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ORIGINAL PAPER

Chelator-enhanced phytoextraction of copper and zinc by sunflower, Chinese cabbage, cattails and reeds

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Abstract The phytoextraction of copper and zinc assisted by the chelators such as ethylene diamine tetraacetic acid, diethylene triamino pentaacetic acid, ethylene diamine disuccinate and citric acid into sunflowers (*H*h can act as effective cation exchangers. The negatively charged chelator complexes prevented binding to the cell walls of the roots and allowed complexes to enter the cells. Organic content contains fewer soil nutrients and has fewer negatively charged functional groups, such as carboxical, phenolical and hydroxyl; these adsorb negative free metal cations and reduce metal mobility, leading to less plant uptake. Regardless of different soils' varying organic content, plant propagation ability can be listed in descending order as follows: cattails > reeds > sunflowers > Chinese cabbage. The mechanism of metal uptake was apoplastic transportation. Metal accumulation levels in different parts of plants are listed in descending order as follows: roots > stems > leaves. This is similar to the findings of most studies.

Keywords Phytoextraction · Chelators · Heavy metals · Sunflower (*Helianthus annuus)* · Chinese cabbage (*Brassica campestris)* · Cattail (*Typha latifolia)* and reed *(Phragmites communis*)

Introduction

Heavy metals, such as Cu and Zn, are detrimental to the environment. For instance, Cu is very toxic to phytoplankton and is commonly employed as an algaecide. The contamination of soil with heavy metals threatens ecosystems and human health. In Taiwan, farmland has been contaminated by swine wastewater in which Cu and Zn are the primary metal contaminants. Cu and Zn are used as fodder additives to prevent swine diarrhea and skin abrasions (Yeh and Wu 2009). Commonly practiced soil remediation approaches, such as excavation, dumping, washing and flushing, are generally costly and harmful to the soil properties, and may not be feasible due to limited landfill space.

Phytoextraction, the use of plants to extract metals from contaminated soil, is seen as a vital green remediation approach; it has drawn a lot of attention for its low energy consumption and high public acceptance (Kos and Lestan 2004). It is an economical and non-invasive alternative to conventional civil engineering techniques for the remediation of contaminated soil (Vangronsveld et al. 2009). The main phytoremediation mechanisms include phytoextraction and phytostabilization. Phytoextraction refers to the extraction of metals from soils and their concentration into harvestable aerial parts; phytostabilization refers to the process of using metal-tolerant plants to reduce the mobility of metals leaching into groundwater. The degree of translocation from the roots to the aerial tissues mainly depends on the species of plant, the types of metal and the soil's metal bioavailability. Phytoextraction can be used in areas with medium to low soil pollution levels where physical–chemical soil remediation techniques are too costly.

The sunflower (*Helianthus annuus*) has shown itself to be effective for reducing soil contamination (Lin et al. 2009). It is also an ideal bioenergy plant. Using sunflowers to clean up polluted soil and then recycling them to produce biofuel is a novel approach. Chinese cabbage

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(*Brassica campestris*), also an energy plant, is one of the plants most commonly used to soil metals (Moreno et al. 2005). The harvested plant can realize oil extraction and be reused as biofuel. Energy plants are the base of industrial non-food crops with a focus on renewable energy crops. The phytoextraction application aims to reduce risk and generate alternative income for agriculture (Thewys et al. 2010). The cattail (*Typha latifolia)* and the reed *(Phragmites communis*) have been studied in regard to mitigating water pollution, particularly in constructed wetlands (Yeh et al. 2009). Both macrophytes possess reliable pollutant mitigation, including organic matter (e.g., BOD, COD and inert organics), nutrients (e.g., nitrogen and phosphorus) and microbial indicators (e.g., *E. coli*). Cattails and reeds generally produce large amounts of biomass and can be harvested for proper final waste disposal.

Four commonly used chelators are ethylene diamine tetraacetic acid (EDTA), diethylene triamino pentaacetic acid (DTPA), ethylene diamine disuccinate (EDDS) and citric acid (CA). EDTA, a synthetic chelator, biodegrades poorly in soil because of its effectiveness at complexing metals. Excess amounts of EDTA may leach into groundwater and cause subsurface water contamination. Unlike EDTA, EDDS, which is an EDTA isomer, is a biodegradable chelator. EDDS is a naturally occurring substance in soil that decomposes easily into less detrimental by-products. DTPA (diethylene triamino pentaacetic acid) is a synthetic ligand that forms stable complexes with most of the metals; it has been used in water treatment to prevent metal precipitation and can be employed for soil metal extraction to enhance mobility. CA is a weak organic acid and an intermediate in the CA cycle, which is also known as the tricarboxylic acid (TCA) cycle, or the Krebs cycle. It occurs in the metabolism of virtually all living things and can also be used as an environmentally benign cleaning agent in processes such as metal complexing.

Ethylene diamine tetraacetic acid, DTPA, EDDS and CA provided shoot concentration levels of Cu and Zn higher than those observed in the control plants. In addition, CA was able to induce the removal of Cu and Zn from the soil without increasing the leaching risk (Nascimento et al. 2006). EDDS degrades quite quickly compared to EDTA and DTPA; therefore, several applications should be scheduled in order to reach the remediation objective. EDDS possesses the ability to maximize shoot metal uptake, which also reduces the risk of metal leaching into groundwater. Research has shown that both EDTA and DTPA significantly increase the contents of copper accumulated in the aboveground parts and the rhizospheres of plants, while DTPA is not as adept at enhancing copper uptake as EDTA. Nevertheless, neither EDTA nor DTPA had serious negative effects on the growth of the plants in the study.

The success of phytoextraction depends on the choice of plant species and metal forms retained in soil. Factors such as soil cation-exchange capacity (CEC), pH and organic content also influence phytoextraction efficiency (Saifullah et al. 2009). Several methods have been employed to facilitate phytoextraction, including the use of a chelator to enhance metal mobility in soils and the use of vegetation to translocate metals from underground tissues to the aerial parts of plants. High biomass production, accelerated metal uptake rates and translocation into aerial parts are critical factors for viable metal phytoextraction.

After a review of the related literature (Table 1), to the best of our knowledge, our research is unprecedented in its investigation of the four chelators (EDTA, DTPA, EDDS and CA), two metals (Cu and Zn) and four plants (sunflowers, Chinese cabbage, cattails and reeds) in high- and low-organic content soils to compare phytoextraction efficiencies. The objectives of this study were to investigate elements of phytoextraction efficiency, including root uptake and aerial transportation assisted by EDTA, DTPA, EDDS and CA in sunflowers, Chinese cabbage, cattails and reeds.

Materials and methods

Organic matter content analysis

Soil samples were collected from a local farm and compost site (22°73′N, 120°28′E), which represented low- and high-organic content soils, respectively; 250 g of soil was air-dried overnight and put into an oven at 103 °C. The dried soil was then put into another oven at 550 °C for further organic content measurement. Organic content is defined as the weight difference between soil dried at 103 °C and at 550 °C.

Total metal content and sequential extraction analysis

High- and low-organic content soils were tested to investigate the organic effects. The chemical and physical properties of the tested soil are presented in Table 1. The high- and low-organic content soils contained 25.49 ± 0.48 and 4.16 ± 0.27 % organic materials, respectively. Initial Cu and Zn concentrations before artificial metal spiking were 23 and 121 mg/kg, respectively, similar to common background soil metal concentrations in Taiwan.

The collected soil was artificially spiked with $CuSO_4$ and $ZnCl_2$, mixed well, and air-dried for 5 days to mimic local contamination levels: 1,000 and 8,000 mg/kg for Cu

Table 1 Plant uptake and transportation in recent study

Plant species	Chelator concentration	Plant uptake concentration (mg/kg)	TF	BCF	Reference
Paulownia t.	EDTA 5 mmol/kg	Cu : EDTA(570, 46)[a]	Cu: 0.08	Cu: 0.27	Doumett et al. (2008)
		Zn : EDTA(750, 149)	Zn :0.2	Zn :0.16	
Zea mays	EDTA 3 mmol/kg	Cu : EDTA(153, 69)[a]	Cu: EDTA(0.45)	Cu: EDTA(0.71)	
		Zn : EDTA(315, 180)	Zn: EDTA(0.57)	Zn: EDTA(0.63)	
Helianthus annuus	EDTA 3 mmol/kg	Cu : EDTA(170, 51)[a]	Cu: EDTA(0.30)	Cu: EDTA(0.78)	
		Zn : EDTA(225, 162)	Zn: EDTA(0.72)	Zn: EDTA(0.45)	
Sedum alfredii	CA 5 mmol/kg	Cu: CA (32, 10, 11, 11)[b]	Cu: CA (0.03)	Cu: CA (0.03)	Sun et al. (2009)
	EDTA 5 mmol/kg	EDTA (25, 12, 12, 12)	EDTA (0.57)	EDTA (0.57)	
		Zn: CA (680, 2,000, 1,950, 1,930)	Zn: CA (2.88)	Zn: CA (12.6)	
		EDTA (380, 2,030, 2,000, 2,030)	EDTA (5.34)	EDTA (10.8)	
Vetiveria zizanioides	EDTA 0.8 mmol/kg	Zn (150, 82)[a]	Zn: 0.55	Zn: 0.85	Xu et al. (2009)

[a] (C_{root}, C_{shoot})

[b] (C_{root}, C_{stem}, C_{leaf}, C_{shoot})

[c] (C_{root}, C_{stem}, C_{leaf})

and Zn, respectively. These levels are higher than those in most current phytoextraction studies (Luo et al. 2005; Meers et al. 2008). Cu and Zn levels were equivalent to 2.5 and 4.0 times the current Taiwan soil pollution control standard, respectively, and required remediation. Plants were transferred to pots which were 17 cm deep and 18 cm in diameter, and filled with 1.5 kg (14 cm) of air-dried soil. EDTA, EDDS and CA with a concentration of 5 mmol/kg were initially applied to each pot at the same time. The fractionation of Cu and Zn was investigated using a sequential extraction technique in which soil samples were placed in plastic bottles and then shaken overnight to ensure proper mixing; they were then subjected to a five-step serial extraction procedure. The sequential chemical extraction procedure used in this study included a series of reagents, which were represented as follows: exchangeable (1 M KNO_3), inorganically bound (0.5 M KF), organically bound (0.1 M $Na_4P_2O_7$), Fe and Mn oxide bound (0.3 M $Na_3C_6H_5O_7$, 1 M $NaHCO_3$ and 0.5 g $Na_2S_2O_4$) and in sulfide (6 M HNO_3) form (Tessier et al. 1979).

After harvest, the plants were washed carefully, air-dried and then dried at 103 °C in an oven. Dried plant samples were divided into roots, stems and leaves for metal accumulation assessment. Some 0.5 g of pretreated plants were digested in a solution containing 11:1 HNO_3/HCl solution using a microwave digestion apparatus (Mars 230/60, CEM Corporation) and diluted to 100 mL with deionized water. Some 0.2 g of air-dried soil had aqua regia rendering added for microwave digestion and 2.5 g of air-dried soil for sequential extraction experiments. Metal analyses were conducted using atomic absorption spectrophotometry (AAS, PerkinElmer).

Data and statistical analysis

Data were evaluated relative to the control in order to determine the statistical differences. Plant metal concentration levels were recorded as mg of metal per kilogram of dry biomass. The bioaccumulation coefficient (BCF; Croots/Csoil or water) was calculated as the metal concentration of the plant divided by the heavy metal concentration in the solution or soil in the hydroponic and pot experiments, respectively. TF (shoots/Croots) was depicted as the ratio of the concentration of metal in the shoot to the concentration in the root; it was calculated by dividing the metal concentration in the shoot by the metal concentration in the root. A schematic diagram of the pot experiment and BCF and TF is shown in Fig. 1. Statistical significance was assessed using a mean comparison test. Differences were determined using Student's t test. A level of $p < 0.05$ was considered to be a statistically significant difference. All of the statistical analyses were performed using Microsoft Office Excel 2007.

Results and discussion

Cu sequential extraction results for the soil

In high-organic content soil, the total Cu and Zn concentrations were 847.22 ± 27.57 and 4,486.86 ± 327.26 mg/kg,

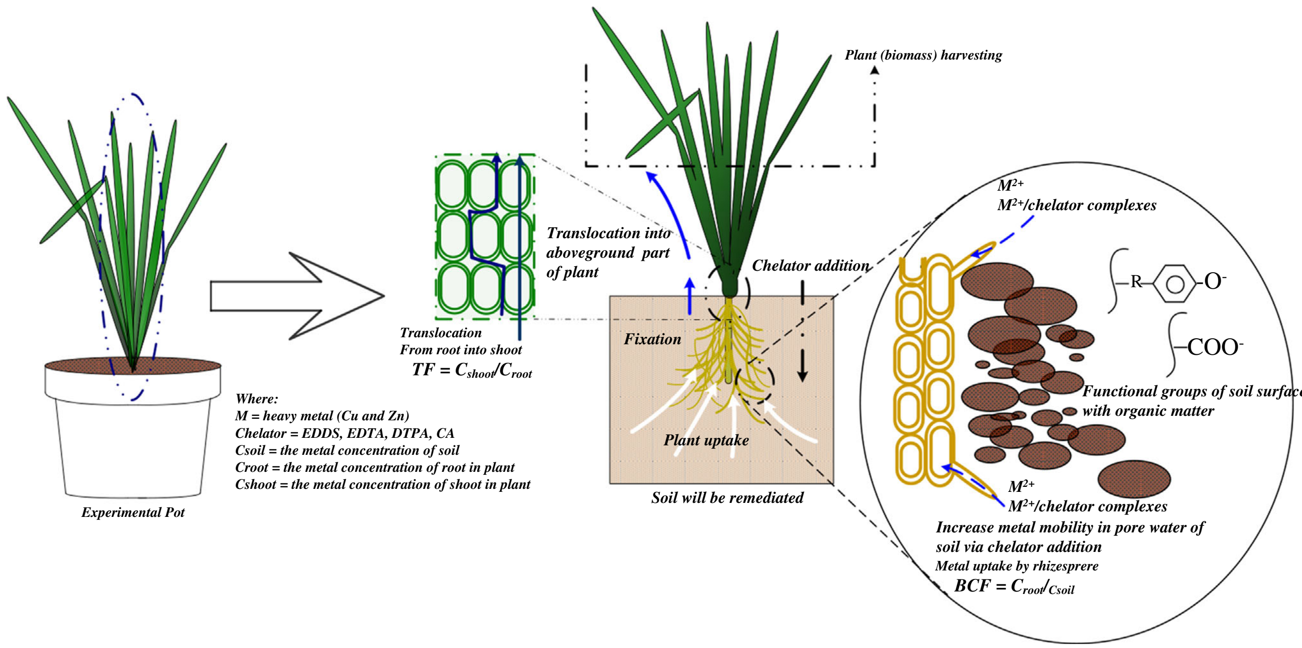

Fig. 1 Schematic diagram of the pot experiment

respectively. In low-organic content soil, the total Cu and Zn concentrations were 872.63 ± 61.34 and 4,393.25 ± 497.09 mg/kg, respectively. The sequential results are shown in Fig. 2. The control low- and high-organic content soils contained 21 and 8 % loosely retained Cu, respectively. This implies that daily water could leach more Cu in low-organic content soil because it contains fewer negative adsorption sites to prevent free cation adsorption. The chelator successfully transferred stable adsorbed metal fraction forms (e.g., organically bound, carbonate bound, Mn oxide bound and sulfide bound) to loosely bound fraction forms (e.g., exchangeable and inorganically bound). In high-organic content soil, EDTA, DTPA, EDDS and CA loosely bound to Cu increased to 34, 36, 24 and 17 %, respectively, while in low-organic content soil, EDTA, DTPA, EDDS and CA loosely bound to Cu increased to 50, 55, 39 and 31 %, respectively. The increasing amounts of loosely bound Cu occurred in descending sequence as follows: DTPA > EDTA > EDDS > CA, which indicates that DTPA was the most effective chelator for transforming stable adsorbed Cu to loosely bound Cu, due to the stability constant commonly expressed as log K; the higher the stability constant, the higher the complexing ability. The stability constants (log K) for DTPA-Cu, EDTA-Cu, EDDS-Cu and CA-Cu were 21.2, 20.5, 18.4 and 7.6, respectively, indicating that DTPA was the most prominent chelator for enhancing Cu complexing, while CA was the least effective (Sorvari and Sillanpaa 1996; Sillanpaa and Oikari 1996; Bostjan and Domen 2004; Polettini et al. 2007).

Zn sequential extraction results of soil

In high-organic content soil, the initially stable retained and loosely bound Zn proportions were 96 and 4 %, respectively, while in low-organic content soil, the initially stable adsorbed and loosely bound Zn proportions were 81 and 19 %, respectively. EDTA, DTPA, EDDS and CA increased loosely bound Zn to 14, 16, 11 and 9 %, respectively, while in low-organic content soil, EDTA, DTPA, EDDS and CA increased loosely bound Zn to 31, 42, 31 and 26 %, respectively. The loosely bound Zn increased in descending sequence as follows: DTPA > EDTA > EDDS > CA. The stability constants log K for DTPA, EDTA, EDDS and CA were 18.3, 16.5, 13.5 and 6.06, respectively.

In high-organic content soil, EDTA, DTPA, EDDS and CA increased the proportion of loosely bound Cu to 34, 36, 24 and 17 %, respectively, while in low-organic content soil, EDTA, DTPA, EDDS and CA increased the proportion of loosely bound Cu to 50, 55, 39 and 31 %, respectively. The loosely bound Cu increased in descending sequence as follows: DTPA > EDTA > EDDS > CA, which indicated that DTPA was the most effective chelator for transforming stable adsorbed Cu to loosely bound Cu. The chief reason for this was the

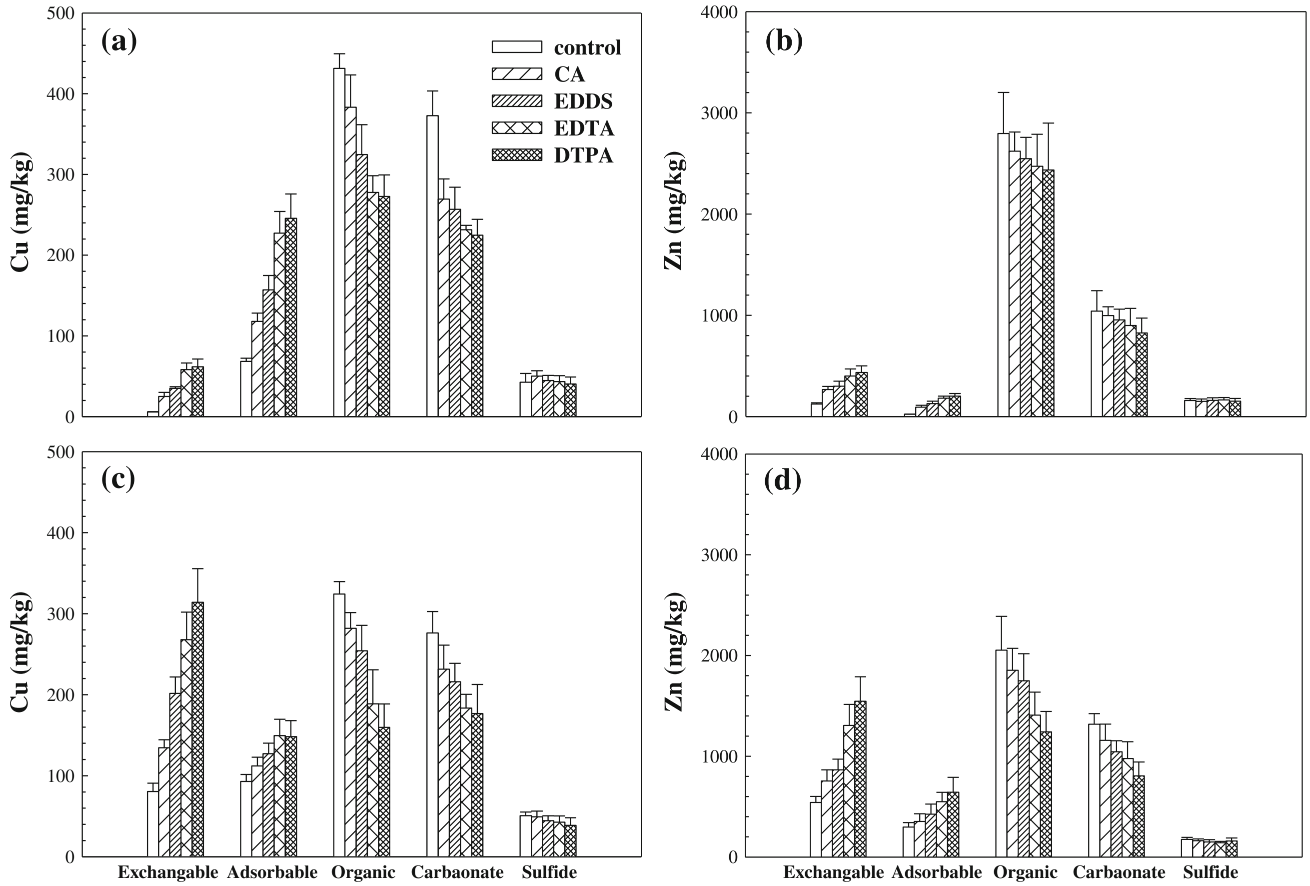

Fig. 2 Comparison of the chemical bonds of Cu and Zn in soils with high and low levels of organic content: **a** Cu and **b** Zn concentrations of high-organic content soil. **c** Cu and **d** Zn concentrations of low-organic content soil

stability constant commonly expressed as (log K); a higher stability constant implies higher complexing ability.

Growth and toxicity symptoms of plants

Regardless of the organic content of soil, plant propagation decreased in the following order: cattails > reeds > sunflowers > Chinese cabbage. The duration between plant wilting and death decreased in the following order: cattails > reeds > sunflowers > Chinese cabbage. Chelators were shown to enhance metal uptake in plants, although adverse effects on growth were also observed. The biodegradable chelators, EDDS and CA, had less negative impact on plants than did the synthetic chelators, DTPA and EDTA. In particular, EDTA demonstrated the most toxic effects. Toxicity symptoms included leaf yellowing, wilting and death. Cattails and reeds displayed the greatest tolerance to metal pollution in terms of total soil metal concentration, while sunflowers displayed the least tolerance to metal pollution. Chinese cabbage demonstrated the least metal tolerance to the two tested metals, Cu and Zn.

Previous studies have investigated the phytotoxicity of EDDS and EDTA. Demonstrated visible symptoms, such as necrosis and chlorosis, were detected for EDDS concentrations of 3.125 mmol/kg and EDTA concentrations of 12.5 mmol/kg (Evangelou et al. 2007). CA is carboxylic acid exuded by plant roots and is commonly found in the rhizosphere where organic acids have the potential to enhance metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals. The presence of organic acids may affect heavy metal desorption, solubility and mobility (Schwab et al. 2008).

Metal uptake and translocation

Sunflowers

High-organic-soil content Metal uptake results of the rhizosphere and the aerial parts of the sunflower are shown in Fig. 3. The chelator induced detectable metal

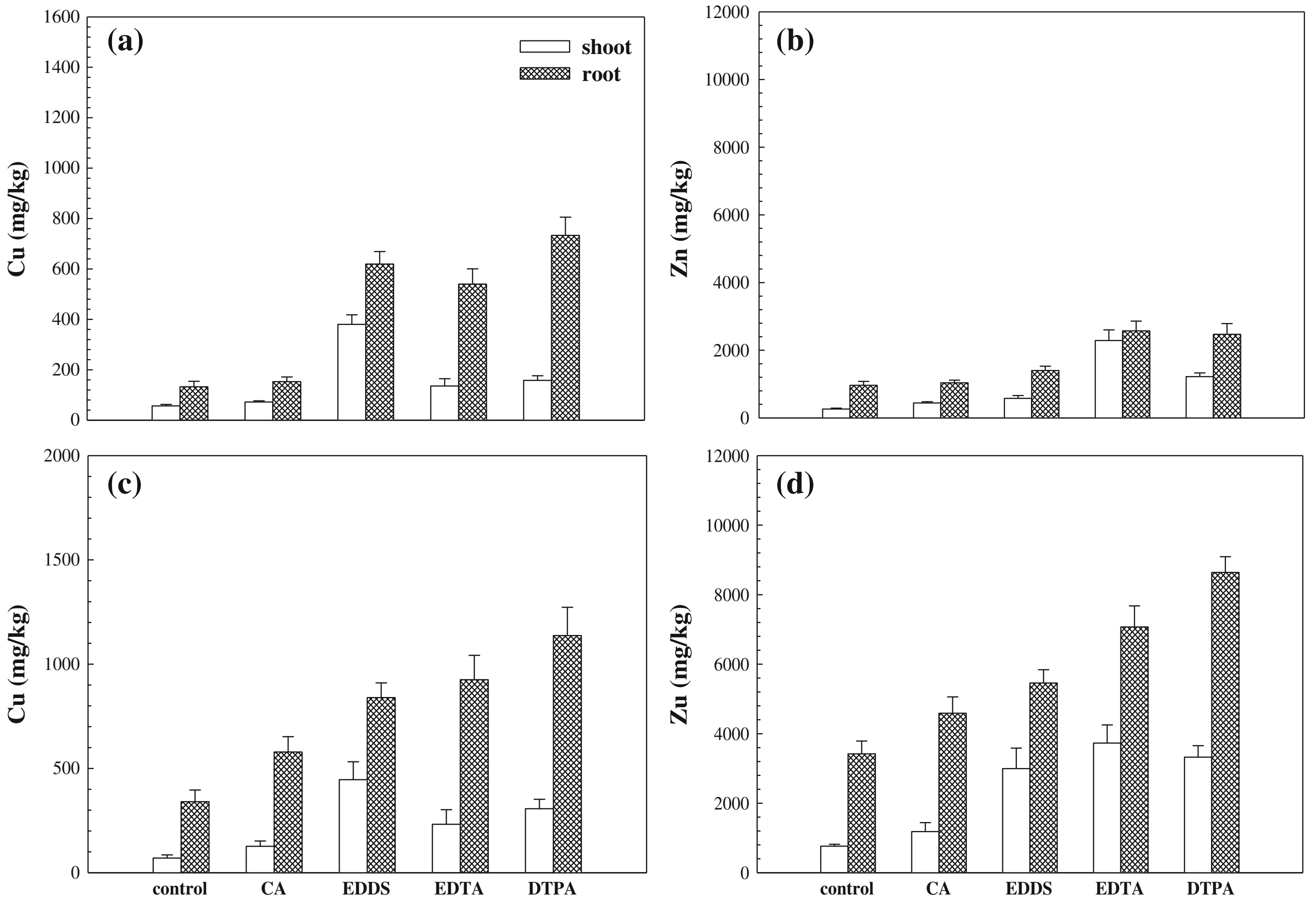

Fig. 3 Comparison of Cu and Zn accumulation by sunflowers planted in high- and low-organic content soils: **a** Cu and **b** Zn accumulated by sunflowers planted in high-organic content soil. **c** Cu and **d** Zn accumulated by sunflowers planted in low-organic content soil

uptake improvement, particularly for the energy plant, the sunflower. In high-organic content soil, the greatest accumulation was in the root areas. The control Cu accumulation levels in the aerial parts and roots were 56.68 ± 5.96 and 132.44 ± 21.74 mg/kg, respectively, while the control Zn accumulation levels in the aerial parts and roots were 263.74 ± 26.13 and 963.77 ± 113.77 mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 2.39- and 4.08-fold, respectively, while Zn accumulation levels increased 8.67- and 2.67-fold, respectively. DTPA increased Cu accumulation levels 2.79- and 5.53-fold, respectively, while Zn increased accumulation levels 4.62- and 2.56-fold, respectively. EDDS increased Cu accumulation levels 6.70- and 4.68-fold, respectively, while Zn increased accumulation levels 2.19- and 1.45-fold, respectively. CA increased Cu accumulation levels 1.27- and 1.16-fold, respectively, while Zn accumulation levels increased 1.67 and 1.08-fold, respectively. Increases in Cu aerial metal levels are listed in descending order as follows: EDDS > DTPA > EDTA > CA, while the rhizosphere metal levels increased in descending sequence. Zn aerial metal levels increased; the amounts of metal uptake in descending order were as follows: EDTA > DTPA > EDDS > CA, while the rhizosphere metal level increased in descending sequence as follows: EDTA > DTPA > EDDS > CA.

Low-organic content soil Low-organic content soil metal uptake results are shown in Fig. 3c, d. Cu accumulation levels of aerial and rhizosphere plant parts were 70.27 ± 15.49 and 340.93 ± 55.61 mg/kg, respectively, while Zn accumulation levels of aerial portions and roots were 765.24 ± 52.20 and $3{,}420.67 \pm 367.56$ mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 3.3- and 2.71-fold, respectively, while Zn accumulation levels increased 4.88- and 2.07-fold, respectively. DTPA increased Cu accumulation levels 4.36- and 3.33-

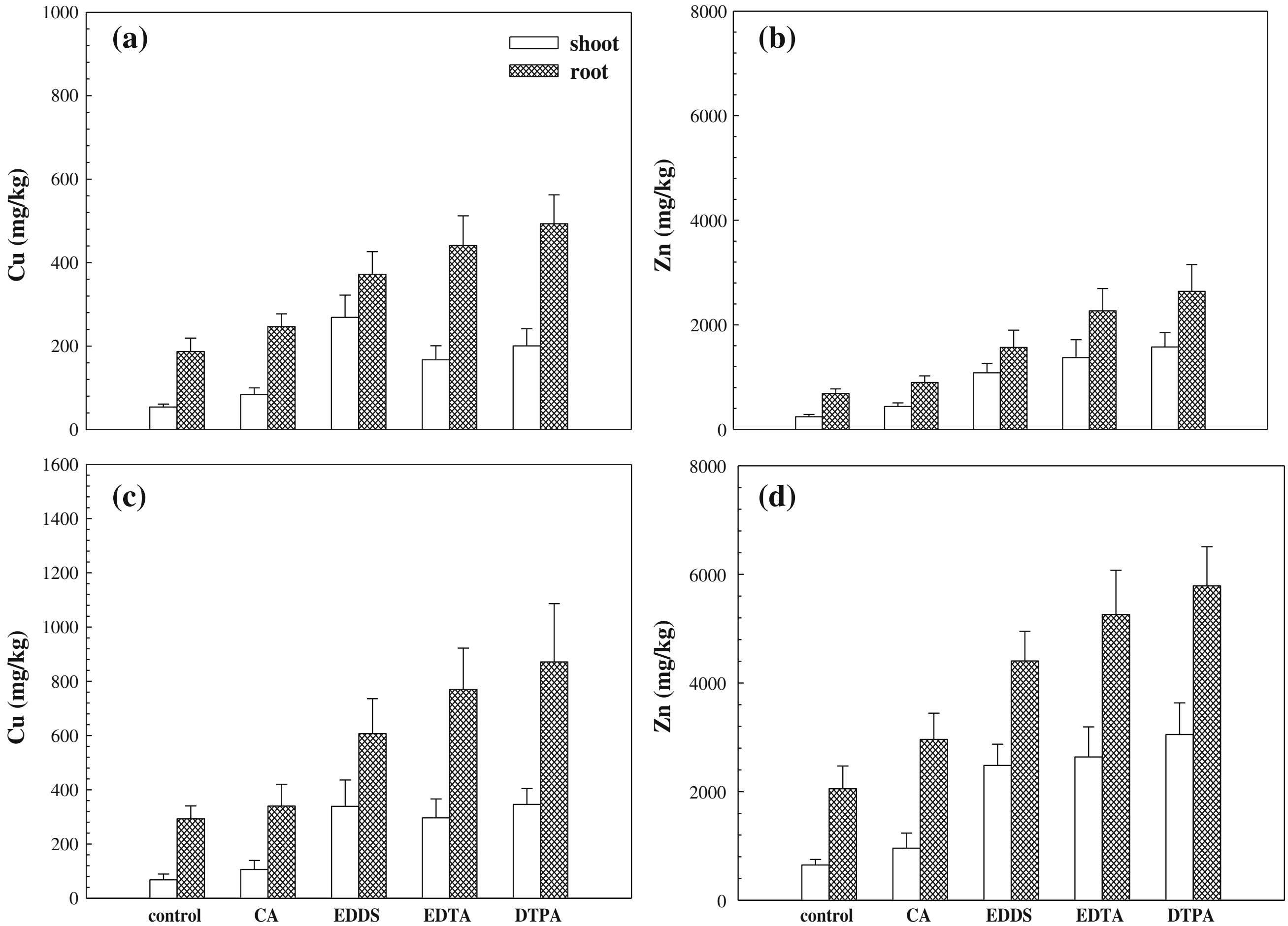

Fig. 4 Comparison of Cu and Zn accumulation by Chinese cabbage planted in high- and low-organic content soils: **a** Cu and **b** Zn accumulated by Chinese cabbage planted in high-organic content soil. **c** Cu and **d** Zn accumulated by Chinese cabbage planted in low-organic content soil

fold, respectively, while Zn accumulation levels increased 4.34- and 2.53-fold, respectively. EDDS increased Cu accumulation levels 6.35- and 2.46-fold, respectively, while Zn accumulation levels increased 3.91- and 1.60-fold, respectively. CA increased Cu accumulation levels 1.81- and 1.70-fold, respectively, while Zn accumulation levels increased 1.55- and 1.34-fold, respectively.

Chinese cabbage

High-organic content soil Metal uptake results are shown in Fig. 4. Most of the metals accumulated in the rhizosphere, and Zn displayed better uptake than Cu did. In the control, Cu accumulation levels of aerial and root plant parts were 54.06 ± 6.92 and 186.88 ± 32.16 mg/kg, respectively, while Zn accumulation levels in the aerial parts and roots were 243.25 ± 41.62 and 686.46 ± 89.67 mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 3.09- and 2.36-fold, respectively, while Zn accumulation levels increased 5.65- and 3.30-fold, respectively. DTPA increased Cu accumulation levels 3.71- and 2.64-fold, respectively, while Zn accumulation levels increased 6.48- and 3.85-fold, respectively. EDDS increased Cu accumulation levels 4.97- and 1.99-fold, respectively, while Zn accumulation levels increased 4.45- and 2.29-fold, respectively. CA increased Cu accumulation levels 1.56- and 1.32-fold, respectively, while Zn accumulation levels increased 1.80- and 1.31-fold, respectively. Chelator Cu uptake increases are listed in descending order as follows: EDDS > DTPA > EDTA > CA. The rhizosphere Cu accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

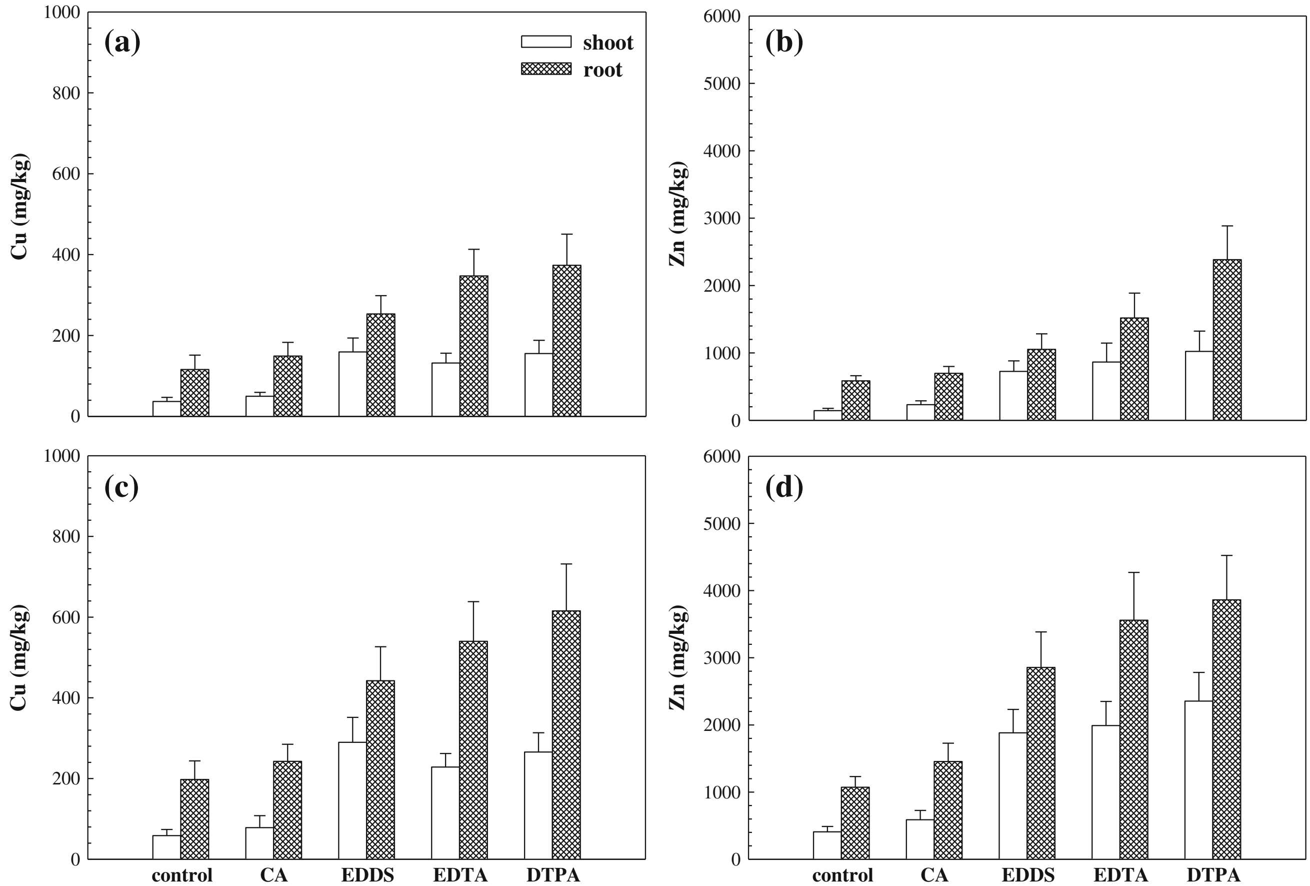

Fig. 5 Comparison of Cu and Zn accumulation of cattails planted in high- and low-organic content soils: **a** Cu and **b** Zn accumulated by cattails planted in high-organic content soil. **c** Cu and **d** Zn accumulated by cattails planted in low-organic content soil

Low-organic content soil Metal uptake results for the roots and aerial parts are shown in Fig. 4c, d. Most of the metals accumulated in the rhizosphere, and Zn showed better uptake performance than Cu did. In the control, the Cu accumulation levels in the aerial plant parts and roots were 67.83 ± 21.29 and 292.82 ± 47.33 mg/kg, respectively, while Zn accumulation levels in the aerial portions and roots were 648.16 ± 103.12 and $2{,}053.17 \pm 418.07$ mg/kg, respectively. Relative to the control, EDTA increased the Cu accumulation levels 4.37- and 2.63-fold, respectively, while Zn accumulation levels increased 4.07- and 2.56-fold, respectively. DTPA increased the Cu accumulation levels 5.10- and 2.98-fold, respectively, while Zn accumulation levels increased 4.72- and 2.82-fold, respectively. EDDS increased Cu accumulation levels 4.97- and 1.99-fold, respectively, while Zn accumulation levels increased 4.45- and 2.29-fold, respectively. CA increased Cu accumulation levels 1.57- and 1.16-fold, respectively, while Zn accumulation levels increased 1.48- and 1.44-fold, respectively. The chelator transferred the stable retained metal to loosely bound fractions to enhance plant metal uptake. The chelator Cu uptake increases are listed in descending order as follows: DTPA > EDDS > EDTA > CA. Rhizosphere Cu accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

The energy plant maize could provide 33,000–40,000 kWh of renewable energy (electrical and thermal) per hectare per year which, if substituted for coal-generated energy, would prevent the generation of up to 21×10^3 kg/ha year CO_2. The sunflower also may potentially serve as an alternative energy crop used for purposes similar to those of maize (Meers et al. 2010).

Cattail

High-organic content soil Metal uptake results for root and aerial parts are shown in Fig. 5. Most of the metals accumulated in the rhizosphere, and Zn displayed better

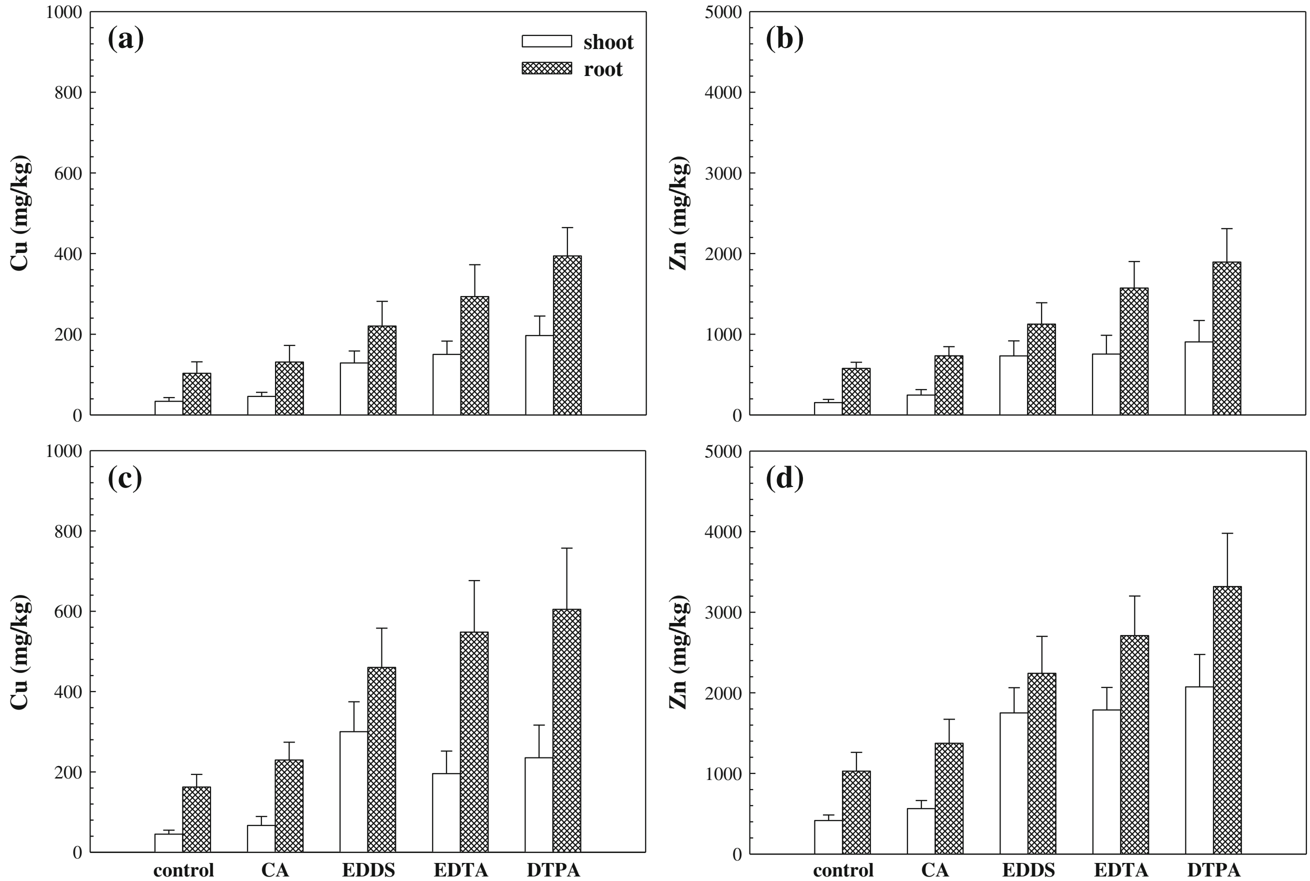

Fig. 6 Comparison of Cu and Zn accumulated by reeds planted in high- and low- organic content soils: **a** Cu and **b** Zn accumulated by reeds planted in high-organic content soil. **c** Cu and **d** Zn accumulated by reeds planted in low-organic content soil

uptake than Cu did. In the control, Cu accumulation levels in the aerial parts and roots were 36.75 ± 10.32 and 115.91 ± 35.46 mg/kg, respectively, while Zn accumulation levels in the aerial and rhizosphere parts were 143.62 ± 33.57 and 585.35 ± 76.24 mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 3.59- and 3.00-fold, respectively, while Zn accumulation levels increased 6.01- and 2.59-fold, respectively. DTPA increased Cu accumulation levels 4.23- and 3.22-fold, respectively, while Zn accumulation levels increased 7.11- and 4.07-fold, respectively. EDDS increased Cu accumulation levels 4.33- and 2.19-fold, respectively, while Zn accumulation levels increased 5.05- and 1.80-fold, respectively. CA increased Cu accumulation levels 1.36- and 1.29-fold, respectively, while Zn accumulation levels increased 1.61- and 1.19-fold, respectively. The chelator Cu uptake improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA. Rhizosphere Cu accumulation is listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

Low-organic content soil Metal uptake results for roots and aerial parts are shown in Fig. 5c, d. Most of the metals accumulated in the rhizosphere, and Zn displayed better uptake performance than Cu did. In the control, Cu accumulation levels of aerial parts and roots were 58.59 ± 15.23 and 197.67 ± 45.96 mg/kg, respectively, while Zn accumulation levels in the aerial portions and roots were 409.36 ± 79.29 and 1,072.83 ± 159.48 mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 3.90- and 2.73-fold, respectively, while Zn accumulation levels increased 4.86- and 3.32-fold, respectively. DTPA increased Cu accumulation levels 4.54- and 3.11-fold, respectively, while Zn accumulation levels increased 5.75- and 3.60-fold, respectively. EDDS increased Cu accumulation levels 4.94- and 2.24-fold, respectively, while Zn accumulation levels increased 4.60- and 2.66-fold, respectively. CA increased Cu accumulation levels 1.34- and

1.23-fold, respectively, while Zn levels increased accumulation levels 1.44- and 1.36-fold, respectively. The chelator transferred the stable retained metal to loosely bound fractions to enhance plant metal uptake. The chelator Cu uptake is listed in descending order as follows: EDDS > DTPA > EDTA > CA. Rhizosphere Cu accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation is listed in descending order as follows: DTPA > EDTA > EDDS > CA.

Reed

High-organic content soil Metal uptake results for roots and aerial parts of sunflowers are shown in Fig. 6. Most of the metals accumulated in the rhizosphere, and Zn displayed better uptake performance than Cu did. In the control, Cu accumulation levels of the aerial parts and roots were 33.82 ± 9.12 and 103.23 ± 28.46 mg/kg, respectively, while Zn accumulation levels in the aerial portions and roots were 153.72 ± 38.57 and 575.97 ± 79.24 mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation levels 4.43- and 2.84-fold, respectively, while Zn accumulation levels increased 4.91- and 2.73-fold, respectively. DTPA increased Cu accumulation levels 5.82- and 3.79-fold, respectively, while Zn accumulation levels increased 5.89- and 3.29-fold, respectively. EDDS increased Cu accumulation levels 3.81- and 2.13-fold, respectively, while Zn accumulation levels increased 4.76 and 1.95-fold, respectively. CA increased Cu accumulation levels 1.35- and 1.27-fold, respectively, while Zn accumulation levels increased 1.58- and 1.27-fold, respectively. The chelator Cu uptake increases are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Rhizosphere Cu accumulations levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

Low-organic content soil Metal uptake results for roots and aerial parts of reeds are shown in Fig. 6c, d. Most of the metals accumulated in the rhizosphere and Zn displayed better uptake performance than Cu did. In the control, Cu accumulation levels in aerial plant parts and roots were EDDS, respectively, while Zn accumulation levels in aerial portions and roots were 45.08 ± 9.87 and 162.36 ± 31.53 mg/kg, respectively. Zn accumulation levels in aerial parts and roots were 415.81 ± 69.08 and $1,028.56 \pm 232.46$, respectively. Relative to the control, EDTA increased Cu accumulation levels in the aerial parts and roots 4.34- and 3.37-fold, respectively, while Zn accumulation levels increased 4.30- and 2.64-fold, respectively. DTPA increased Cu accumulation levels 5.22- and 3.72-fold, respectively, while Zn accumulation levels increased 4.99- and 3.23-fold, respectively. EDDS increased Cu accumulation levels 6.66- and 2.83-fold, respectively, while Zn accumulation levels increased 4.21- and 2.18-fold, respectively. CA increased Cu accumulation levels 1.48- and 1.41-fold, respectively, while Zn accumulation levels increased 1.35- and 1.34-fold, respectively. The chelator attached soil metal to a soluble metal complex, which is mobile in a soil solution. It was also shown in this study that chelators transfer stable retained metal to loosely bound fractions. The chelator Cu uptake increases are listed in descending order as follows: EDDS > DTPA > EDTA > CA. Rhizosphere Cu accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. Root Zn accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

The metal complex translocation mechanism has been indicated as the passive apoplastic transportation system (Komárek et al. 2010). The addition of chelators has generally prevented metal precipitation and formed metal complex compounds. The apoplast is the free diffusional space outside the plasma membrane that has a high content of carboxylic groups, which can act as effective cation exchangers. The negatively charged chelator complexes prevent binding with cell walls in the roots and allow complexes to enter the cells. Metal–chelator complexes were subsequently translocated to the aerial part of the plant through passive apoplastic pathways.

Bioconcentration factor (BCF) and translocation factor (TF)

Sunflowers

High-organic content soil Bioconcentration factor and TF were employed to evaluate soil metal transfer to the rhizosphere and further uptake transference to the aerial parts of plants. BCF indicates plant soil metal adsorption levels, while TF refers to the amount of metal uptake and transfer from the roots to the aerial parts of the plant.

In the control, Cu and Zn BCF values were 0.17 ± 0.03 and 0.21 ± 0.03, respectively, while Cu and Zn TF values were 0.43 ± 0.04 and 0.28 ± 0.05, respectively. BCF results indicated that EDTA, DTPA and EDDS performed relatively effective in terms of rhizosphere metal adsorption, while the lowest stability constant CA demonstrated less root metal retention.

Low-organic content soil In the control, the Cu and Zn BCF values were 0.39 ± 0.06 and 0.78 ± 0.08, respectively, while Cu and Zn TF values were 0.21 ± 0.03 and 0.22 ± 0.02. EDTA BCF values increased 2.72- and 2.06-fold relative to the control. EDDS increased 2.46- and 1.59-fold relative to the control. DTPA increased 3.33- and 2.53-fold relative to the control.

Ethylene diamine tetraacetic acid, DTPA and EDDS all demonstrated significant metal transfer, while CA was less effective. TF values for CA were 0.22 and 0.26. EDDS increased 2.52- and 2.50-fold relative to the control. The primary mechanisms for inducing metal transfer from the rhizosphere to the aerial parts of the plant were metal–chelator complexes. Negatively charged, these are able to intrude into the Casparian strip, enter the plant and induce an increase in metal transfer, as was indicated by the TF values. The Casparian strip is a band of cell wall material. The Casparian strip appears to form a barrier at which the apoplastic flow is forced to pass through the selectively permeable plasma membrane into the cytoplasm (thus the symplast) rather than continuing along the cell wall. It influences metal–chelator complex translocation in plants (Luo et al. 2005).

Chinese cabbage

High-organic content soil In the control, the BCF values of Cu and Zn were 0.21 ± 0.02 and 0.16 ± 0.01, respectively. Rhizosphere accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. BCF results indicated that EDTA, DTPA and EDDS performed relatively effective in terms of rhizosphere metal adsorption, while the lowest stability constant, CA, demonstrated less root metal retention ability.

In the control, the TF values of Cu and Zn were 0.28 ± 0.04 and 0.35 ± 0.05, respectively. EDDS demonstrated the most prominent metal translocation to the upper parts of the plants. Its TF values increased 2.60- and 2.00-fold relative to the control. Chelator translocation improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA.

Low-organic content soil In the control, Cu and Zn BCF values were 0.33 ± 0.04 and 0.58 ± 0.07, respectively. The Cu and Zn BCF values were higher than those of the high-organic content soil, which were 0.21 ± 0.02 and 0.16 ± 0.01, respectively. Low-organic content soil contains more loosely bound metal, which leads to more soluble metal, causing the lower BCF. EDTA, DTPA and EDDS all demonstrated significant metal transfer, while CA was less effective. In the control, TF values were 0.24 ± 0.02 and 0.27 ± 0.01 for Cu and Zn, respectively, and 0.32 ± 0.01 and 0.35 ± 0.04 for CA. EDDS showed that the most effective metal translocation occurred where the TF values were 0.58 and 0.59 for Cu and Zn, respectively. The primary mechanisms for inducing metal transfer from the rhizosphere to the aerial parts of the plant were the metal–chelator complexes, which are negatively charged and able to intrude into the Casparian strips and enter into plants to induce an increase in metal transfer, which is indicated by TF values (Luo et al. 2005).

Cattails

High-organic content soil In the control, Cu and Zn BCF values were 0.14 ± 0.01 and 0.13 ± 0.02, respectively. Rhizosphere accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA. BCF results indicated that EDTA, DTPA and EDDS were relatively effective in terms of rhizosphere metal adsorption, while the lowest stability constant, CA, demonstrated less root metal retention ability.

Cu and Zn TF values were 0.63 ± 0.09 and 0.70 ± 0.10 according to the EDDS application, respectively, while the Cu and Zn TF values for the control were 0.29 ± 0.06 and 0.24 ± 0.04, respectively. EDDS demonstrated the most prominent metal translocation to the upper parts of the plant with the TF values, showing a 2.17- and 2.92-fold increase relative to the control. The chelator translocation improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA.

Low-organic content soil In the control, Cu and Zn while Cu and Zn BCF values were 0.23 ± 0.02 and 0.24 ± 0.01. In the control, Cu and Zn while Cu and Zn TF values were 0.29 ± 0.04 and 0.38 ± 0.06. EDDS demonstrated the most prominent metal translocation to the upper parts of the plant in low-organic content soil. The TF values increased 2.28- and 1.74-fold relative to the control. The chelators' translocation improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA.

Reeds

High-organic content soil Bioconcentration factor and TF results are shown in figure. BCF results indicated that EDTA, DTPA and EDDS performed relatively effective in terms of rhizosphere metal adsorption, while the lowest stability constant, CA, demonstrated less root metal retention ability. In the control, BCF values were

0.12 ± 0.01 and 0.14 ± 0.01, respectively. Rhizosphere accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

In the control, the TF values of Cu and Zn in reeds were 0.32 ± 0.04 and 0.27 ± 0.02, respectively. EDDS indicated that the most prominent metal translocation was to the upper parts of reed plants, and the TF values were increased 1.84- and 2.40-fold relative to the control condition. The chelator translocation improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA.

Low-organic content soil BCD results indicate that EDTA, DTPA and EDDS were relatively effective in terms of rhizosphere metal adsorption, while the lowest stability constant, CA, demonstrated less root metal retention ability. In the control, BCF values were 0.19 ± 0.01 and 0.23 ± 0.01. Rhizosphere accumulation levels are listed in descending order as follows: DTPA > EDTA > EDDS > CA.

In the control, Cu and Zn while Cu and Zn TF values were 0.26 ± 0.02 and 0.40 ± 0.04, respectively. EDDS demonstrated the most prominent metal translocation to the upper parts of the plant, where the TF values were 0.67 ± 0.06 and 0.78 ± 0.11, respectively. Translocation improvement is listed in descending order as follows: EDDS > DTPA > EDTA > CA.

In summary, metals accumulate in different parts of plants in different amounts. They are listed in descending order from most to least as follows: roots > stems > leaves. This finding is consistent with those of other studies (Evangelou et al. 2007). Researchers also found similar results while investigating the metal accumulation in wetlands macrophytes (Yeh et al. 2009).

A higher extraction capability can be predicted on the basis of the stability constant. For instance, the stability constants (log K) of Zn for EDDS, EDTA, DTPA and CA were 13.5, 16.5, 18.3 and 6.1, respectively, and the stability constants (log K) of Cu for EDDS, EDTA, DTPA and CA were 18.4, 20.5, 21.2 and 7.6, respectively (Sillanpaa and Oikari 1996; Sorvari and Sillanpaa 1996; Bostjan and Domen 2004; Polettini et al. 2007; Meers et al. 2008). Similar extraction results have been found by other researchers (Polettini et al. 2007). EDTA was more effective than EDDS in preventing metal precipitation. This can be predicted by the higher stability constant of EDTA metal complexes. The speed of EDDS biodegradation might be a factor in selecting a feasible chelator for phytoextraction improvement (Epelde et al. 2008).

Biodegradable chelators EDDS and CA performed reliably in this study. EDTA and DTPA also enhance metal mobility, but potential groundwater contamination concern remains an issue.

Effect of soils with different organic contents

The organic makeup of soil has been demonstrated to be an important factor that influences plant metal uptake and metal bioavailability. High-organic content soils provide more substrates for plant propagation. Great biomass production induced prominent plant uptake. Low-organic content soil has fewer nutrients but also has fewer negative-charged functional groups, such as carboxical, phenolical and hydroxyl; these might adsorb negative free metal cations and reduce metal mobility, leading to less plant uptake. High-organic content soil has fewer negative functional groups, indicating that negative chelator–metal complexes can be easily translocated to the aerial parts of plants.

The organic content of soil might influence heavy metal retention by forming stable complexes. Organic matter contributes to a decrease in contaminated soil's metal availability for plants (Halim et al. 2003).

The prediction of daily metal removal, based on the pot experiment results' 9:1 ratio weight, was used to calculate the aerial and root parts, respectively. In high-organic content soil, sunflowers' daily uptakes of Cu and Zn were 5.5 and 27.8 mg/kg per day, respectively. Chinese cabbage's daily uptakes of Cu and Zn were 9.6 and 41.0 mg/kg per day, respectively. Cattails' daily uptakes of Cu and Zn were 1.5 and 6.3 mg/kg per day, respectively. Reeds' daily uptakes of Cu and Zn were 1.4 and 6.5 mg/kg per day, respectively. In low-organic content soil, sunflowers' daily uptakes of Cu and Zn were 12.1 and 128.8 mg/kg per day, respectively. Chinese cabbage's daily uptakes of Cu and Zn were 12.9 and 112.6 mg/kg per day, respectively. Cattails' daily uptakes of Cu and Zn were 4.1 and 26.4 mg/kg per day, respectively. Reeds' daily uptakes of Cu and Zn were 3.2 and 26.5 mg/kg per day, respectively.

Regardless of the amount of organic material in the soil, the energy plants, sunflowers and Chinese cabbage, demonstrated more effective metal uptake than did the wetlands macrophytes, cattails and reeds. Previous research showed promising phytoextraction effects from using sunflower and Chinese cabbage (Lin et al. 2009; Moreno et al. 2005). EDTA, DTPA and EDDS all demonstrated prominent metal uptake improvement, while CA results were less promising.

Nevertheless, chelator-assisted phytoextraction using sunflowers, Chinese cabbages, cattails and reeds to remove Cu and Zn was demonstrated. The prospects of future in situ applications using this green remediation are good. However, possible chelator–metal complex leaching and contamination of groundwater are pivotal issues to be taken into serious consideration.

Conclusion

Sunflowers, Chinese cabbage, cattails and reeds were shown to be effective plants for chelator-enhanced phytoextraction. Organic content was shown to be a critical factor influencing metal uptake. Organic content has fewer soil nutrients and has negatively charged functional groups, such as carboxical, phenolical and hydroxyl; these adsorb negative free metal cations and reduce metal mobility, leading to less plant uptake. Recently, sludge from the activated sludge processors in sewage treatment plants was meant to be reused as soil amendment fertilizer. The metal transfer from various media, including soil, groundwater and plants, was the foremost concern. Sewage sludge generally contains various levels of organic matters. The results of this study can be referenced by in situ operation engineers to field manage real-site sludge reuse operations.

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