

## The Effect of Varying Soil Organic Levels on Phytoextraction of Cu and Zn uptake, enhanced by chelator EDTA, DTPA, EDDS and Citric Acid, in Sunflower (*Helianthus annuus*), Chinese Cabbage (*Brassica campestris*), Cattail (*Typha latifolia*), and Reed (*Phragmites communis*)

T.Y. Yeh\*, C.F. Lin, C.C. Chuang and C.T. Pan

Department of Civil and Environmental Engineering, National University of Kaohsiung, Taiwan

### Abstract

Focusing on the influence of organic soil contents on the Phytoextraction of Cu and Zn, assisted by chelators EDTA, DTPA, EDDS, and citric acid, in Sunflower (*Helianthus annuus*), Chinese Cabbage (*Brassica campestris*), Cattail (*Typha latifolia*), and Reed (*Phragmites communis*), this study demonstrates prominent Cu and Zn uptake enhancement. Soil organic concentration has been shown to be a critical factor in metal uptake and bioavailability in plants. Organic content has less soil nutrients, and less negatively charged functional groups, such as carboxylic, phenolic and hydroxyl groups. This allows adsorption of negative free metal cations, and reduces metal mobility. Regardless of various soil organic contents, this study ranks Cattail, Reed, Sunflower, and Chinese Cabbage, in descending order of propagation efficacy. The mechanism of metal is apoplastic transportation. In plant cells, the apoplast is the free diffusional space outside the plasma membrane. It contains high concentrations of carboxylic groups which act as effective cation exchangers. The negatively charged chelator complexes are prevented from being bound to the cell walls of the roots, and allow complexes to enter into the cells. Metal chelator complexes are subsequently translocated to the aerial part of plant via the passive apoplastic pathway. Metal is seen to accumulate in the roots, stems, and leaves, in descending order of concentration; a result similar to most other research conclusions.

**Keywords:** Phytoextraction; chelators; Heavy metals; Sunflower (*Helianthus annuus*); Chinese cabbage (*Brassica campestris*); Cattail (*Typha latifolia*); Reed (*Phragmites communis*)

### Introduction

Heavy metals Cu and Zn are detrimental to the environment (for example, Cu is very toxic to phytoplankton, and is commonly employed as an algacide). The continued heavy metal contamination of soil is threatening human health and ecosystems. In Taiwan, farmland has been contaminated by swine wastewater, with Cu and Zn being the primary metal contaminants. This is because Cu and Zn are used as fodder additives in order to prevent swine diarrhea and skin abrasions [1]. Common soil remediation approaches, such as soil excavation and dumping, or soil washing/flushing, are generally costly and harmful to soil properties, and may simply be infeasible due to limited landfill space.

Phytoextraction, the use of plants for the extraction of metals from contaminated soils, is regarded as a vital green remediation approach, and has attracted a great deal of interest due to its low energy consumption and high public acceptance [2]. It is an economical and non-invasive alternative relative to conventional civil engineering techniques for contaminated soil remediation [3]. Phytoremediation mechanisms mainly include phytoextraction and phytostabilization. Phytoextraction refers to the extraction of metals from soils by concentrating them into the harvestable aerial parts of plants, while phytostabilization refers to the introduction to the soil of metal tolerant plants in order to reduce the mobility of metals leaching into groundwater. The degree of translocation from roots to aerial tissues mainly depends on the species of plant, type of metal, or soil metal bioavailability. Phytoextraction can be used in areas with medium to low soil pollution levels, where physical chemical soil remediation techniques are too costly.

Sunflowers (*Helianthus annuus*) have been demonstrated to be a viable plant for soil contamination phytoextraction [4]. It is also

an ideal bioenergy plant. Using sunflowers to clean up polluted soil, and then recycling them to produce bio-fuel, is a novel approach. Chinese cabbage (*Brassica campestris*), also an energy plant, has been demonstrated to be an efficient phytoextraction plant [5], from which oil can be extracted to be re-used as bio-fuel. Energy plants are industrial non-food crops which are cultivated as renewable energy crops, with the intentions of risk reduction and alternative agricultural income generation [6]. Authors have studied cattail (*Typha latifolia*) and reed (*Phragmites communis*) with regard to their ability to mitigate water pollution, particularly in constructed wetland areas [7]. Both macrophytes reliably mitigate pollution of various kinds, including organic matter (e.g. BOD, COD, and inert organics), nutrients (e.g. nitrogen and phosphorus), and microbial indicators (e.g. E-coli). Cattail and reed generally produce enormous biomass and can be harvested for proper final waste disposal.

Four commonly used chelators, namely ethylenediaminetetraacetic acid (EDTA), diethylene triamine penta acetate (DTPA), ethylene diamine disuccinate (EDDS), and citric acid (CA), are introduced below:

EDTA, a synthetic chelator, is poorly biodegraded in soils although

**\*Corresponding author:** Prof. T. Y. Yeh, National University of Kaohsiung, Department of Civil and Environmental Engineering, Kaohsiung 811, Taiwan, Tel: 886-7-591-9536; Fax: 886-7-591-9376; E-mail: [tyyeh@nuk.edu.tw](mailto:tyyeh@nuk.edu.tw)

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it is effective at complexing metals. Excess amounts of EDTA may leach to groundwater and cause subsurface water contamination. EDDS is an EDTA isomer, and unlike EDTA, it is a biodegradable chelator. EDDS occurs naturally in soil and is easily decomposed into less detrimental byproducts. DTPA is a synthetic ligand that forms stable complexes with most metals. It is been utilized in water treatment to prevent metal precipitates, and can be borrowed for soil metal extraction in order to enhance metal mobility. CA is a weak organic acid, and an intermediate in the CA cycle also known as the tricarboxylic acid cycle (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, for example, metal complexing.

The success of phytoextraction depends on the choice of plant species and metal forms retained in the soil. Factors including soil Cation Exchange Capacity (CEC) and pH, and organic content also influence phytoextraction efficiency [8]. Several methods have been employed to facilitate phytoextraction, including using chelators to enhance metal mobility in soils, and using proper vegetation to translocate metals from the underground tissues to the aerial parts of plants. Large biomass production and accelerated metal uptake rate and translocation into aerial plant parts are critical elements of viable metal phytoextraction.

Having reviewed recent studies (Table 1), our research is, to the best of our knowledge, unprecedented. We investigate four chelators (EDTA, DTPA, EDDS and CA), two metals (Cu and Zn), and four plants (sunflower, Chinese cabbage, cattail, and reed) in high and low organic content soil in order to compare phytoextraction efficiency. The objectives of this study are to investigate the phytoextraction

efficiency, including root uptake and aerial transportation assisted by EDTA, DTPA, EDDS, and CA, in sunflower, Chinese cabbage, cattail, and reed.

## Materials and Methods

### Organic matter content analysis

Soil samples were collected from local farmland and a compost site (22°73'N, 120°28'E), representing low and high organic soil, respectively. 250 g of soil was air dried overnight, placed in an oven at 103°C, and then weighed. The dried soil was then placed in a 550°C oven for further organic content measurement. The organic content is defined as the weight difference between 103°C and 550°C, which represent the inorganic and organic portions of the soil samples.

### Total metal content and sequential extraction analysis

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

Collected soil was artificially spiked with CuSO<sub>4</sub> and ZnCl<sub>2</sub>, and mixed well, and air dried for 5 days to mimic the local contaminated levels, which were 1,000 and 8,000mg/kg, for Cu and Zn, respectively, which is higher than in most current phytoextraction studies [9,10]. Cu and Zn levels were equivalent to 2.5 and 4.0 times that of the current

Plant species	Chelator concentration	Plant uptake concentration (mg/kg)	TF	BCF	Reference
<i>Paulownia t.</i>	EDTA 5mmol/kg	Cu : (570, 46) <sup>a</sup> Zn :(750, 149) Pb :(750, 149)	Cu: 0.08 Zn :0.2 Pb: 0.1	Cu: 0.27 Zn :0.16 Pb: 0.06	Doumett et al. (2008)[24]
<i>Cynara cardunculus</i>	EDDS 10 mmol/kg EDTA 10 mmol/kg	Pb: EDDS (4165, 310) <sup>a</sup> EDTA (6695, 1332)	EDDS: 0.02 EDTA: 0.20	EDDS: 0.83 EDTA: 1.34	Epelde et al. (2008)[21]
<i>Sedum alfredii</i>	CA 5 mmol/kg EDTA 5 mmol/kg	Cu: CA (32, 10, 11, 11) <sup>b</sup> EDTA (25, 12, 12, 12) Pb: CA (39, 18, 18, 18) EDTA (68, 39, 43, 40) Zn: CA (680, 2000, 1950, 1930) EDTA (380, 2030, 2000, 2030)	Cu: CA (0.03) EDTA (0.57) Zn: CA (2.88) EDTA (5.34) Pb: CA (0.45) EDTA (0.61)	Cu: CA (0.03) EDTA (0.57) Zn: CA (12.6) EDTA (10.8) Pb: CA (0.29) EDTA (0.7)	Sun et al. (2009)[25]
<i>Vetiveria zizanioides</i>	EDTA 0.8 mmol/kg	Zn (150, 82) <sup>a</sup>	Zn: 0.55	Zn: 0.85	Lin et al. (2009)[4]
<i>Vetiveria zizanioides</i>	EDDS 5 mmol/kg	Cu: EDDS (1818, 1459, 361) <sup>c</sup> CA (926, 56, 15) EDTA (2080, 954, 86) Zn: EDDS (16388, 12412, 12036) CA (14444, 12420, 10821) EDTA (12899, 9891, 12552)	Cu:EDDS (0.51) CA (0.04) EDTA (0.25) Zn: EDDS (0.7) CA (0.82) EDTA (0.86)	Cu:EDDS(1.97) CA (0.88) EDTA (2.22) Zn: EDDS(1.95) CA (1.67) EDTA (1.5)	Zaier H et al. (2010)[26]
<i>Vetiveria zizanioides</i>	CA 5 mmol/kg EDTA 5 mmol/kg	Pb: EDDS (4343, 280, 197) CA (4914, 388, 103) EDTA (4632, 1878, 340)	Pb: EDDS (0.06) CA (0.05) EDTA (0.24)	Pb: EDDS(0.63) CA (0.67) EDTA (0.58)	

**Table 1:** Plant uptake and transportation in recent study.

Soil properties	Low organic soil	High organic soil
pH (H <sub>2</sub> O)	6.58 ± 0.44	6.32 ± 0.08
Organic matter (%)	4.16 ± 0.27	25.49 ± 0.48
Clay (%) < 2µm	15.19	0.14
Silt (%) 2-50µm	78.69	7.17
Sand (%) 50-2000µm	6.12	92.69
Background Cu concentration (mg/kg)	23.26 ± 4.72	23.57 ± 3.51
Background Zn concentration (mg/kg)	121.55 ± 6.34	125.34 ± 26.71

**Table 2:** General properties and background metal concentrations in high and low organic soils.

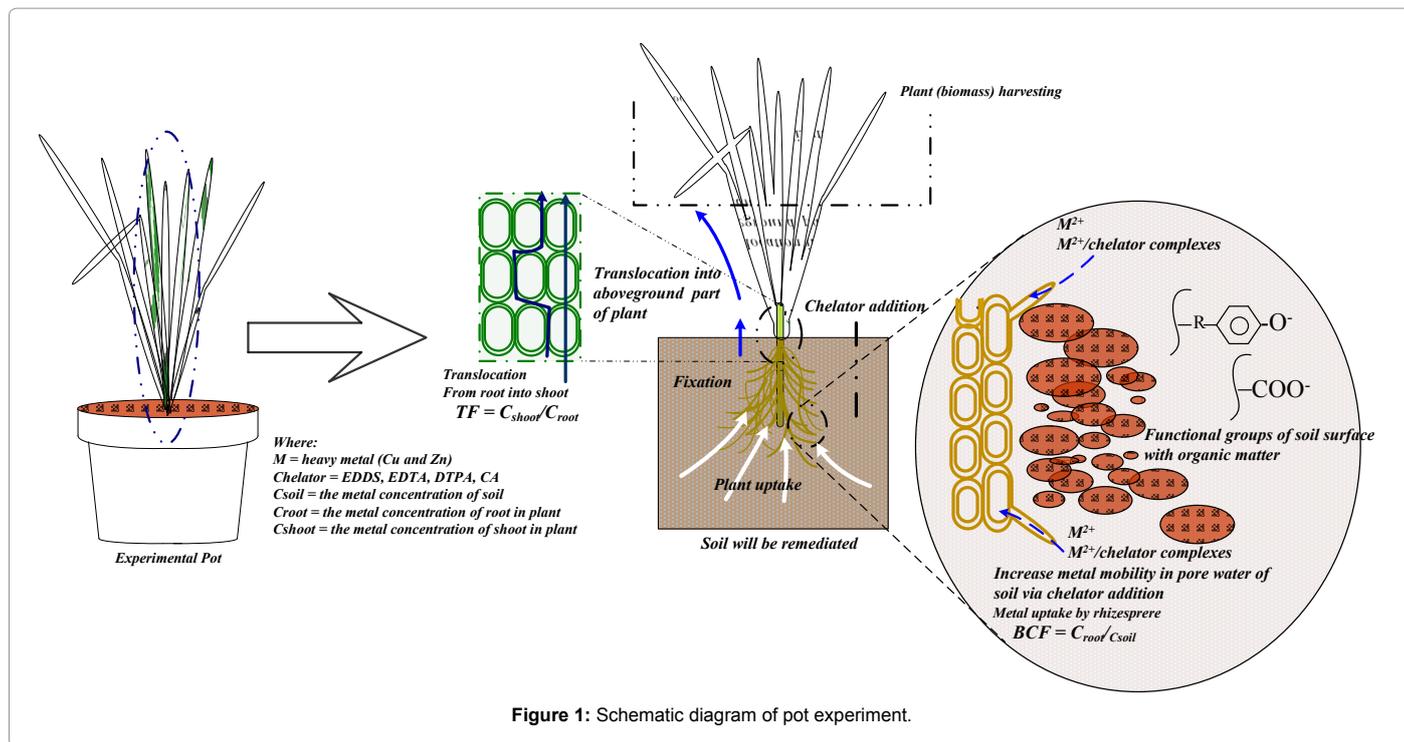


Figure 1: Schematic diagram of pot experiment.

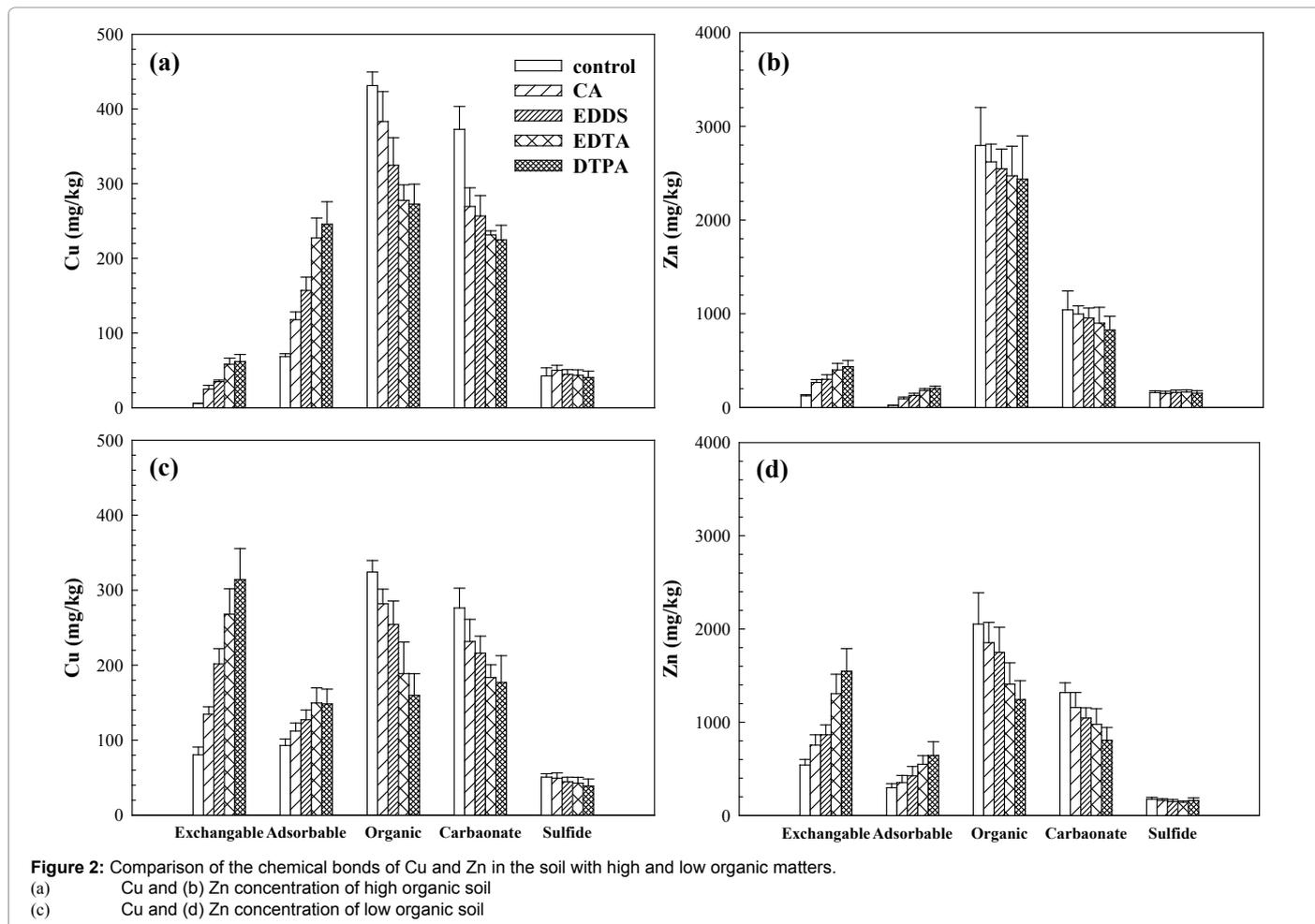


Figure 2: Comparison of the chemical bonds of Cu and Zn in the soil with high and low organic matters.  
 (a) Cu and (b) Zn concentration of high organic soil  
 (c) Cu and (d) Zn concentration of low organic soil

soil pollution control standard in Taiwan, respectively, and required remediation. Plants were transferred to pots 17cm deep and 18cm in diameter, which were filled with 1.5kg (14cm) of air dried soil. 5mmol/kg of the same concentrations of EDTA, EDDS, and CA with were applied to each pot at once initially. The fractionation of Cu and Zn was measured by a sequential extraction technique in which soil samples were placed in a plastic bottle and shaken to ensure proper mixing overnight, then subjected to a five-step serial extraction procedure. The sequential chemical extraction procedure used in this study includes a series of reagents which are represented as exchangeable (1 M KNO<sub>3</sub>), adsorbable bound (0.5 M KF), organic bound (0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>), carbonate bound (0.1M EDTA), and sulfide (6 M HNO<sub>3</sub>) forms, respectively [11].

### Metal content analysis

After being harvested, the plants were careful washed and air dried, and then dried in a 103°C oven. The dried plant matter was separated into root, stem, and leaf samples for metal accumulation assessment. 0.5 g of pretreated plant matter was digested in a solution of 11:1 HNO<sub>3</sub>: HCl via a microwave digestion apparatus (Mars 230/60, CEM Corporation), and diluted in 100ml of de-ionized water. 0.2g of air dried soil adding *aqua regia* rendering for microwave digestion and 2.5 g of dried for sequential extraction experiments. Metal analyses were conducted by means of atomic absorption spectrophotometry (AAS, Perkin Elmer).

### Data and Statistical analysis

Data were evaluated relative to the control in order to investigate the statistical differences. Plant metal concentration was recorded as mg of metal per kilogram of dry biomass. Bioconcentration factor (BCF, *Croot/Csoil*) was calculated as the metal concentration in plant matter divided by the heavy metal concentration in the soil for pot experiments, respectively. Translocation factor (TF, *Cshoot/Croot*) was depicted as the ratio of concentration of metal in shoots to its concentration in roots. It was calculated by dividing the metal concentration in shoot material by the metal concentration in root material. Schematic diagrams of the pot experiments are shown in Figure 1. Statistical significance was assessed using the mean comparison test. Differences were determined by Student's *t* test. A level of  $p < 0.05$  was considered to be a statistically significant difference among all comparisons. All statistical analysis was performed with Microsoft Office EXCEL 2007.

## Results and Discussion

### Cu Sequential extraction results of soil

For high organic soil, total Cu and Zn concentration was  $847.22 \pm 27.57$  mg/kg, and  $4486.86 \pm 327.26$  mg/kg, respectively, while for low organic soil, total Cu and Zn concentration was  $8872.63 \pm 61.34$  mg/kg and  $4393.25 \pm 497.09$  mg/kg, respectively. The sequential results are shown in Figure 2. For control, low organic and high organic soil had loosely retained 8 and 21%, Cu, respectively. This fact implied that daily watering could leach more Cu from low organic soil due to the lower negative adsorption site and prevention of free cation adsorption. Chelator was successfully transfer stable absorbed metal fractions (i.e., organically bound, carbonate bound and sulfide bound) forms to loosely bound fractions (i.e., exchangeable and adsorbable bound) forms. For high organic soil, EDTA, DTPA, EDDS, and CA increased loosely bound Cu to 34, 36, 24, and 17%, respectively, while for low organic soil, EDTA, DTPA, EDDS, and CA increased loosely bound Cu to 50, 55, 39, and 31%, respectively. The increased loosely bound Cu amounts corresponded to DTPA > EDTA > EDDS > CA, in descending

order, which indicated that DTPA was the most effective chelator at transforming stable absorbed Cu to loosely bound Cu. The reason for this is the stability constant commonly expressed as log *K*. The higher the stability constant indicated, 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 effective Cu complexing enhancement chelator, while CA was the least effective [2,12-14].

### Zn Sequential extraction results of soil

For high organic soil, initial stable retained and loosely bound Zn amounts were 96 and 4%, respectively, while for low organic soil, initial stable absorbed and loosely bound Zn amounts were 81 and 19%, respectively. In high organic soil EDTA, DTPA, EDDS, and CA increased loosely bound Zn to 14, 16, 11, and 9%, respectively, while in low organic soil EDTA, DTPA, EDDS, and CA increased loosely bound Zn to 50, 55, 39, and 31%, respectively. The increased loosely bound Zn amounts corresponded to DTPA > EDTA > EDDS > CA in descending order. The stability constants (log *K*) for DTPA-Zn, EDTA-Zn, EDDS-Zn and CA-Zn were 18.3, 16.5, 13.5 and 6.06, respectively [10,12-14].

For high organic soil, EDTA, DTPA, EDDS, and CA increase loosely bound Cu to 34, 36, 24, and 17%, respectively while for low organic soil EDTA, DTPA, EDDS, and CA increase loosely bound Cu to 50, 55, 39, and 31%, respectively. The increased loosely bound Cu amounts was in the descending sequence DTPA > EDTA > EDDS > CA which indicated that DTPA was the most effective chelator to transform stable adsorped Cu to loosely bound Cu. The reason mainly related the stability constant (log *K*). The higher stability constant implied the higher complex ability.

### Growth and toxicity symptoms in plants

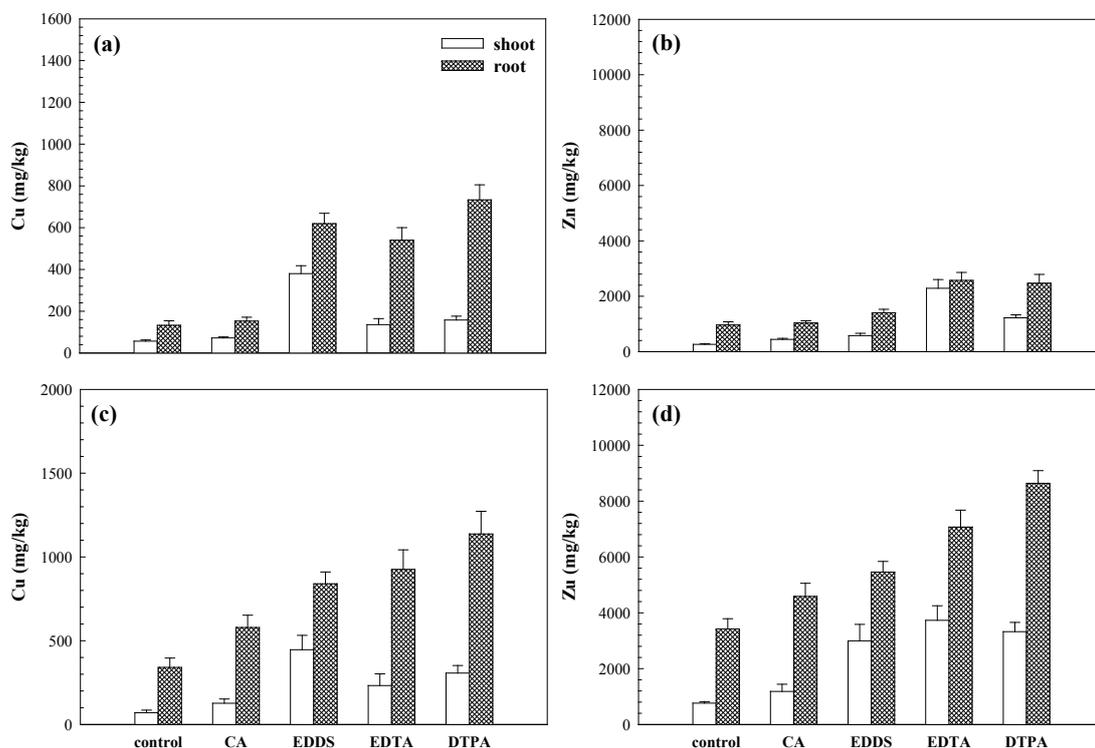
Regardless of the soil's organic contents, plant propagation occurred in descending order: cattail > reed > sunflower > Chinese cabbage. In terms of the duration between the plant wilting and death, plants placed in decreasing order: Cattail > reed > sunflower > Chinese cabbage. Chelators were demonstrated to enhance plant metal uptake, however adverse growth effects were also observed. Biodegradable chelators EDDS and CA had less negative effects on the plants than synthetic chelators DTPA and EDTA. EDTA in particular demonstrated the most unpleasant toxic effect. Toxicity symptoms included yellowing leaves, wilting and death. Cattail and reed showed the highest metal pollution endurance, in terms of total soil metal concentration, while sunflowers show the lowest metal sustainability. Chinese cabbage showed the lowest metal resistance toward two tested metals: Cu and Zn.

In a previous study the phytotoxicity of EDDS and EDTA was investigated, and visible symptoms, such as necrosis and chlorosis, were detected under a concentration of 3.125 mmol/kg of EDDS, and a concentration of 12.5 mmol/kg of EDTA [15]. CA is carboxylic acid exuded by plant roots, 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 these organic acids may affect heavy metal adsorption, solubility, and mobility [16].

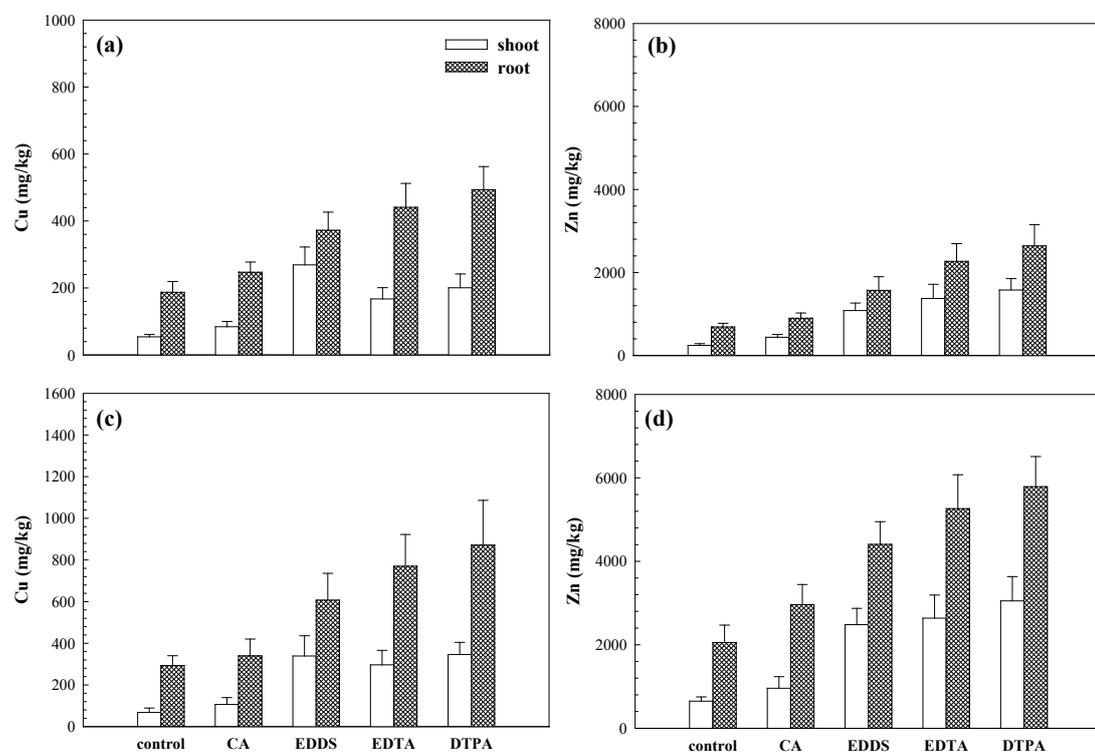
### Metal uptake and translocation

#### Sunflower

**High soil organic content:** Metal uptake results of the rhizosphere and aerial parts of sunflowers are shown in Figure 3. Chelator induced



**Figure 3:** Comparison of Cu and Zn accumulation by sunflower planted in the soil with high and low organic matters  
 (a) Cu and (b) Zn accumulated by sunflower planted in high organic soil.  
 (c) Cu and (d) Zn accumulated by sunflower planted in low organic soil.



**Figure 4:** Comparison of Cu and Zn accumulation by Chinese cabbage planted in the soil with high and low organic matters.  
 (a) Cu and (b) Zn accumulated by Chinese cabbage planted in high organic soil.  
 (c) Cu and (d) Zn accumulated by Chinese cabbage planted in low organic soil.

detectable metal uptake enhancement occurs, particularly for energy plants sunflower. For high organic soil, the highest concentration was found in the root areas. For control, Cu accumulation levels in the aerial and root parts were  $56.68 \pm 5.96$  and  $132.44 \pm 21.74$  mg/kg, respectively, while Zn accumulation in the aerial and root portions were  $263.74 \pm 26.13$  and  $963.77 \pm 113.77$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 2.39 and 4.08, respectively, while Zn levels increased by multiples of 8.67 and 2.67, respectively. DTPA increased Cu accumulation by multiples of 2.79 and 5.53, respectively, while Zn accumulation increased by multiples of 4.62 and 2.56, respectively. EDDS increased Cu accumulation by multiples of 6.70 and 4.68, respectively, while Zn accumulation increased by multiples of 2.19 and 1.45, respectively. CA enhanced Cu levels by multiples of 1.27 and 1.16, respectively, while Zn levels increased by multiples of 1.67 and 1.08, respectively. Cu aerial metal levels were increased, in descending order, by EDDS > DTPA > EDTA > CA, while rhizosphere metal levels increased in descending sequence. Zn aerial metal levels were increased, in comparison to the amounts of metal uptake possessed, in descending order, by EDTA > DTPA > EDDS > CA, while rhizosphere metal levels were increased, in descending order, by EDTA > DTPA > EDDS > CA.

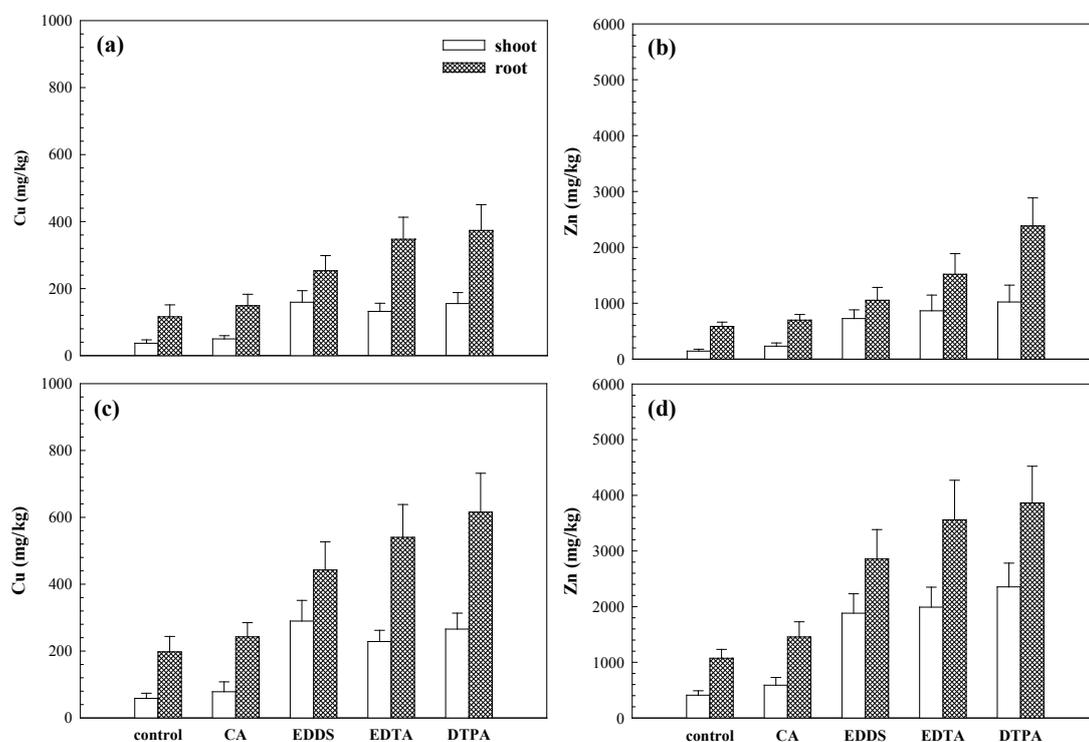
**Low soil organic content:** Low organic soil metal uptake results are shown in Figure 3c and d. Cu accumulation levels in aerial and rhizosphere plant parts were  $70.27 \pm 15.49$  and  $340.93 \pm 55.61$  mg/kg, respectively, while Zn accumulation in aerial and root portions were  $65.24 \pm 52.20$  and  $3420.67 \pm 367.56$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 3.3 and 2.71, respectively, while Zn levels increased by multiples of 4.88 and 2.07, respectively. DTPA increased Cu accumulation by multiples of 4.36

and 3.33, respectively, while Zn increased by multiples of 3.91 and 1.60, respectively. EDDS increased Cu accumulation by multiples of 6.35 and 2.46, respectively, while Zn increased by multiples of 3.91 and 1.60, respectively. CA enhanced Cu levels increased by multiples of 1.81 and 1.70, respectively, while Zn levels increased by multiples of 1.55 and 1.34, respectively.

#### Chinese cabbage

**High soil organic content:** Metal uptake results are shown in Figure 4. Most metal accumulated in the rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels in aerial and root plant parts were  $554.06 \pm 6.92$  and  $186.88 \pm 32.16$  mg/kg, respectively, while Zn accumulation in aerial and root portions were  $243.25 \pm 41.62$  and  $686.46 \pm 89.67$  mg/kg, respectively. Relative to control, EDTA increased Cu accumulation by multiples of 3.09 and 2.36, respectively, while Zn levels increased by multiples of 5.65 and 3.30, respectively. DTPA increased Cu accumulation by multiples of 3.71 and 2.64, respectively, while Zn increased by multiples of 6.48 and 3.85, respectively. EDDS increased Cu accumulation by multiples of 4.97 and 1.99, respectively, while Zn increased by multiples of 4.45 and 2.29, respectively. CA enhanced Cu levels by multiples of 1.56 and 1.32, respectively, while Zn levels increased by multiples of 1.80 and 1.31, respectively. Cu uptake enhancement was increased, in descending order, by EDDS > DTPA > EDTA > CA. The rhizosphere Cu accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA, while root area Zn accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA.

**Low soil organic content:** Metal uptake results of root and aerial parts are shown in Figure 4c and 4d. Most metal accumulated in the



**Figure 5:** Comparison of Cu and Zn accumulation by cattail planted in the soil with high and low organic matters. (a) Cu and (b) Zn accumulated by cattail planted in high organic soil (c) Cu and (d) Zn accumulated by cattail planted in low organic soil

rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels of aerial and root plant parts were  $67.83 \pm 21.29$  and  $292.82 \pm 47.33$  mg/kg, respectively, while Zn accumulation of aerial and root portions were  $648.16 \pm 103.12$  and  $2053.17 \pm 418.07$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 4.37 and 2.63, respectively, while Zn levels increased by multiples of 4.07 and 2.56, respectively. DTPA increased Cu accumulation by multiples of 5.10 and 2.98, respectively, while Zn increased by multiples of 4.72 and 2.82, respectively. EDDS increased Cu accumulation by multiples of 4.97 and 1.99, respectively, while Zn increased by multiples of 4.45 and 2.29 folds, respectively. CA enhanced Cu levels by multiples of 1.57 and 1.16, respectively, while Zn levels increased by multiples of 1.48 and 1.44, respectively. The Chelators transferred the stable retained metal to loosely bound fractions, which enhanced plant metal uptake. Cu uptake enhancement was increased, in descending order, by chelators DTPA > EDDS > EDTA > CA. Rhizosphere Cu accumulation was enhanced, in decreasing order, by DTPA > EDTA > EDDS > CA, while root area Zn accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA.

The energy plant maize could yield 33,000 to 40,000KWh of renewable energy (electrical and thermal) per hectare per year, which, by substitution of fossil fuel energy, would imply a reduction of up to  $21 \times 10^3$  kg/ha<sup>-1</sup>/year<sup>-1</sup> of CO<sub>2</sub>, if used as a substitute for a coal fed power plant. The authors also suggest that sunflowers could potentially serve as similar alternative crops, for similar purposes [17].

#### Cattail

**High soil organic content:** Metal uptake results of root and aerial parts are shown in Figure 5. Most metal accumulated in the rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels of aerial and root plant parts were  $36.75 \pm 10.32$  and  $115.91 \pm 35.46$  mg/kg, respectively, while Zn accumulation of aerial and rhizosphere portions was  $143.62 \pm 33.57$  and  $585.35 \pm 76.24$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 3.59 and 3.00, respectively, while Zn levels increased by multiples of 6.01 and 2.59, respectively. DTPA increased Cu accumulation by multiples of 4.23 and 3.22, respectively, while Zn increased by multiples of 7.11 and 4.07, respectively. EDDS increased Cu accumulation by multiples of 4.33 and 2.19, respectively, while Zn increased by multiples of 5.05 and 1.80, respectively. CA enhanced Cu levels by multiples of 1.36 and 1.39, respectively, while Zn levels increased by multiples of 1.61 and 1.19, respectively. Cu uptake enhancement was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA. The rhizosphere Cu accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA, while root area Zn accumulation was enhanced in descending order, by DTPA > EDTA > EDDS > CA.

**Low soil organic content:** Metal uptake results of root and aerial parts are shown in Figures 5c and 5d. Most metal accumulated in the rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels of aerial and root plant parts were  $58.59 \pm 15.23$  and  $197.67 \pm 45.96$  mg/kg, respectively, while Zn accumulation of aerial and root portions were  $409.36 \pm 79.29$  and  $1072.83 \pm 159.48$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 1.44 and 1.36, respectively, while Zn levels increased by multiples of 4.86 and 3.32, respectively. DTPA increased Cu accumulation by multiples of 4.86 and 3.32, respectively, while Zn increased by multiples of 5.75 and 3.60, respectively. EDDS increased Cu accumulation by multiples of 1.44 and 1.36, respectively, while Zn increased by multiples of 1.44 and 1.36, respectively. CA

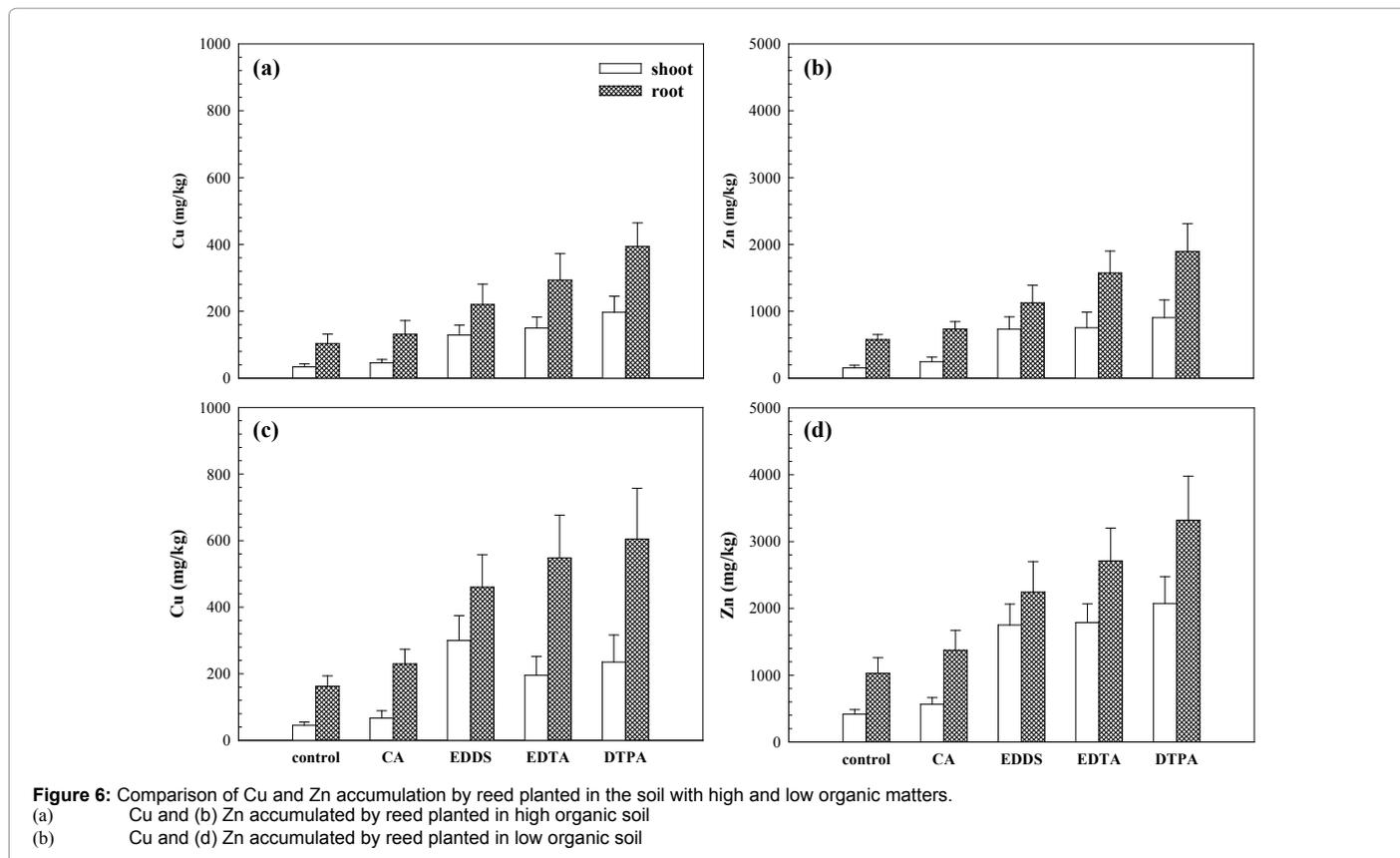
enhanced Cu levels by multiples of 1.34 and 1.23, respectively, while Zn levels increased by multiples of 1.44 and 1.36, respectively. Chelators transferred the stable retained metal to loosely bound fractions, which enhanced plant metal uptake. Cu uptake was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA. The rhizosphere Cu accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA, while root area Zn accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA.

#### Reed

**High soil organic content:** Metal uptake results of root and aerial parts of sunflowers are shown in Figure 6. Most metal accumulated in the rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels of aerial and root plant parts were  $33.82 \pm 9.12$  and  $103.23 \pm 28.46$  mg/kg, respectively, while Zn accumulation of aerial and root portions were  $153.72 \pm 38.57$  and  $575.97 \pm 79.24$  mg/kg, respectively. Relative to the control, EDTA increased Cu accumulation by multiples of 4.43 and 2.84, respectively, while Zn levels increased by multiples of 4.91 and 2.73, respectively. DTPA increased Cu accumulation by multiples of 5.82 and 3.79, respectively, while Zn increased by multiples of 5.89 and 3.29, respectively. EDDS increased Cu accumulation by multiples of 3.81 and 2.13, respectively, while Zn increased by multiples of 4.76 and 1.95, respectively. CA enhanced Cu levels by multiples of 1.35 and 1.27, respectively, while Zn levels increased by multiples of 1.58 and 1.27, respectively. Cu uptake was in enhanced, in descending order, by chelators DTPA > EDTA > EDDS > CA. The rhizosphere Cu accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA, while root area Zn accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA.

**Low soil organic content:** Metal uptake results of root and aerial parts of reed are shown in Figure 6c and 6d. Most metal accumulated in the rhizosphere, and there was a better uptake of Zn than of Cu. For control, Cu accumulation levels of aerial and root plant parts were  $45.081 \pm 9.87$  and  $162.36 \pm 31.53$  mg/kg, respectively, while Zn accumulation of aerial and root parts were  $415.81 \pm 69.08$  and  $1028.56 \pm 232.46$ , respectively. Relative to the control, EDTA increased Cu accumulation of aerial and root plant parts by multiples of 4.34 and 3.37, respectively, while Zn levels increased by multiples of 4.30 and 2.64, respectively. DTPA increased Cu accumulation by multiples of 5.22 and 3.72, respectively, while Zn increased by multiples of 4.99 and 3.23, respectively. EDDS increased Cu accumulation by multiples of 6.66 and 2.83, respectively, while Zn increased by multiples of 4.21 and 2.18, respectively. CA enhanced Cu levels by multiples of 1.48 and 1.41, respectively, while Zn levels increased by multiples of 1.35 and 1.34, respectively. The chelators attached soil metal to soluble metal-complexes, which are mobile in the soil solution. They then transferred the stable retained metal to loosely bound fractions, which enhanced plant metal uptake, as demonstrated in this study. Cu uptake was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA. The rhizosphere Cu accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA, while root areas Zn accumulation was enhanced, in descending order, by DTPA > EDTA > EDDS > CA.

The metal-complex translocation mechanism, as indicated, is passive apoplastic transportation [18]. In this study, the addition of chelators generally prevented metal precipitation and facilitated the formation of metal complex compounds. The apoplast, in plant cells, is the free diffusional space outside the plasma membrane, which has



a high concentration of carboxylic groups, which can act as effective cation exchangers. The negatively charged chelator complexes were prevented from being bound to the cell walls of the roots, and allowed complexes to enter into the cells. Metal chelator complexes were subsequently translocated to the aerial part of plant *via* the passive apoplastic pathway.

The application of EDTA, DTPA, EDDS, and CA resulted in shoot concentrations of Cu and Zn which were 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 [19]. EDDS degraded relatively quickly, compared to EDTA and DTPA, and therefore should schedule several applications in order to reach the remediation objective. EDDS maximized shoot metal uptake, also reducing the risk of metal leaching into groundwater. Our research has demonstrated that both EDTA and DTPA significantly increase the amount of copper accumulated in the above ground parts and rhizospheres of plants, while DTPA is less effective, relative to EDTA, at enhancing copper uptake. Nevertheless, neither EDTA nor DTPA had serious negative effects on the growth of plants in their study [20].

### Bioconcentration factor (BCF) and translocation factor (TF)

#### Sunflower

**High soil organic content:** The results of BCF and TF of sunflowers in high organic soil are shown Figure 7. BCF and TF were employed to evaluate the soil metal transfer to rhizosphere and further uptake transferred to the aerial parts of the plants. BCF indicated the plant soil metal adsorption levels, while TF referred to the amounts of metal uptake and further transfer from root zones to aerial parts of the plants.

For control, Cu and Zn BCF values were  $0.17 \pm 0.03$  and  $0.21 \pm 0.03$ , respectively, while Cu and Zn TF values were  $0.43 \pm 0.04$  and  $0.28 \pm 0.05$ , respectively. BCF results indicated that EDTA, DTPA, and EDDS yielded relatively effective results in terms of rhizosphere metal adsorption, while CA had the lowest stability constant, demonstrating less effective root metal retention ability.

**Low soil organic content:** The BCF and TF results of sunflowers in low organic soil are shown Figure 8. For control, Cu and Zn BCF values were  $0.39 \pm 0.06$  and  $0.78 \pm 0.08$ , respectively, while Cu and Zn TF values were  $0.21 \pm 0.03$  and  $0.22 \pm 0.02$ , respectively. EDTA increased BCF values by multiples of 2.72 and 2.06, relative to the control. EDDS increased BCF values by multiples of 2.46 and 1.59, relative to the control. DTPA increased BCF values by multiples of 3.33 and 2.53, relative to the control.

EDTA, DTPA and EDDS all demonstrated significant metal transfer, while CA was less effective. TF values for CA were 0.56 and 0.01. EDDS increased by multiples of 2.52 and 2.50, relative to the control. The primary induction mechanism of metal transfer from the rhizosphere to aerial parts of the plants was the metal-chelator complexes, which are negatively charged, able to penetrate the casparian strip and enter into the plant, inducing increased metal transfer. This is 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 continue along the cell wall. It influences the metal-chelator complex translocation in plants [9].

### Chinese cabbage

**High soil organic content:** The BCF results of Chinese cabbage in high organic soil are shown in Figure 7. For control, BCF values of Cu and Zn were  $0.21 \pm 0.02$  and  $0.16 \pm 0.01$ , respectively. Rhizosphere accumulation was enhanced, in decreasing order, by DTPA > EDTA > EDDS > CA. BCF results indicated that EDTA, DTPA, and EDDS performed relatively effectively in terms of rhizosphere metal adsorption, while CA had the lowest stability constant, demonstrating less effective root metal retention ability.

For control, the TF values of Cu and Zn were  $0.28 \pm 0.04$  and  $0.35 \pm 0.05$ , respectively, which are shown in Figure 7c and d. EDDS demonstrated the most prominent metal translocation to upper parts of plant, with TF values increased by multiples of 0.73 and 0.70, relative to the control. Chelators translocation was enhanced, in descending order, by EDDS > DTPA > EDTA > CA.

**Low soil organic content:** The Cu and Zn BCF values of Chinese cabbage in high organic soils are shown in Figure 7. For control, Cu and Zn BCF values were  $0.14 \pm 0.01$  and  $0.13 \pm 0.02$ , respectively. These BCF values were higher than those of high organic soil, which were  $0.21 \pm 0.02$  and  $0.16 \pm 0.01$ , respectively. Low organic soil has more loosely bound metal, leading to more soluble metal, inducing the lower BCF. EDTA, DTPA and EDDS all demonstrated significant metal transfer, while CA was less effective. For control, TF values were  $0.24 \pm 0.02$  and  $0.27 \pm 0.01$  for Cu and Zn, respectively, and  $0.32 \pm 0.01$  and  $0.35 \pm 0.04$  for CA. EDDS showed the most effective metal translocation, where the TF values were 0.58 and 0.59 for Cu and Zn, respectively. The primary mechanism inducing metal transfer from the rhizosphere

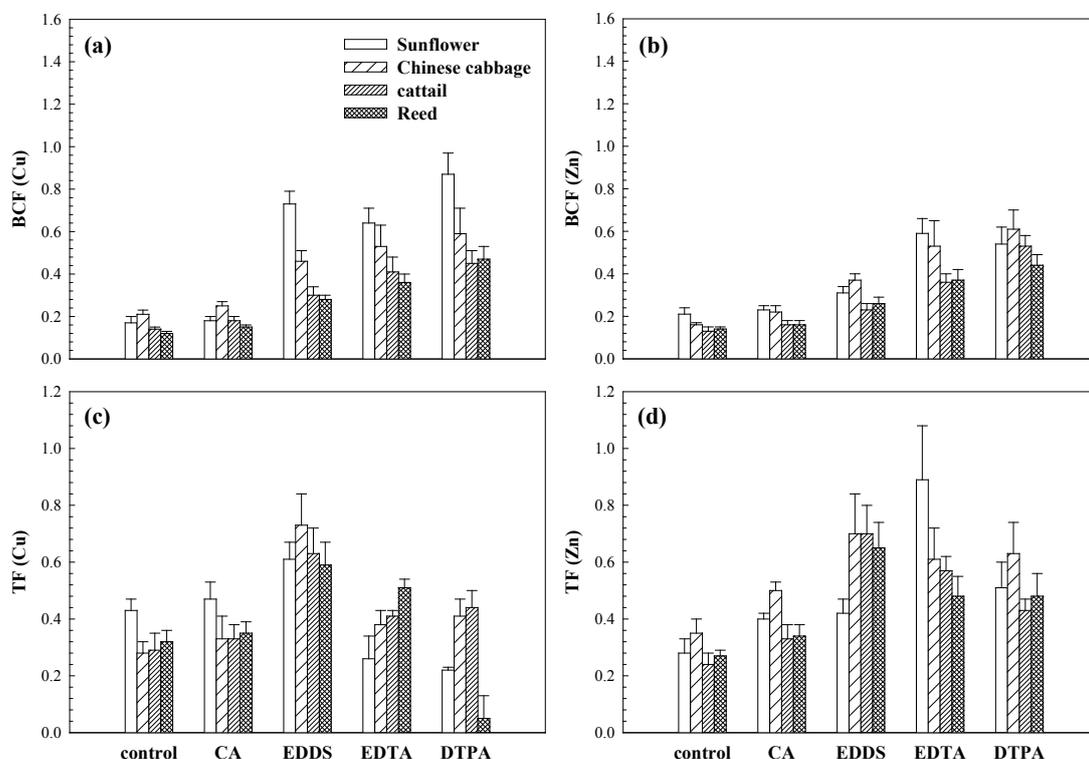
to aerial parts of the plant was the metal-chelator complexes, which are negatively charged, able to penetrate the casprian strip, and enter into the plant, inducing increased metal transfer, indicated by the TF values [9].

### Cattail

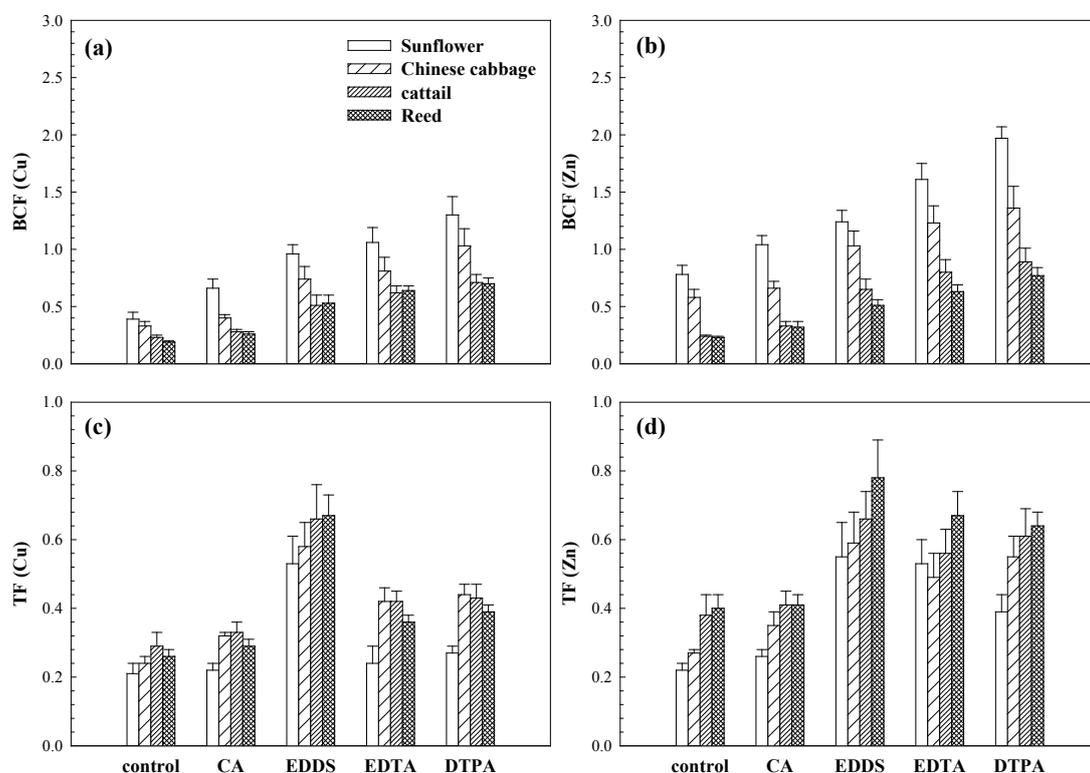
**High soil organic content:** The Cu and Zn BCF values of cattails in high organic soil are shown in Figure 7. For control, Cu and Zn BCF values were  $0.14 \pm 0.01$  and  $0.13 \pm 0.02$ , respectively. Rhizosphere accumulation was enhanced, in decreasing order, by DTPA > EDTA > EDDS > CA. BCF results indicated that EDTA, DTPA, and EDDS performed relatively effectively in terms of rhizosphere metal adsorption, while CA, with the lowest stability constant, demonstrated less effective root metal retain ability.

Cu and Zn TF values were  $0.63 \pm 0.09$  and  $0.70 \pm 0.10$  after EDDS application, respectively, while the Cu and Zn TF values of the control were  $0.29 \pm 0.06$  and  $0.24 \pm 0.04$ , respectively. EDDS demonstrated the most prominent metal translocation to upper parts of the plant: the TF values were increased by multiples of 0.73 and 0.70, relative to the control. Translocation was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA.

**Low soil organic content:** The results of Cu and Zn BCF and TF values of cattails in low organic soils are shown in Figure 8. For control, Cu and Zn BCF values were  $0.23 \pm 0.02$  and  $0.24 \pm 0.01$ , respectively, while the Cu and Zn BCF values of cattail after EDDS application were  $0.51 \pm 0.09$  and  $0.65 \pm 0.09$ , respectively. The Cu and Zn TF values of the control were  $0.29 \pm 0.04$  and  $0.38 \pm 0.06$ , respectively, while Cu and Zn TF values after EDDS application were  $0.66 \pm 0.10$  and  $0.66$



**Figure 7:** Comparison of BCF and TF values of sunflower, Chinese cabbage, cattail and reed in high organic soil. (a) Cu and (b) Zn BCF values of plants (c) Cu and (d) Zn TF values of plants



**Figure 8:** Comparison of BCF and TF values of sunflower, Chinese cabbage, cattail and reed in low organic soil.  
 (a) Cu and (b) Zn BCF values of plants  
 (c) Cu and (d) Zn TF values of plants

$\pm 0.08$ , respectively. EDDS demonstrated the most effective metal translocation to upper parts of the plant in the low organic soil, and the TF values of Cu and Zn were increased by multiples of 1.53 and 1.16, respectively, relative to the control. Translocation was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA.

#### Reed

**High soil organic content:** BCF and TF results are shown in Figure 7. BCF results indicated that EDTA, DTPA, and EDDS performed relatively effectively in terms of rhizosphere metal adsorption, while CA, with the lowest stability constant, demonstrated less effective root metal retention ability. For control, BCF values were  $0.12 \pm 0.01$  and  $0.14 \pm 0.02$ , respectively. Rhizosphere accumulation was enhanced, in decreasing order, by DTPA > EDTA > EDDS > CA.

For control, TF values of Cu and Zn in reed were  $0.32 \pm 0.04$  and  $0.27 \pm 0.02$ , respectively. EDDS demonstrated the most effective metal translocation to upper parts of the plant, and the TF values were increased by multiples of 1.84 and 2.40, relative to the control condition. Translocation was enhanced, in descending order, by chelators EDDS > DTPA > EDTA > CA.

**Low soil organic content:** BCF results indicated that EDTA, DTPA, and EDDS performed relatively effectively in terms of rhizosphere metal adsorption, while CA, with the lowest stability constant, demonstrated less effective root metal retention ability. For control, BCF values were  $0.19 \pm 0.01$  and  $0.23 \pm 0.01$ . Rhizosphere accumulation was enhanced, in decreasing order, by DTPA > EDTA > EDDS > CA.

For control, Cu and Zn TF values were  $0.26 \pm 0.02$  and  $0.40$

$\pm 0.04$ , respectively. EDDS demonstrated the most effective metal translocation to upper parts of the plant. The TF values of reed in the EDDS condition were  $0.67 \pm 0.06$  and  $0.78 \pm 0.11$ , respectively. Translocation was enhanced, in descending order, by EDDS > DTPA > EDTA > CA.

In summary, metal accumulation in different parts of the plants decreased in descending order: roots > stems > leaves. This effect is consistent with other research [15]. Some researchers also found similar facts while investigating the metal accumulation in wetland macrophytes [7].

A higher extraction capability can be predicted on the basis of the stability constant. For instance, the stability constants ( $\log K$ ) of Zn with EDDS, EDTA, DTPA and CA were 13.5, 16.5, 18.3, and 6.1, respectively, while Cu was 18.4, 20.5, 21.2, and 7.6, respectively [2,12-14]. Similar extraction results have been demonstrated by other authors [14]. EDTA was more effective than EDDS in preventing metal precipitation. This result can be predicted by the higher stabilization constant of EDTA metal complexes. The speed of EDDS biodegradation might be a factor in selecting a feasible chelator for phytoextraction enhancement [21].

Biodegradable chelators EDDS and CA performed reliably in this study, while EDTA and DTPA, though they also enhanced metal mobility, were always accompanied by the concern of potential groundwater contamination.

#### Effect of different soil organic contents

A critical, soil organic concentration, has demonstrated as an important factor to influence plant metal uptake and metal

bioavailability. High organic soils have provided more substrate for plant propagation. Great biomass production was induced prominent plant uptake. Low organic content though has less soil nutrients but also has less negative charged functional groups such as carboxylic, phenolic and hydroxyl [22]. These facts might adsorb negative free metal cation and reduce metal mobility leading to less plant uptake while high organic soil has less negative functional groups indicating negative chelator-metal complexes can be easily translocate to aerial parts of plants.

Soil organic matter might influence heavy metal retention in soils *via* the formation of stable complexes. Organic matter added to contaminated soils decreases metal availability to plants [23].

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

Regardless of organic levels, energy plants sunflower and Chinese cabbage demonstrated more prominent metal uptake than wetland macrophytes cattail and reed. Previous researches have demonstrated promising phytoextraction effects using sunflower and Chinese cabbage [4,5]. EDTA, DTPA, and EDDS all demonstrated prominent metal uptake enhancement, while CA results were less promising.

Nevertheless, chelator assisted phytoextraction using sunflower, Chinese cabbage, cattail, and reed, in order to remove Cu and Zn has been demonstrated. We are optimistic about future *in-situ* applications using this green remediation. However, possible chelator-metal complexes contaminating groundwater is a serious concern.

## Conclusion

Sunflower, Chinese cabbage, cattail, and reed have been demonstrated as valid plants for chelator enhanced phytoextraction. Organic content has been demonstrated as a critical factor influencing metal uptake. Organic content has less soil nutrients and has less negatively charged functional groups, such as carboxylic, phenolic and hydroxyl groups. These facts lead to negative free metal cation adsorption, and reduce metal mobility. Recently, sludge from activated sludge processors of sewage treatment plants was intended for reuse 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 matter where the results of this study can be referenced by in-situ operation engineers to manage field real site sludge reuse operations.

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