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Effect of Organic Ligands on Accumulation of Copper in Hyperaccumulator and Nonaccumulator *Commelina communis*

Haiou Wang · Guangrong Zhong

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Abstract Better understanding of copper uptake and accumulation regulation in plants is critical to the phytoremediation of copper contaminated soil. This study employed a 30-day pot experiment to assess the relationship between organic ligands and copper accumulation in plants. Hyperaccumulator and nonaccumulator varieties of *Commelina communis* were used, different organic ligands were applied, and the data of copper accumulation in shoots were collected. The six organic ligands included ethylenediaminetetraacetic acid and organic acids (formic acid, citric acid, malic acid, tartaric acid, and succinic acid). The results showed that organic ligands added to culture increased the copper accumulation both varieties. The results of the copper accumulation in shoots agreed with the study of the root uptake kinetics of copper influx. The addition of organic acids could increase copper accumulation in shoots because the copper influx in roots was increased. The results also indicated that the copper influx of hyperaccumulator roots was higher than that of nonaccumulator roots. This is one of the mechanisms by which a hyperaccumulator could amass large amounts of copper in its shoots. In this accumulation process, little effect on the leaf relative water content was in the hyperaccumulator and nonaccumulator of leaves and normal physiological condition of plants.

Keywords Organic acids · EDTA · Influx · Copper · Hyperaccumulator

Abbreviations

EDTA	Ethylenediaminetetraacetic acid
RWC	Leaf relative water content
Tris	Tris(hydroxymethyl)aminomethane
MES	Morpholineethanesulfonic acid

H. Wang (✉) · G. Zhong
Department of Biological Science and Engineering, School of Chemical and Biological Engineering,
University of Science and Technology Beijing, Beijing 100083, People's Republic of China
e-mail: wanghaiou@ustb.edu.cn

Introduction

Large areas of land are contaminated with heavy metals due to emissions from many industrial processes. Toxic heavy metals pose a special threat. There is much concern that such heavy metals will enter the food chain via plant uptake or contaminate ground water supplies by leaching through the soil. Plants have a remarkable ability to absorb and accumulate metals and organic compounds from contaminated soil [1, 2]. Worldwide, substantial effort has been directed to the removal of heavy metals from environment through chemical and physical remediation processes, but these are expensive and, therefore, have limited applicability in many areas. Alternative and environmental friendly technologies are thus urgently needed to combat global heavy metal contamination. Among various technologies available so far or under development currently, phytoremediation is considered to be the most environmentally friendly and cost effective [3].

Recently, there has been an increased interest in the development of phytoextraction of heavy metals [4, 5]. Copper in soils is not only an essential plant nutrient but also one of the heavy metals related to environment quality. Cupric ions (Cu^{2+} ions) at toxic concentrations (above 0.01 mM in the incubation medium [6]) interfere with numerous physiological processes [7, 8]. Copper in soil solution is usually less than 5% of the total soil copper. Thus, to plants, the copper bioavailability in soil is limited. A key to copper phytoextraction is to increase and maintain copper concentration in the soil solution. There are many indications that organic acids are involved in heavy metal tolerance, transport, and storage in plants [9, 10]. Probing into the relationship of organic ligands and copper accumulation in copper hyperaccumulator and nonaccumulator plants will be conducive to a further understanding of the translocation and transformation regulations of this element and thus helpful to remedying copper contaminated soil. Ligands (such as low molecular weight organic acids and EDTA) have been applied in soils and nutrient solutions to increase the solubility of metal cations in plant growth media and are reported to have significant effects on metal accumulation in plants [11]. Ligands have been also used to remove heavy metals from contaminated soils in soil washing remediation techniques [12]. Low molecular weight organic acids are common substances in nature, playing a role in many metabolic reactions. In soils, organic acids come from the breakdown of plant residues and exudation from plant roots [13, 14]. Organic acid can bind elements such as metals, and their role as detoxification agents has been widely discussed [15]. They are capable of forming complexes with metal ions and modify the mobility of the metals in the rhizosphere [16, 17]. Recently, synthetic ligands have been used to trigger Pb hyperaccumulation in a number of plant species grown on Pb-contaminated soils [12]. However, there is little information in the literature concerning the mechanism of how these compounds affect copper accumulation in plants.

Commelina communis was discovered to accumulate large amounts of copper from contaminated soils (containing copper 7789 mg/kg), with copper concentrations reaching as high as 0.1% in its above ground biomass [18]. This process also has the potential to greatly increase plant biomass. However, very little work has been done to clarify how organic acids affect copper uptake of hyperaccumulator and nonaccumulator plant varieties.

The objective of this study was to examine copper accumulation in *C. communis* hyperaccumulator and nonaccumulator under different copper concentrations and various organic ligands in culture. We characterized copper influx in root and accumulation in shoots of hydroponically grown seedlings of hyperaccumulator and nonaccumulator varieties of *C. communis*. Results should provide critical information on *C. communis*' ability to tolerate and extract copper and to translocate copper to its above ground biomass, shedding further light on its applicability for remediating copper-contaminated soils.

Materials and Methods

Plant Culture

In this experiment, hyperaccumulator varieties and nonaccumulator varieties of *C. communis* were collected from contaminated areas in Hubei, China and examined. When the length of *C. communis* seedlings collected were 15 cm, those were placed into solutions of 1/10 strength Hoagland's medium (pH 6.0) and then permitted to grow for a period of 8 weeks in a night/day incubator at 18/25°C. The nutrient solution was replaced every 3 days with 1/10 strength Hoagland's medium and a constant volume of the nutrient solution was maintained by addition of distilled water on the other days. After 8 weeks, *C. communis* were then transferred to solutions of modified Hoaglands medium containing various concentrations of copper over a 30-day period.

Solution Preparation

Different kinds of organic ligand or copper concentrations were used to examine their effect on copper accumulation in *C. communis*. The experiments applied different organic ligands such as EDTA, formic acid, malic acid, citric acid, tartaric acid, and succinic acid. The concentration of each applied organic ligands was 5 mM. Modified Hoaglands medium and each organic acids solution were prepared by adding copper at 0.01 μM, 0.1, 0.2, 0.4, 0.6, and 0.8 mM as nitrate, respectively, and then were adjusted to pH 6.0 with 1 mM NaOH.

C. communis were harvested after 0, 1, 2, 4, and 30 days and washed thoroughly with distilled water before analysis.

Leaf Relative Water Content

Leaf relative water content (RWC) was estimated by collecting 0.5 g fresh leaf sample, keeping in water for 4 h, and reweighing (saturated weight) followed by drying in an hot air oven till constant weight was achieved [19].

$$\mathrm{RWC}[\%] = [(\text{fresh weight} - \text{dry weight})/(\text{saturated weight} - \text{dry weight})] \times 100$$

Determination of Copper in Shoots

Shoots were dried at 60°C for 48 h and 0.100 g of leaf sample was digested with 3 ml of $HNO_3/HClO_4/HF$ (3:1:1, *v*/*v*) under high-pressure conditions prior to Cu determination by inductively coupled plasma mass spectrometry (Plasma Quad III, Fisons Instruments, UK) [20]. A solution of ^{115}In at 10 ng/ml in 2% HNO_3 was used as an internal standard to compensate for matrix suppression and signal drifting during analysis.

Effect of Organic Acid on Concentration-Dependent Kinetics of Copper Influx

Plants were grown in hydroponic vessels (three seedlings in each vessel) with 1/10 strength nutrient solution for 4 weeks. Eight different concentrations of copper (0.5–100 μM) were used to study the influx kinetics of copper for each organic acid, separately. Each concentration was replicated three times. After 20 min of uptake, the seedlings were quickly rinsed with distilled water, and then transferred to vessels containing ice-cold desorption solutions (2 mM Tris–MES, 5 mM $CaCl_2$). Following a

15-min desorption period, seedlings were harvested, their roots were excised and weighed, and copper was quantified via gamma detection [21].

Statistical Analysis

All results were the means of three replicates. Data analyses were performed using SPSS 11.5 for windows (SPSS Inc., USA) for *t* test, one-way ANOVA and LSD test. Differences at $P<0.05$ were considered significant.

Results

Effect of Organic Ligands and Copper Concentrations on RWC

The variation of RWC should be viewed as one of the parameters, which reflected plant intracellular change. The results in Table 1 showed that RWC in hyperaccumulator and nonaccumulator varieties of *C. communis* were very similar. It is easily seen that RWC of both plants remained stable over the 30-day experimental period ($P<0.05$). At the end of the experiment, the RWC values of two plants still showed no significant variation, no matter what copper concentration or organic ligand treatments were applied ($P<0.05$). Thus, only data of the highest copper concentration treatment (0.8 mM) were shown in Table 1, others were omitted.

Effect of Organic Ligands and Copper Concentrations on Copper Accumulation

Copper accumulations in shoots were observed within 30 days of the experiment under organic ligands and copper treatments. After organic ligands were applied, time-dependent copper accumulation was examined by assaying copper concentration in shoots at various times. The copper accumulation trends in shoots of hyperaccumulators and nonaccumulators were similar ($P<0.05$; from Tables 2, 3, 4, 5, 6, 7). The copper concentrations in two varieties' shoots treated with organic ligands increased more than control plants. There existed a time-dependent relationship between copper treatment concentrations and shoot copper accumulation ($P<0.05$). Each applied copper concen-

Table 1 Relative water content (RWC,%) of two varieties of *C. communis* in nutrient solution containing 0.8 mM copper (n=3)

0.8 mM	RWC of hyperaccumulator					RWC of nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	95.74	95.36	95.11	94.24	95.18	94.02	94.17	94.03	92.70	92.61
EDTA	95.74	94.29	94.65	94.97	95.34	94.10	94.02	94.05	93.12	92.94
Tartaric acid	95.74	94.30	94.73	94.15	94.62	93.42	93.72	92.93	93.44	93.81
Succinic acid	95.74	95.45	95.57	95.44	94.31	93.23	93.18	92.83	92.80	93.30
Citric acid	95.74	92.09	92.61	93.68	94.17	94.08	94.15	93.04	93.31	92.80
Malic acid	95.74	95.14	95.35	95.08	97.92	93.23	93.63	93.06	92.49	92.29
Formic acid	95.74	92.74	94.28	95.67	95.38	93.32	93.44	92.61	92.83	92.34

Values were the means

Table 2 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.1 μM copper (n=3, μg/g, dry mass)

Organic acids	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.87	1.78	3.12	16.55	102.99	0.87	1.17	2.23	13.25	22.32
EDTA	0.85	1.89	3.20	22.24	168.96	0.87	3.86	4.65	15.77	25.15
Tartaric	0.83	5.26	8.37	31.28	228.55	0.87	3.67	4.88	20.86	65.93
Succinic	0.88	1.52	2.63	32.45	251.24	0.87	2.24	2.68	18.88	27.92
Citric	0.87	3.67	4.74	36.71	214.55	0.87	1.82	3.62	15.75	56.21
Malic	0.97	2.92	7.44	21.01	226.13	0.87	6.91	7.79	13.56	78.13
Formic	0.84	3.43	8.59	22.41	211.37	0.87	6.29	8.17	11.45	50.13

Values were the means

trations and organic ligand type could increase the copper accumulations in both varieties' shoots ($P < 0.05$). The rate of copper accumulation in both hyperaccumulators and nonaccumulators increased slowly in 4 days of copper application. The copper accumulation observed after 30 days of copper application was even greater.

EDTA cannot be synthesized in plants, so it was selected as a representative of synthetic ligands. From our results, EDTA could increase copper accumulation in both hyperaccumulators and nonaccumulators (from Tables 2, 3, 4, 5, 6, 7). The increase in copper concentrations in hyperaccumulator shoots was up to 251.3 μg/g. DW more than that in the control plants, which were hyperaccumulators without EDTA. While the increased range of copper concentrations in nonaccumulator shoots was up to 29.5 μg/g. DW more than that in the control plants, which were nonaccumulators without EDTA.

The high efficiency of anionic chelators in promoting uptake, translocation, and accumulation of inorganic pollutants has been shown in a comparative study on the effects of organic acid on uranium desorption from soil to soil solution [22]. In our experiment, formic, tartaric, succinic, malic, and citric acids were selected as the representatives of low molecular weight organic acids. It is reported that organic acids can be exuded from roots [15]. Tables 2, 3, 4, 5, 6, and 7 showed that all organic acids added to the culture could

Table 3 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.1 mM copper (n=3, μg/g, dry mass)

Organic ligands	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.90	2.32	4.68	21.08	242.64	0.88	1.58	3.95	14.30	22.93
EDTA	0.98	5.27	9.34	36.86	493.89	0.88	4.16	5.32	25.05	34.35
Tartaric	0.89	4.06	8.43	38.93	650.92	0.88	4.29	5.32	31.01	68.86
Succinic	0.88	2.82	3.13	39.34	583.88	0.88	2.13	4.27	33.33	35.27
Citric	0.87	4.20	10.51	40.99	599.27	0.88	2.73	4.97	24.86	71.11
Malic	0.85	3.06	5.97	23.78	593.91	0.88	6.05	7.75	21.85	81.14
Formic	0.91	3.97	12.09	27.25	522.83	0.88	4.51	8.61	17.32	58.50

Values were the means

Table 4 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.2 mM copper (n=3, μg/g, dry mass)

Organic ligands	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.90	2.96	5.83	23.84	331.07	0.90	2.56	4.47	13.65	23.89
EDTA	0.91	4.53	10.88	38.40	498.60	0.90	4.59	5.86	28.57	39.54
Tartaric	0.92	6.85	9.25	45.05	598.56	0.90	4.78	6.21	47.02	71.98
Succinic	0.89	5.84	15.44	49.78	564.82	0.90	2.10	4.91	48.61	56.05
Citric	0.88	4.25	15.21	51.69	578.75	0.90	2.77	4.59	15.38	78.50
Malic	0.95	5.23	7.07	26.85	560.61	0.90	6.39	7.61	16.56	96.51
Formic	0.93	4.68	13.36	25.02	479.01	0.90	4.29	6.85	11.36	59.54

Values were the means

increase copper accumulation. The copper accumulation in hyperaccumulator shoots responded to the copper concentrations in solution and treatment time. After 30 days' treatment, the copper concentrations in hyperaccumulator shoots had increased 100 times more than before treatment. However, the increase of copper accumulations in shoots was observed to be similar under the higher copper concentrations (>0.4 mM) treatment as under the lower concentration treatments.

Effect of Organic Acid on Copper Uptake

The concentration dependent kinetics of copper influx showed a saturable (hyperbolic) component and a linear component for both varieties (Fig. 1a). To mathematically resolve these curves, we applied a Michaelis–Menten model combined with a linear component using SigmaPlot 12.0 (SPSS, Inc., Chicago). The linear and saturable components are shown separately in Fig. 1b. This procedure to fit the curves was used by Lasat et al. [21] to resolve the kinetic of influx of Zn in *T. caerulescens*. The saturable component was believed to represent the apoplastically bound fraction that was not removed by the desorption procedure. The linear kinetic component is generally

Table 5 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.4 mM copper (n=3, μg/g, dry mass**)**

Organic ligands	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.94	3.02	6.77	28.36	472.99	0.92	3.86	5.25	23.70	28.09
EDTA	0.93	4.30	9.08	34.77	491.83	0.92	5.19	6.28	45.70	57.55
Tartaric	0.92	5.97	7.76	49.79	675.53	0.92	4.74	4.92	52.01	78.14
Succinic	0.96	6.16	16.10	46.74	760.39	0.92	3.04	4.72	57.31	68.53
Citric	0.91	5.05	11.00	55.34	785.37	0.92	4.26	5.73	23.81	63.38
Malic	0.90	7.48	10.53	29.57	781.49	0.92	7.76	8.55	27.17	87.12
Formic	0.92	5.76	10.89	30.63	592.13	0.92	4.82	6.89	17.33	78.65

Values were the means

Table 6 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.6 mM copper (n=3, μg/g, dry mass)

Organic ligands	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.94	3.44	7.25	41.07	611.35	0.94	4.49	6.01	26.52	38.85
EDTA	0.95	4.87	14.87	50.56	658.72	0.94	3.52	6.69	47.82	53.51
Tartaric	0.96	3.92	8.90	56.09	935.78	0.94	5.80	6.35	62.33	131.22
Succinic	0.90	6.89	15.33	48.87	948.91	0.94	3.48	5.12	61.11	70.79
Citric	0.97	6.31	7.28	61.57	986.90	0.94	3.32	5.82	20.35	60.49
Malic	0.98	7.67	10.76	35.60	951.31	0.94	5.57	9.65	27.22	116.07
Formic	0.94	8.73	11.92	33.26	857.61	0.94	5.13	6.11	24.66	104.98

Values were the means

considered as true transport across the plasma membrane [21]. In all cases, the model fitted closely the experimental data as demonstrated by R^2 values of about 0.99 (Table 8).

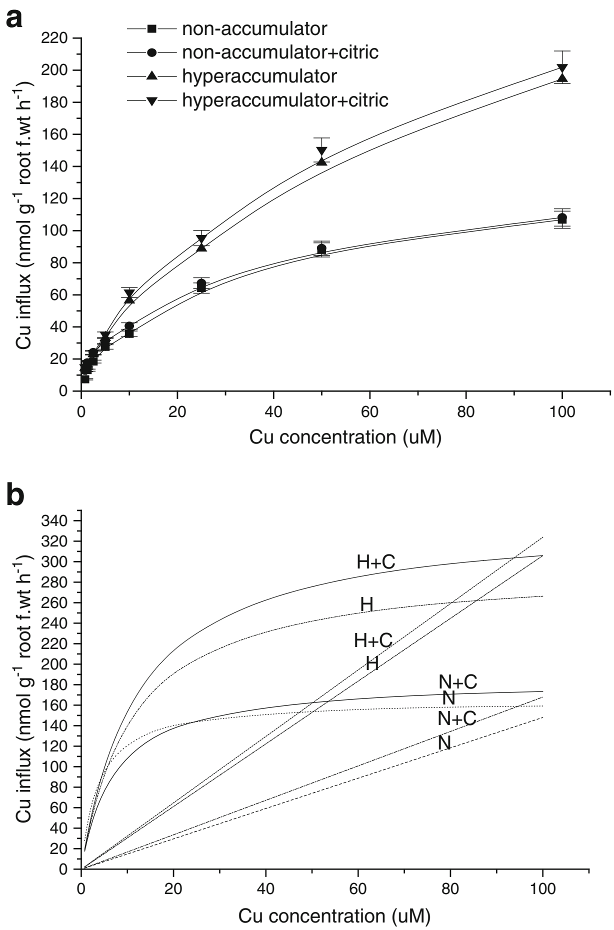
Plants grown for 4 weeks in 1/10 strength Hoagland solution were transferred into a series of copper solution with different concentrations. Copper influx was observed for two plants with or without applied citric acid (Table 8). The 20-min uptake period showed significant differences in terms of unidirectional copper influx rate in the roots of the two varieties. The copper influx was larger in hyperaccumulator than that in nonaccumulator (Fig. 1a). Also, the rate of copper influx was increased by citric acid addition: 16% in hyperaccumulator and 13% in nonaccumulator. The data revealed that the kinetics K_m of the two hyperaccumulators were similar (11.2±2.5 and 12.4±4.9 μM for control and citric-treated plants, respectively), but V_{max} were different (296.2±73.4 and 344.0±81.5 nmol/gh for control and citric-treated plants, respectively). The kinetics K_m of the two nonaccumulators were also similar (3.7±1.2 and 7.2±2.1 μM for the control and the citric-treated plants, respectively). The V_{max} values of the two nonaccumulators were different (165.1±21.7 and 185.8±26.9 nmol/g h, for the control and the citric-treated plants, respectively). In the saturable component of the copper influx, no significant differences were observed as a result of the citric acid addition to the

Table 7 Copper concentrations in shoots of two varieties of *C. communs* in nutrient solution containing 0.8 mM copper (n=3, μg/g, dry mass)

Organic ligands	Hyperaccumulator					Nonaccumulator				
	0 day	1 day	2 day	4 day	30 day	0 day	1 day	2 day	4 day	30 day
Control	0.92	4.80	9.87	40.96	632.06	0.95	4.56	6.83	29.65	48.67
EDTA	0.96	5.11	13.56	56.07	674.16	0.95	5.02	7.11	55.71	65.82
Tartaric	0.95	5.10	9.88	63.21	999.66	0.95	5.11	6.99	64.32	171.17
Succinic	0.90	6.80	9.26	51.74	973.06	0.95	6.26	7.07	65.79	74.05
Citric	0.91	6.97	9.82	65.56	975.38	0.95	5.20	7.27	19.05	60.61
Malic	0.93	8.01	18.63	44.95	991.49	0.95	11.92	14.56	30.58	181.59
Formic	0.97	7.66	9.79	40.44	902.56	0.95	4.71	7.12	29.67	139.28

Values were the means

Fig. 1 Concentration-dependent kinetics of Cu uptake in roots of nonaccumulator and hyperaccumulator varieties of *C. communis* grown in 1/10 strength nutrient solution for 4 weeks. Data points represent mean Values ± SE, n=3. The lines in **a** represent the best fit of the data using a Michaelis-Menten plus linear model. **b** show the dissected Michaelis-Menten and linear components separately



plants. Similarly, the angular coefficients characterizing the linear component of the kinetic of influx curves did not vary significantly for the citric acid treatments.

Discussion

Some studies have reported that organic acids would constitute a major component of root exudates [15]. Certain synthetic and natural chelating agents were found to greatly facilitate metal uptake by roots as well as metal transport to plant aerial parts [11]. Thus, in the practice of phytoremediation, the accumulation of metals in aerial tissues of plants can be enhanced through the application of synthetic and/or natural chelators to the substrate.

High-lead concentrations in shoot tissue of a lead hyperaccumulator were obtained from lead-containing soils, amended with synthetic ligands, such as EDTA [12, 23, 24]. Tissue accumulation levels were proportional to lead and EDTA concentrations in the soil. Ligands with higher efficiency in enhancing soil lead desorption were found to have higher efficiency in total lead accumulation in plant shoot. Those research results were

Table 8 Parameters of the linear component and Michaelis–Menten model used for the kinetic of influx curves in Fig. 1a and b

Ecotype	V_{max} Nmol/g·h (root fresh wt)	K_m μM	r^2
Nonaccumulator	165.1±21.7	3.7±1.2	0.99
Nonaccumulator + citric acid	185.8±26.9	7.2±2.1	0.99
Hyperaccmulator	296.2±73.4	11.2±2.5	0.99
Hyperaccumulator + citric acid	344.0±81.5	12.4±4.9	0.99

Eight different concentrations of Cu (0.5 to 100 μM) were used to study the influx kinetics of Cu over a 20-min uptake period. Values are means ± SE

supported by our experiment. However, the transport of synthetic ligand-metal complexes across the membrane is not widely accepted. In our experiment, EDTA was less effective in causing copper accumulation than other organic acids (from Tables 2, 3, 4, 5, 6, 7). This could be explained by the fact that EDTA transport sites in cell membranes are fewer than those organic acids which could be produced by plant itself, even though the capacity of EDTA to complex copper is higher than those of organic acids (see Table 9). These results agree with the work of [25].

Organic acids, commonly present in root secretions, had the capacity to complex copper in solution (Table 9), and the addition of organic acids increased copper accumulations in shoots (from Tables 2, 3, 4, 5, 6, 7). The influx study results indicated that the copper uptake of both the hyperaccumulator and nonaccumulator plants was affected by organic acids (Fig. 1). Research of the accumulation of several different cations in roots demonstrates that concentration-dependent cation uptake kinetics were biphasic, with an initial rapid component followed by a slower, linear phase of uptake [21]. The rapid component is generally interpreted to represent accumulation in the apoplasm, whereas the slower, linear phase is thought to be due to transport across the plasma membrane. The citric acid was found to increase V_{max} for copper by 16% and 13% in hyperaccumulator and nonaccumulator varieties, respectively, suggesting an increased density of copper transporters on the plasma membranes in root cells of the hyperaccumulator. However, comparison of the values of K_m in the two varieties indicated no significant difference in affinity after organic acid was applied. The lower values of V_{max} and K_m for copper in nonaccumulator varieties compared with the hyperaccumulator (Table 8) suggest a distinct difference in uptake of copper. The higher uptake rate of copper in hyperaccumulator varieties (Fig. 1) might be attributed to differences in some physiological and morphological attributes of the roots systems compared to the nonaccumulator. The physiological attributes include concentration of transporters in the plasma membrane, and the morphological attributes include root

Table 9 Low molecular weight organic acids used in this study

Organic acid	Citric acid	Formic acid	Malic acid	Succinic acid	Tartaric acid	EDTA
Log K	5.90	1.38	3.42	2.61	3.39	18.80

Log K values were adopted from Serieant and Dempsey [26] length, root diameter, and root hairs. Longer and slimmer roots will result in a higher surface area per unit mass of roots and can cause higher uptake compared with the root mass with lower surface area. Characteristics of uptake kinetics can be considered as one of the important criteria for selecting a variety to be used in areas of polluted soil.

The results of the accumulation experiment in shoots reported above are in agreement with root uptake studies of the kinetics of copper influx. The copper uptake influx of hyperaccumulator roots was higher than that of nonaccumulator roots. This is one of the mechanisms by which a hyperaccumulator can accumulate a lot of copper in its shoots, and the applied organic acid could increase copper accumulation in shoots because the copper influx of roots was increased.

There are, however, hyperaccumulators in which highly accumulated copper does not appear to be a major osmotic solute. It has been suggested that the capacity of plant cells to adjust their osmotic effect is a critical attribute for the accumulation of copper. Furthermore, it implies an ability to maintain metabolic activity in the presence of large amounts of copper. The RWC of leaves were not affected by copper accumulation in leaf tissue. These results showed that the normal physiological condition hyperaccumulators had little effect on the accumulation process. The nonaccumulators also experienced a little of effect because there is a minimal amount of osmotic stress to accrue low copper concentrations in their shoots.

The success of phytoremediation depends on several factors. Plants must produce sufficient biomass while accumulating high concentrations of metal. The metal-accumulating plants also need to be responsive to agricultural practices to allow repeated planting and harvesting of the metal-rich tissues. In addition, these plants should preferentially accumulate environmentally important toxic metals. Known metal accumulators do not meet these criteria. The ability to cultivate a high biomass plant with a high content of toxic metals on a contaminated soil will be a determining factor in the success of phytoremediation. Therefore, enhancing metal accumulation in existing high yielding crop plants without diminishing their yield is the most feasible strategy in the development of phytoremediation [25].

This experiment clearly demonstrated the effectiveness of *C. communis* in absorbing large amounts of copper from culture solutions, and the translocation of this copper to its aboveground biomass. The RWC results showed that the normal growth of the plant was not prevented and that the treatments imposed had no toxic effects. The nontoxic effects, in spite of copper accumulation in the plants, may be ascribed to the fact that organic ligands not only enhance the uptake of copper but also reduce their toxicity in plants. The hyperaccumulator of *C. communis* is a useful plant by itself because it has several useful characters for phytoremediation as Wang et al. [27]. Results from this study demonstrate that organic ligands are a necessary amendment in triggering copper hyperaccumulation in plants.

The organic ligands could increase the copper accumulation process in hyperaccumluators and nonaccumulators. The copper accumulation process had little effect on RWC of the two varieties. Thus, when *C. communis* will be used in the supply remediation of copper soils with the addition of organic acids, the high copper accumulation in plant leaves will not affect normal physiological conditions of plants.

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