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Phytoremediation potential of Cd and Zn by wetland plants, *Colocasia esculenta* L. Schott., *Cyperus malaccensis* Lam., and *Typha angustifolia* L. grown in hydroponics

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Abstract

Cadmium and zinc phytoremediation potential of wetland plants, *Colocasia esculenta, Cyperus malaccensis,* and *Typha angustifolia*, was investigated. Plants were grown for 15 days in nutrient solutions containing various concentrations of Cd (0, 5, 10, 20, 50 mg l^{-1}) and Zn (0, 10, 20, 50, 100 mg l^{-1}). *T. angustifolia* was tolerant to both metals as indicated by high RGR when grown in 50 mg l^{-1} Cd and 100 mg l^{-1} Zn solutions. All these plants accumulated more metals in their underground parts and > 100 mg kg^{-1} in their aboveground with TF values < 1. Only *C. esculenta* could be considered a Zn hyperaccumulator because it could concentrate > 10,000 mg kg^{-1} in its aboveground parts with TF > 1. *T. angustifolia* exhibited highest biomass production and highest Cd and Zn uptake, confirming that this plant is a suitable candidate for treating of Cd contaminated soil/sediments.

Key words

Cadmium, *Colocasia esculenta*, *Cyperus malaccensis*, *Typha angustifolia*, Zinc

Introduction

Cadmium accumulation in soil and water is becoming a major environmental problem due to the high toxicity of Cd and its high mobility from soil or water to plants and further to food chain (Tang *et al*., 2009). Mae Tao river basin in Mae Sot district, Tak province, western Thailand is surrounded by agricultural land (rice paddies and fields of soybeans, corn, and sugarcane). Recently, Cd pollution of the paddy fields and implication for public health has been demonstrated in the area (Simmons *et al*., 2005). Paddy soils were contaminated with Cd from the use of irrigation waters from the Mae Tao river basin that receive Cd either via natural runoff and/or uncontrolled discharges from Zn mining (Phaenark *et al*., 2009). High concentrations of Cd has been found in rice grains and people living in this area have high levels of Cd in their urine and suffer from kidney problems due to rice consumption (Simmons *et al*., 2005; Swaddiwudhipong *et al*., 2007). Fish sampled from Mae Tao creek was reported to have Cd concentration within Thailand safe limit for consumption of <2 mg kg^{-1} (Department of Pollution Control, 2004). However, Krissanakriangkrai *et al.* (2009) examined Cd levels in various fish species and reported high Cd level (>0.2 mg kg^{-1} allowable contamination level) (Joint FAO/WHO, 2006) in swamp eel (*Fluta alba*), the most popular fish consumed by the local people of the area.

Wetlands are often considered as sinks for contaminants such as heavy metals. Hence, sediments in Mae Tao river basin were highly likely to be contaminated by Cd and Zn due to anthropogenic sources, natural runoff, or discharges from Zn mine. There is an urgent need for the remediation of Cd-contaminated water and sediments. Several physical and chemical processes have been identified for remediation of contaminated soil and water. Management techniques such as isolation, cleansing and inerting are three general categories of conventional treatment for Cd contaminated soil/sediment (Kashem *et al*., 2008). For heavy metal removal from water, chemical processes such as precipitation, solvent extraction, ion-exchange, adsorption is employed. But these conventional procedures are expensive and frequently inefficient. Therefore, alternative methods of heavy metal removal based on biological process such as phytoremediation has received attention in recent years. Phytoremediation, in which plant hyperaccumulators or accumulators are used to take up pollutants, has become a promising soil/sediment remediation technique (McGrath *et al*., 2002). Bunluesin *et al*. (2004) and Kashem *et al*. (2008) reported phytoremediation potential of Cd and Pb by some wetland plants such as *Typha*, *Colocasia*, *Cyperus*, *Phragmites* grown in hydroponics. Hydroponic study provides potential to examine metal tolerance and magnitude of metal accumulation in plant species with greater precision than soil studies (Kashem *et al*., 2008). Hence, in the present study, common local wetland plant species such as narrow leaf cattail (*Typha angustifolia*), taro (*Colocasia esculenta*) and Malacca galingale (*Cyperus malaccensis*) were selected for phytoremediation of Cd and Zn using hydroponic experiment.

Materials and Methods

Ten surface sediment and water samples from the Mae Tao river basin (average temperature of 25.6 ºC and annual rainfall 1,450.1 mm yr^{-1}) were collected by grab sampler for heavy metal screening. Sediments were oven dried at 65 ºC for 72 hr, then ground into fine powder and sieved through a 2-mm nylon mesh sieve. Water samples were filtered through millipore filter (0.4 μm), acidified with 1% HNO_3 and stored at 10 ºC for further analysis.

Three plant species, *C. esculenta*, *C. malaccensis* and *T. angustifolia* were collected from non-contaminated sites and grown in greenhouse under controlled conditions (27-29 ºC, 15,000 lux, 12/12 hrs light/dark cycle, 70% relative humidity) for 3 months. The plants were transferred into plastic containers filled with 20% Hoagland's solution and acclimatized for another two weeks. Selected healthy uniform plants were then treated with different concentrations of Cd ($CdCl_2$: 5, 10, 20, 50 mg l^{-1}) and Zn ($ZnSO_4$: 10, 20, 50, 100 mg l^{-1}) in 700 ml of Hoagland's solution without aeration. There were 3 replicates (1 plant/replicate) for each treatment. Plants grown in Hoagland's solution without metals served as control. The solutions were changed every five days and plants were harvested on day 15.

For sediment metal analysis, 0.5 g of sediment samples was digested in aqua regia (3:1 $HCl:HNO_3$) using an open tube digestion method (McGrath and Cunliffe, 1985). Plant samples from hydroponic experiment were thoroughly washed with tap water and deionized water, separated into above- and belowground parts, and oven-dried (65 ºC) to a constant weight. After measuring dry weight, the plants were ground into powder and sieved through a 2-mm mesh sieve. The plant samples (1 g each) were digested in 2:1 $HNO_3:HClO_4$ using open tube digestion method (Simmons *et al*., 2003). The concentrations of metals (Cd, Zn) in sediment and plant were determined by flame atomic absorption spectrophotometer (Perkin Elmer 300), using a mixture of air and acetylene as carrier gas.

Bioconcentration factor (BCF) of each plant species was determined by dividing the metal concentration in plant tissue at harvest (mg kg^{-1}) by initial concentration of metal in external solution (mg l^{-1}) (Zayed *et al*., 1998). Translocation factor (TF) was determined by dividing the metal concentration in aboveground part by metal concentration in the belowground part (Baker, 1981). Plant relative growth rates were calculated according to Hunt's equation:

$$RGR = \ln W_2 - \ln W_1 / T_2 - T_1$$

Where RGR is relative growth rate (g g^{-1} d^{-1}), and W_1, T_1 and W_2, T_2 are initial and final dry weights and times for each treatment, respectively (Hunt, 1978). The mean values of dry biomass and metal accumulation were calculated and subjected to the analysis of variance (ANOVA) and least significant difference (LSD) on SPSS for Windows Program. All tests were significant at $P < 0.05$.

Results and Discussion

Water in the Mae Tao river basin contained < 0.05 mg l^{-1} Cd and 10.9 mg l^{-1} Zn. Sediments from the Mae Tao river basin showed high concentration of total Cd (47.8 mg kg^{-1}) but low concentration of Zn (91.2 mg kg^{-1}). The DTPA extractable concentrations of both metals were relatively low (15.2 mg kg^{-1} Cd, 31.4 mg kg^{-1} Zn). These concentrations of metals indicated that sediments were highly contaminated with Cd than Zn. According to the US-EPA's sediment standard, Cd concentration of 1.2 mg kg^{-1} and Zn concentration of 150 mg kg^{-1} represent concentrations which caused 10% adverse biological effects (Long *et al*., 2005). Recent studies reported on Cd concentrations in surface water and sediment of Mae Tao creek (Department of Pollution Control, 2004; Krissanakriangkrai *et al*., 2009). Cd levels in surface water ranged from 0-0.005 mg l^{-1}, which did not excreed Thailand standard level or US-EPA standard criterion (0.01 mg l^{-1}; US-EPA, 1986). Cd concentrations in sediments showed variation from low contamination (≤ 3.5 mg kg^{-1}) to high contamination (≥ 35 mg kg^{-1}). These concentrations highly exceeded the US-EPA sediment quality guidelines for sediment of 0.6 mg kg^{-1} (US-EPA, 1999).

All three species of plants (*C. esculenta, C. malaccensis,* and *T. angustifolia*) exposed to various concentrations of Cd and Zn under hydroponic conditions for 15 days exhibited 100% survival. The relative growth rates (RGR) of *C. malaccensis* decreased significantly when exposed to Cd (10 – 50 mg l^{-1}) (Fig. 1A), while there were no clear trends for RGR of *C. esculenta* as related to initial Cd concentrations. *T. angustifolia* exhibited higher RGR when exposed to higher Cd concentrations indicating their tolerance to Cd. No significant changes in RGR were observed in any of the three species when Zn concentration was increased from 10 to 100 mg l^{-1} (Fig. 1B). The highest RGRs' (0.036 – 0.059 g g^{-1} d^{-1}) were observed in *T. angustifolia*.

High Cd and Zn level was observed in the belowground than in the aboveground parts, with highest Cd accumulation

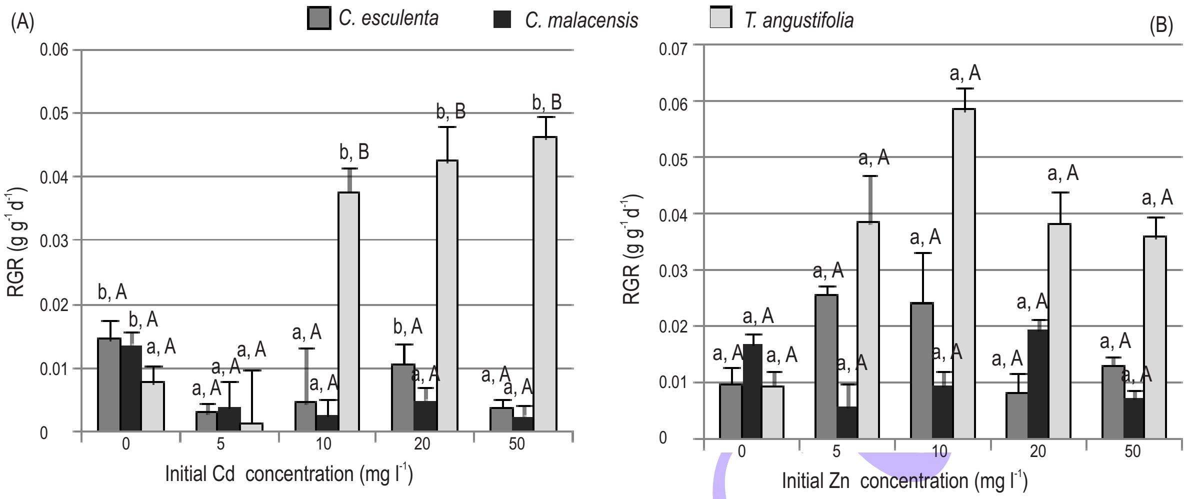

Fig. 1 : Relative growth rates of wetland plants treated with various concentrations of Cd (A) and Zn (B) under hydroponics for 15 days. Data are given as means ± S.D. Small letters indicated significant differences among plant species, and capital letters indicated significant differences among metal concentrations for each plant species. Bars with the same letters were not significantly different ($P < 0.05$, ANOVA; LSD mean comparison test)

Table 1 : Dry biomass, Cd accumulation and uptake, BCF and TF of *C. esculenta*, *C. malaccensis* and *T. angustifolia* grown in hydroponics for 15 days

Plant	Initial Cd conc. (mg l^{-1})	Initial dry biomass (g plant^{-1})	Final dry biomass (g plant^{-1})	Cd accumulation (mg kg^{-1})		Cd uptake (µg plant^{-1})	BCF	TF
				Aboveground	Belowground			
C. esculenta	0	4.2±0.7a,A	5.3±0.2b,A	0.0±0.1a,A	0.0±0.0a,A	0.0	0.0	0.0
	5	3.2±0.9a,A	3.3±1.7a,A	671.7±22.9a,B	3,222.6±89.3a,B	13.0	778.9	0.2
	10	3.4±2.6a,A	3.6±0.3a,A	3,591.8±145.1b,C	5,585.0±80.3a,B	33.0	917.7	0.6
	20	6.5±1.7b,A	7.6±0.2a,B	3,272.6±301.5b,C	6,207.0±115.5a,B	72.0	474.0	0.4
	50	5.3±2.5a,A	5.6±0.2a,A	3,241.3±252.3b,C	15,409.6±420.7b,C	103.8	373.0	0.3
C. malaccensis	0	1.4±0.2a,A	1.8±0.4b,A	0.0±0.0a,A	0.0±0.0a,A	0.0	0.0	0.0
	5	2.8±0.1a,A	3.0±1.2a,A	520.6±11.3a,B	2,819.0±243.7a,B	5.0	667.9	0.2
	10	4.6±1.3a,B	4.8±0.3a,B	2,231.3±219.6b,C	4,221.6±78.3a,C	14.4	645.3	0.5
	20	2.9±0.5a,A	3.1±1.8a,A	2,223.7±231.8b,C	5,613.3±111.13a,C	22.7	391.8	0.4
	50	3.1±0.5a,A	3.2±1.7a,A	2,983±176.9b,C	8,605.5±323.4a,D	36.7	231.8	0.3
T. angustifolia	0	5.8±1.7a,A	6.5±1.8b,A	0.0±0.2a,A	0.0±0.1a,A	0.0	0.0	0.0
	5	6.6±0.5a,A	6.7±0.4a,A	254.0±19.9a,B	1,718.4±71.1a,B	7.3	533.1	0.1
	10	8.3±1.6b,B	14.6±5.5b,B	406.2±15.1a,B	2,773.9±361.3a,BC	46.4	217.8	0.1
	20	9.4±1.5b,B	17.9±0.5b,B	1,557.4±49.8a,C	4,063.4±200.5a,C	100.4	314.6	0.9
	50	9.1±1.9b,B	18.1±2.7b,B	1,601.6±60.4a,C	11,131.5±424.0b,D	230.9	702.2	0.1

Values followed by the same letter did not differ, small letters illustrated differences between plants species ($P < 0.05$, LSD), and capital letters illustrated differences among initial Cd concentrations for each plant species ($P<0.05$, LSD)

(8,605.5 – 15,409.6 mg kg^{-1}) when plants were exposed to 50 mg l^{-1} Cd solution (Table 1). Similarly, all the three plant species also accumulated high level of Cd (> 1,000 mg kg^{-1}) when exposed to 10-50 mg l^{-1} solution (Table 1), indicating their hyperaccumulation capability (Cd > 100 mg kg^{-1}; Reeves and Baker, 2000). However, highest total Cd uptake was found in *T. angustifolia* (230.9 µg plant^{-1}), followed by *C. esculenta* (103.8 µg plant^{-1}) and *C. malaccensis* (36.7 µg plant^{-1}) at 50 mg l^{-1} Cd. Bioconcentration value (BCF) close to 1,000 (917.7) was observed in *C. esculenta* exposed to 10 mg l^{-1} Cd solution. All translocation factor (TF) values for Cd were below 1 indicating low ability of plants to translocate Cd from belowground to aboveground parts (Table 1).

Both aboveground and belowground parts of all the three plant species showed an increase in Zn accumulation with increased Zn concentration in nutrient solution (Table 2). *C. esculenta* accumulated > 10,000 mg kg^{-1} Zn in the aboveground part, indicating Zn hyperaccumulation (Reeves and Baker, 2000). However, highest Zn uptake was found in *T. angustifolia* exposed

Table 2 : Dry biomass, Zn accumulation and uptake, BCF and TF of *C. esculenta*, *C. malaccensis* and *T. angustifolia* grown in hydroponics for 15 days

Plant	Initial Zn conc (mg l^{-1})	Initial dry biomass (g plant^{-1})	Final dry biomass (g plant^{-1})	Zn accumulation (mg kg^{-1})		Zn uptake (µg plant^{-1})	BCF	TF
				Above ground	Below ground			
C. esculenta	0	2.1±0.2a,A	2.4±0.2a,A	0.0±0.2a,A	0.0±0.3a,A	0.0	0.0	0.0
	10	0.3±0.2a,A	0.5±0.1a,A	7,008.2±287.6b,B	8,287.3±472.2b,B	7.6	1,529.3	1.1
	20	1.4±0.1a,A	2.0±0.1a,A	7,211.7±156.8b,B	5,293.8±238.1a,B	24.6	625.3	1.6
	50	2.2±0.8a,A	2.5±0.2a,A	9,395.7±183.4b,B	6,207±115.5a,B	46.2	369.8	1.3
	100	1.6±0.8a,A	2.0±0.0a,A	13,902.9±534.2b,C	19,451.5±619.7a,C	65.8	334.4	0.8
C. malaccensis	0	12.5±3.6b,A	16.0±0.2b,A	0.0± 0.1a,A	0.0±0.1a,A	0.0	0.0	0.0
	10	12.8±2.1b,A	13.9±0.2b,A	36.2±5.6a,B	3,393.9±155.3a,B	47.8	343.0	0.0
	20	14.6±3.5b,A	16.8±0.4b,A	130.6±15.3a,C	6,063.7±876.4a,B	104.3	309.7	0.0
	50	8.0±2.6b,A	10.7±0.4b,A	1,459.1±128.0b,D	10,658.2±1,590.4a,C	130.1	242.3	0.1
	100	11.5±3.8b,A	12.7±0.7b,A	1,108±77.5a,D	12,210.6±1,493.0a,C	169.6	133.2	0.1
T. angustifolia	0	8.9±2.5b,A	10.2±1.4b,A	0.0±0.2 a,A	0.0±0.1a,A	0.0	0.0	0.0
	10	7.1±3.2b,A	12.7±0.5b,A	11.9±1.2 a,B	3,019.5±235.4a,B	38.4	303.1	0.2
	20	7.7±1.4a,A	18.5±0.5b,A	471.3±25.2 a,C	8,589.9±358.5a,B	76.7	453.1	0.1
	50	7.4±3.1b,A	13.2±0.8b,A	710.9±36.2 a,C	21,952.6±323.5b,C	298.4	453.3	0.1
	100	5.3±1.9ab,A	9.1±0.3b,A	1,819.3±267.6a,D	19,782.4±696.1a,C	195.9	216.0	0.1

Values followed by the same letter did not differ, small letters illustrated differences between plants species ($P < 0.05$, LSD), and capital letters illustrated differences among initial Zn concentrations for each plant species ($P < 0.05$, LSD).

to 50 and 100 mg l^{-1} Zn (195.9 – 298.4 µg plant^{-1}). BCF values for all the three species were < 1,000, except for *C. esculenta* in 10 mg l^{-1} Zn solution (1,529.3). Only *C. esculenta* could translocate Zn from belowground to the aboveground parts (TF>1; Table 2).

Hydroponic study revealed that *C. esculenta, C. malaccensis* and *T. angustifolia* were tolerant to Cd and Zn indicated by their increases in relative growth rates at higher metal concentration. In addition, Cd accumulation in all the three plants increased with Cd and Zn levels in the nutrient solution but the magnitude of metal increase varied among the plant parts and species used. *T. angustifolia* was most tolerant to Cd and Zn, followed by *C. malaccensis* and *C. esculenta*. Several studies have demonstrated the metal tolerance capacity of *Typha* species (*T. latifolia, T. angustifolia*) to various heavy metals such as Cd, Zn, Pb (Bunluesin *et al.*, 2004; Demirezen and Aksoy, 2004; Deng et al. 2006; Tang *et al.*, 2009; Yadav and Chandra, 2011). Jiang *et al.* (2001) indicated that metal tolerant plants must be able to exclude absorption of excess metals or detoxify metals after they have been absorbed. The present study demonstrated the metal exclusion ability of *T. angustifolia*.

Even though metal concentrations (Cd, Zn) increased with increased metal level in solution *T. angustifolia* maintained its shoot metal accumulation at higher metal levels. Highest metal accumulation (10x of those in shoots) occurred in roots or underground part. Exclusion of metals from the aboveground tissues has also been suggested in *T. angustifolia* (Yadav and Chandra, 2011). In addition, Tang *et al.* (2009) also suggested the protective mechanism of *T. angustifolia* for Cd was retention of Cd in roots, cell wall binding with metals and metal ion compartmentation in vacuoles.

Wetland plants are generally non-hyperaccumulators of metals. However, the present study demonstrated that under hydroponics, all the three plants, *C. esculenta, C. malaccensis* and *T. angustifolia* were Cd hyperaccumulators according to their aboveground levels attaining Cd > 100 mg kg^{-1} (Reeves and Baker, 2000). Only *C. esculenta* was Zn hyperaccumulator with aboveground Zn levels > 10,000 mg kg^{-1} (Reeves and Baker, 2000) and BCF value > 1,000. Yadav and Chandra (2011) studied heavy metal accumulation in *T. angustifolia* and *C. esculenta* grown in distillery and tannery effluent polluted natural wetland site in Unnao, India. These plants were reported as potential phytoremediators of heavy metals (Fe, Mn, Cr, Zn, Pb, Cu, Ni, Cd) from wastewater. However, since *C. esculenta* can also be consumed as root vegetable, accumulation of metals in edible part could have direct impact on heath of local inhabitants. While none of these three plants met the criterion of TF > 1 for Cd, *C. esculenta* exhibited TF values > 1 for Zn translocation. The results of Cd accumulation in wetland plants were similar to those reported by Bunluesin *et al.* (2004) and Kashem *et al.* (2008) for plants grown under hydroponic conditions (> 100 mg kg^{-1} Cd accumulation in the aboveground parts of *C. esculenta*, *Colocasia antiquorum*, *Cyperus strigosus*, and *T. angustifolia*).

T. angustifolia was the best choice for Cd and Zn phytoremediation among the plants studied as it fulfilled all the criteria of potential phytoremediation like accumulation capability, (2) metal tolerance, and (3) total uptake of metal (Reeves and

Baker, 2000). This is in agreement with Yadav and Chandra (2011) who reported that *T. angustifolia* showed more tolerance to heavy metal stress and higher potential for Cd accumulation than *C. esculenta*. *T. angustifolia* showed strong tolerance to Cd and Zn in nutrient solution, with relatively high RGR, strong metal accumulation capability and highest Cd and Zn uptake. In addition, plant was widely distributed, fast growing, had well-developed root-shoot system, producing high biomass, and usually forming mono-dominant stands in metal contaminated sediment and possessed low nutrient requirements. *T. angustifolia* also demonstrated the ability to serve as a biological indicator of Cd as it accumulated high concentration of metal which correlated very well with metal concentration in the solution.

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