95^c
	Root Mn content	721 ± 30	1110 ± 23^a	1294 ± 32^b	1600 ± 62^c	1053 ± 8^a	1206 ± 21^b	1288 ± 29^c	1048 ± 35^a	961 ± 20^{ab}	933 ± 42^b
	TF	21.11	12.72	11.49	10.27	15.01	13.44	12.86	13.34	12.57	10.81
	En (%)	–	-7	-2	8	4	7	9	-8	-21	-34

Notes: 1 Except for TF and En, values are: mean ± DE; 2 For En, positive values suggest increase in accumulation capacity, while negative ones indicate decrease; 3 Different lower case letters in the same row denote that different treatment of the same chelating agent in confidence level of $p < 0.05$ will be significantly different.

and DNA of plant cells, leading to lipid peoxidation and oxidation stress.

Effects of chelating agents on the SOD activity in *Polygonum pubescens* Blume: An active substance originated from life entities, SOD can remove the toxic substances generated during the metabolic processes of organisms and help organisms function properly. Table-5 shows the effects of chelating agents on the SOD activity of Mn-stressed *Polygonum pubescens* Blume. The data demonstrated that similar to transport factor (Table-4), the higher the Mn concentration in soil, the higher the SOD activity in *Polygonum pubescens* Blume. This suggested that *Polygonum pubescens* Blume can still remove the reactive oxygen substances in it after chelating agents were added. Therefore, *Polygonum pubescens* Blume can be used in chelate-induced phytoextraction as a Mn hyperaccumulator.

Effects of chelating agent on the biomass of *Polygonum pubescens* Blume: As shown in Table-6, the biomass of most *Polygonum pubescens* Blume in treatments with chelating agent application decreased when compared with that in control group. EDTA showed a more pronounced effect on the plants than ascorbic acid and tartaric acid. In treatments with EDTA addition, the shoot biomass decreased prominently by even 70 %. This may be related to the biotoxicity caused by increases in the concentrations of soluble Mn and chelating agents. In their studies on the remediation potential of oilseed rape for Pb-contaminated soils with the aid of EDTA. Chen *et al.*[16] found that seedling growth was clearly inhibited by the moderate addition of EDTA at 7.5 mmol/kg and the seedlings wilted after 7 days. This suggested that increase in soluble heavy metal would lead to stronger toxicity to plants and affect plant growth. Specifically in this study, the plants in treatments I and II especially E10 with incorporation of chelating agents exhibited intoxication signs such as yellow leaves, leaf chlorosis and small plants. Also, soil acidity, Al^{3+} (Al toxicity), Mn^{2+} (Mn toxicity), Fe^{2+} and soil fertility are influencing factors in plant growth. For example, the soils selected for this study had a highest pH of 5.23, after chelating agents were incorporated, soil pH dropped and became more unfavourable for plant growth. In addition, for the same chelating agent of the same concentration, different biomasses of *Polygonum pubescens* Blume were obtained in different soils. Higher biomass as a whole was harvested in the soil from recovery zone than in the other 3 types of soils. This may be explained by the higher soil fertility in recovery zone. Therefore, how to regulate the soluble heavy metal concentration by controlling the incorporated amount of chelating agents and how to improve soil fertility are the key to increase the biomass of *Polygonum pubescens* Blume.

Effects of chelator incorporation on the total amount of Mn transported by *Polygonum pubescens* Blume: As can be seen in Table-7, the addition of chelating agents effectively

TABLE-5
EFFECTS OF CHELATING AGENTS ON THE SOD ACTIVITY (U g^{-1} FW^{-1}) IN *Polygonum pubescens* Blume

Soil	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Mine tailing soil	282±13	237±15^a	241±16^b	203±11^c	275±18^b	239±12^a	232±13^b	233±12^a	239±13^b	251±17^c
Soil from recovery zone	191±10	215±11^a	243±20^c	231±16^b	225±9^a	205±13^b	204±14^b	248±18^a	251±19^c	349±24^d
I	200±9	205±19^a	231±14^b	264±20^c	222±17^b	210±14^b	201±12^b	250±20^c	279±21^c	285±22^c
II	219±11	231±12^a	238±17^b	234±13^a	247±19^b	239±21^c	224±17^a	232±19^a	238±21^b	252±23^c

TABLE-6
EFFECTS OF CHELATING AGENTS ON THE SHOOT AND ROOT DRY WEIGHTS (g/pot) OF *Polygonum pubescens* Blume

Soil	Item	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Mine tailing soil	Shoot	9.83 ± 0.43	6.04 ± 0.28^{a}	6.42 ± 0.28^{a}	4.81 ± 0.35^{b}	9.78 ± 0.29^{a}	9.06 ± 0.19^{a}	7.29 ± 0.32^{b}	9.07 ± 0.26^{a}	5.23 ± 0.14^{b}	3.52 ± 0.24^{c}
	Root	2.06 0.11	0.89 ± 0.06^{a}	0.85 ± 0.04^{a}	0.68 0.02^{b}	1.53 ± 0.04^{a}	1.30 ± 0.03^{b}	1.08 ± 0.03^{c}	1.07 ± 0.05^{a}	0.83 ± 0.02^{b}	0.63 ± 0.02^{c}
	Change	–	-39 /-57	-35 /-59	-51 /-67	0 /-26	-8 /-37	-26 /-48	-8 /-48	-47 /-60	-64 /-69
Soil from recovery zone	Shoot	11.74 ± 0.14	7.06 ± 0.29^{a}	8.88 ± 0.17^{b}	9.24 ± 0.34^{b}	10.95 ± 1.02^{a}	14.50 ± 0.62^{b}	14.16 ± 0.58^{b}	9.50 ± 0.58^{a}	6.09 ± 0.14^{b}	3.48 ± 0.07^{b}
	Root	1.19 ± 0.05	1.12 ± 0.04^{a}	1.51 ± 0.02^{b}	0.80 ± 0.03^{c}	1.00 ± 0.04^{a}	1.27 ± 0.06^{b}	1.43 ± 0.07^{c}	0.95 ± 0.04^{a}	0.61 ± 0.03^{b}	0.41 ± 0.02^{c}
	Change	–	-40 /-6	-24 /27	-21 /-33	-7 /-16	24 /7	21 /21	-19 /-20	-48 /-49	-70 /-66
I	Shoot	12.09 ± 0.23	9.27 ± 0.26^{a}	8.69 ± 0.12^{a}	7.45 ± 0.29^{b}	7.82 ± 0.18^{a}	7.62 ± 0.18^{ab}	7.16 ± 0.21^{b}	5.10 ± 0.21^{a}	4.37 ± 0.21^{b}	3.89 ± 0.19^{b}
	Root	1.05 ± 0.03	0.64 ± 0.03^{a}	0.58 ± 0.01^{ab}	0.53 ± 0.02^{b}	0.56 ± 0.02^{a}	0.55 ± 0.02^{a}	0.53 ± 0.01^{a}	0.65 ± 0.05^{a}	0.56 ± 0.05^{a}	0.41 ± 0.03^{b}
	Change	–	-23 /-39	-28 /-45	-38 /-50	-35 /-47	-37 /-48	-41 /-49	-58 /-38	-64 /-47	-68 /-61
II	Shoot	7.53 ± 0.11	7.54 ± 0.24^{a}	6.37 ± 0.22^{b}	5.38 ± 0.24^{c}	6.15 ± 0.21^{a}	5.78 ± 0.38^{a}	5.41 ± 0.24^{a}	3.61 ± 0.06^{a}	3.18 ± 0.21^{a}	2.52 ± 0.07^{b}
	Root	0.75 ± 0.02	0.71 ± 0.02^{a}	0.62 ± 0.04^{ab}	0.58 ± 0.03^{b}	0.66 ± 0.03^{a}	0.62 ± 0.03^{ab}	0.56 ± 0.03^{b}	0.47 ± 0.01^{a}	0.41 ± 0.01^{b}	0.36 ± 0.01^{c}
	Change	–	0 /-5	-15 /-18	-28 /-22	-18 /-12	-23 /-17	-28 /-25	-52 /-37	-58 /-45	-67 /-52

Notes: 1 Positive values indicate that yield increased, while negative values indicate that yield decreased; 2 Different lower case letters in the same row denote that different treatment of the same chelating agent in confidence level of $p < 0.05$ will be significantly different.

enhanced Mn movement by *Polygonum pubescens* Blume in the soils from mine tailing and recovery zone. The total transport amount of Mn in shoots increased by as high as 343 % and 366 % in the soils from mine tailing and recovery zone, respectively. However, in treatment I and II, the total transport amount of Mn in shoots decreased. And compared with the other two chelating agents, EDTA exerted a more apparent effect on the extraction of Mn by *Polygonum pubescens* Blume from the soils in these two treatments with a largest drop in transport amount. Especially, the transport amount of Mn in the shoots in treatment E10 decreased significantly ($p < 0.05$) by 81 % and 78 % in treatments I and II, respectively, indicating that the incorporation of chelating agents in treatments I and II with relatively high free Mn content did not enhance Mn uptake by *Polygonum pubescens* Blume but enhanced biotoxicity instead because of the increase in exchangeable Mn.

TABLE-7
EFFECTS OF CHELATING AGENTS ON THE TOTAL TRANSPORT AMOUNT (mg/pot) OF MANGANESE BY *Polygonum pubescens* Blume

Soil	Item	CK	A2	A5	A10	T2	T5	T10	E2	E5	E10
Mine tailing soil	Shoots	19.83 ± 0.44	16.00 ± 0.24^{a}	24.05 ± 1.27^{b}	36.54 ± 2.79^{c}	25.34 ± 0.33^{a}	27.19 ± 0.75^{b}	26.54 ± 0.49^{ab}	64.28 ± 1.41^{a}	84.89 ± 3.99^{b}	87.76 ± 4.58^{b}
	Roots	0.58 ± 0.04	0.91 ± 0.03^{a}	1.37 ± 0.06^{b}	2.14 ± 0.05^{c}	1.15 ± 0.06^{a}	1.73 ± 0.04^{b}	1.60 ± 0.06^{b}	1.84 ± 0.06^{a}	2.38 ± 0.05^{b}	5.15 ± 0.09^{c}
	Change	–	-19 /58	21 /137	84 /272	28 /100	37 /201	34 /179	224 /219	328 /313	343 /796
Soil from recovery zone	Shoots	3.87 ± 0.20	2.24 ± 0.01^{a}	5.63 ± 0.19^{b}	6.16 ± 0.11^{c}	3.50 ± 0.17^{a}	5.16 ± 0.02^{b}	5.47 ± 0.10^{b}	5.83 ± 0.25^{a}	15.14 ± 0.50^{b}	18.03 ± 0.54^{c}
	Roots	0.18 ± 0.02	0.15 ± 0.00^{a}	0.28 ± 0.01^{b}	0.20 ± 0.00^{c}	0.13 ± 0.00^{a}	0.18 ± 0.01^{b}	0.25 ± 0.02^{c}	0.20 ± 0.00^{a}	0.13 ± 0.02^{b}	0.12 ± 0.01^{b}
	Change	–	-42 /-14	45 /60	59 /11	-10 /-27	33 /3	41 /41	50 /12	291 /-26	366 /-32
I	Shoots	138.38 ± 0.07	87.44 ± 0.61^{a}	99.91 ± 0.09^{b}	100.61 ± 1.30^{b}	69.98 ± 0.65^{a}	74.92 ± 0.16^{b}	88.90 ± 0.46^{c}	44.49 ± 1.56^{a}	31.32 ± 0.91^{b}	25.90 ± 0.87^{c}
	Roots	0.65 ± 0.02	0.37 ± 0.02^{a}	0.33 ± 0.02^{a}	0.49 ± 0.03^{b}	0.23 ± 0.00^{a}	0.29 ± 0.00^{b}	0.40 ± 0.02^{c}	0.32 ± 0.04^{a}	0.30 ± 0.01^{a}	0.25 ± 0.01^{a}
	Change	–	-37 /-44	-28 /-50	-27 /-26	-49 /-65	-46 /-56	-36 /-38	-68 /-51	-77 /-55	-81 /-62
II	Shoots	114.44 ± 0.56	106.34 ± 4.41^{a}	94.68 ± 2.39^{b}	88.40 ± 2.66^{b}	97.13 ± 1.00^{a}	93.79 ± 7.54^{a}	89.58 ± 3.25^{a}	50.40 ± 1.39^{a}	38.47 ± 3.09^{b}	25.38 ± 0.95^{c}
	Roots	0.54 ± 0.04	0.79 ± 0.03	0.80 ± 0.03	0.93 ± 0.02	0.70 ± 0.04	0.75 ± 0.05	0.73 ± 0.02	0.50 ± 0.03	0.40 ± 0.02	0.33 ± 0.02
	Change	–	-7 /46	-17 /47	-23 /72	-15 /29	-18 /38	-22 /34	-56 /-8	-66 /-26	-78 /-38

Notes: 1 Positive values indicate an increase in the extraction amount, while negative values indicate an decrease; 2 Different lower case letters in the same row denote that different treatment of the same chelating agent in confidence level of $p < 0.05$ will be significantly different.

Phytoremediation efficiency is primarily decided by heavy metal content in shoots, plant biomass and growth rate[16,17]. The total transport amount of a heavy metal by a plant is co-influenced by the heavy metal content (dry weight basis) and plant biomass. Take this study as an example, the increase in the total transport amount of Mn by *Polygonum pubescens* Blume in mine tailing zone and recovery zone benefited from the increase in Mn content (dry weight basis). In treatment I and II, the addition of chelating agents caused decreases in both Mn content (dry weight basis) and biomass which in turn led to drop in Mn transport by *Polygonum pubescens* Blume. Therefore, when trying to enhance the remediation potential for heavy metals of hyperaccumulators, only to raise heavy metal content or only biomass is not enough. Instead, their combined action should be focused on selecting an optimal regulation scheme. In this study, the incorporation of 5 mmol/kg EDTA in mine tailing soil led to an increase in the extraction amount by shoots by 328 % without causing severe toxicity to plants. However, although high concentration (10 mmol/kg) of EDTA led to an increase in the extraction amount in shoots by 343 %, biomass dropped apparently and leaching risk was also increased. Therefore, under indoor experimental conditions, for Mn contamination in mining areas, 5 mmol/kg EDTA is a relatively appropriate dose for enhancing the transport capacity and remediation efficiency of *Polygonum pubescens* Blume.

Conclusions

• Incorporation of chelating agents in Mn mine tailing soils can increase the mass proportion of exchangeable Mn. Compared with ascorbic acid and tartaric acid, EDTA has a stronger activation ability in Mn mine tailing soils. The exchangeable Mn and carbonate-bound Mn are the forms that can be utilized by *Polygonum pubescens* Blume.

• EDTA-complexed heavy metal can be easily taken up by *Polygonum pubescens* Blume. Transport factor and En increase significantly after EDTA application. In contrast, ascorbic acid- and tartaric acid-complexed heavy metal is weakly taken up by *Polygonum pubescens* Blume. Therefore, the coupling of EDTA and *Polygonum pubescens* Blume can enhance the effective phytoremediation of Mn mine tailing soils.

• Stressed by chelating agents and Mn, *Polygonum pubescens* Blume can still remove the reactive oxygen substances in leaves, indicating that *Polygonum pubescens* Blume can be used in chelate-induced phytoextraction as a Mn hyperaccumulator.

• Soil soluble Mn can be raised by increasing chelating agent. However, excessive soluble Mn can cause biotoxicity of plants. Taken the transport ability, the total transport amount of heavy metal by plants, biomass and growth rate together, 5 mmol/kg EDTA can give the highest Mn phytoremediation efficiency in mine tailing area by *Polygonum pubescens* Blume.

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