App. Envi. Res. 36 (3): 65-76

Applied Environmental Research

Journal homepage : http://www.tci-thaijo.org/index.php/jer

Effect of EDTA and DTPA on Cadmium Removal from Contaminated Soil with Water Hyacinth

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Article history
Submitted: 26 January 2014/ Accepted: 26 March 2014/ Published online: 16 June 2014

Abstract:
The effects of ethylenediaminetetraacetic acid (EDTA) and diethylene triaminepentaacetic acid (DTPA) on cadmium (Cd) uptake by water hyacinth (*Eichhornia crassipes*) in Cd-contaminated soil was studied. Experimental samples were separated into 4 treatment groups: 1) Untreated control, 2) EDTA addition, 3) DTPA addition, and 4) mixture of EDTA and DTPA (1:1) addition. The plants were harvested at 20, 40, 60, 80 and 100 days. Cd levels were measured in soil samples, water samples and two parts of the plant: shoot (stem and leaves) and root. The results showed that Cd accumulation in plants with added EDTA and DTPA were higher than the control set, indicating that EDTA and DTPA addition increased Cd uptake by water hyacinth. Cd accumulations in the root in all groups were significantly (p<0.05) higher than that in the shoot. In EDTA added sets, Cd accumulation in the root was higher than in shoots and were measured at 160.91 and 13.37 mg/kg at 100 days, respectively. This research indicates that DTPA was most suitable for increasing the cadmium removal capacity of water hyacinth and offers a suitable phytoremediation technique to help clean contaminated sites.

Keywords: EDTA; DTPA; cadmium removal capacity; phytoremediation

Introduction

Hazardous waste contamination is one of the many increasing problems stemming from industrial activities, particularly mining, which frequently releases hazardous materials into soil, water, sediment and groundwater. These environmental effects are critically important due to their potential long-term damage on both human health and the life cycle of plants and animals.

Mae Sot District, Tak Province, in northern Thailand was found to have high levels of cadmium contamination in the stream sediment in Mae Tao creek. The amount of cadmium was measured at 6.07 to 33.93 mg/Cd per kg of sediment [1]. Many researchers have studied this problem and searched for methods to reduce the cadmium concentration in this area [2,3].

At presently there are numerous remediation technologies used to clean up heavy metal contamination in water, soil and sediments. These techniques include *in situ* physical and chemical processes (soil flushing, solidification and stabilization), thermal processes, *ex situ* physical and chemical processes (soil washing, chemical reduction and oxidation), and other processes including excavation and off-site disposal [4]. However, most of these treatments are costly [5]. Thus, removal of heavy metals by plants has been proposed due to its low cost and high efficiency. Phytoremediation uses selected plant species and varieties to reduce, remove, degrade or immobilize contaminant toxins in soil, sediment, sludge and groundwater [6,7,8]. Plants be selected to treat soils for various contaminants such as heavy metals, inorganic waste, pesticides, explosives, petroleum oils, hydrocarbons, polycyclic aromatic hydrocarbons and wastewater from garbage dumps [7]. This field is growing fast and is of special relevance to the prevailing economic context in Thailand. Phytoremediation is also environmentally friendly; the use of plants reduces the need for additional chemicals, and plants can absorb toxins from contaminated soil, accumulate them in root tissue and later translocate them to shoots and leaves. Plant metabolism can be used as a means to degrade and reduce pollutant levels using dehalogenase and oxygenase enzymes. Pollutants concentrating in leaves and shoots can then be removed by harvesting the above ground plant tissue, and incinerating or burying the harvest [8,9]. Metals can be reclaimed from the ash, further reducing hazardous waste and generating recycling revenues. Phytoremediation technology has been advocated as an innovative and cost-effective alternative to conventional methods used at hazardous waste sites [4, 7].

In this research, *Eichhorniacrassipes* (Mart.) *Solms*. (water hyacinth) was used to determine its ability to reduce soil heavy metal levels. *Eichhorniacrassipes* is a monocot weed species that grows naturally as a persistent weed in the study area as well as all over Thailand. Its high levels of xylem and phloem flows may lead to increased uptake of heavy metals. The objectives of this present study were to 1) investigate the possibility of using EDTA and DTPA to increase cadmium removal from contaminated soil and water by *Eichhornia crassipes* (Mart.) *Solms.*; 2) study the relationship between the cadmium removal capacities in plant and cadmium or available cadmium in soil; and 3) determine the cadmium accumulation in shoots and roots of the plants.

Materials and methods

1) Soil preparation: Soil samples were collected at a depth of 0-30 cm at a site in Mae Sot district, Tak Province, Thailand. The soil properties are shown in (Table 1).

2) Pot preparation: 33 pots (30 cm. x 35 cm. high) were twice washed with 10% nitric acid solution, then rinsed with distilled water.

3) The Ethylenedinitrilotetraacetic acid ($C_6H_{16}N_2O_8$); EDTA and Diethylenetriamine-pentaacetic acid ($C_{14}H_{23}N_3O_{10}$); DTPA in the various concentrate proportions were diluted with deionized water to 0.5, 1 and 2 mg/L (ppm) and added at a ratio of 1:1 in 10 L of deionized water/pot. Cadmium contaminated soil, 5 kg soil per pot, was added to the pots. The chelating agents were applied at 3 concentration levels as follows:

3.1) Experiment set 1; control pot without added chelating agent and using contaminated soil.
3.2) Experiment set 2; the addition of EDTA at 3 doses of 0.5, 1 and 2 mg/L and using contaminated soil.
3.3) Experiment set 3; the addition of DTPA at 3 doses of 0.5, 1 and 2 mg/L and using contaminated soil.
3.4) Experiment set 4; the addition of both EDTA and DTPA (1:1) at 3 doses of 0.25, 0.5 and 1 mg/L and using contaminated soil.

4) *Eichhorniacrassipes* preparation: Water hyacinths (5 plants per 1 pot) were selected from an uncontaminated area in the Bangpakong River, Nahmuang Sub-District, Muang District, Chachoengsao Province.

5) Planting and maintenance : A rotator machine was placed in each pot and the water level in each pot was maintained at 10 L until the end of the experiment.

6) Sample collection
6.1) Water samples were collected from each pot in 100 ml bottles. The bottles had 2 or 3 drops of 65% nitric acid added to analyze total cadmium.
6.2) Soil samples were collected from each pot in 100 g lots and stored in zip lock bags during harvesting at 20, 40, 60, 80 and 100 days of the experiment. The soil samples were separated into 2 parts; the first parts were oven-dried at 103^{o}C for 2-3 days to a constant weight and used to determine dry matter yield. Then these soil samples were crushed to pass through a 2 mm sieve to determine the total cadmium concentration in the soil. The second samples were open air dried for 2-3 days and analyzed for available cadmium in the soil.
6.3) Plant samples were collected, washed with tap water twice and rinsed with distilled water before being separated into 2 parts: roots and shoots. These samples were oven-dried at 70^{o}C for 2-3 days to get a constant weight and to determine dry matter yield.

7) Sample analysis
7.1) Water samples were analyzed for total cadmium using USEPA method 3051A[10].
7.2) Soil samples and plant samples were analyzed for total cadmium using USEPA method 3052[11].

The sample from USEPA method 3051A and USEPA method 3052 was made up with 50 ml of deionized water and preserved at 4^{o}C until analysis. The total digested solution was analyzed by atomic absorption spectrometer (AAS).

Results and discussion

1) Growth rate of water hyacinth

The study examined the effect of different concentrations of EDTA and DTPA on water hyacinth growth rate raised in cadmium-contaminated soil in Mae Sot. The effects of addition of combinations of chelating agent EDTA and DTPA were examined at 3 concentration levels; 0.5, 1 and 2 mg/L (ppm). The study also compared the effect of EDTA and DTPA in stimulating cadmium removal from contaminated soil in Mae Sot District, Tak Province, Thailand.

Table 1 Soil properties

Soil properties	Methods	Value	Unit
pH	1:1 soil/water ratio	7.5	
Conductivity	1:1 soil/water ratio	0.18	dS/m
Soil moisture	% moisture = [(wet weight – dry weight) x 100] / wet weight	8	%
Organic matter	$K_2Cr_2O_7$ digestion	2.56	%
Nitrogen	Kjedahl	0.13	%
Phosphorus	Molybdenum blue	8.33	mg/kg
Potassium	Digested with Na_2CO_3	56	mg/kg
CEC	Ammonium acetate	7.4	meq/100g
Soil texture	Hydrometer method	loam	
Cd	US EPA-3052	94.64	mg/kg

Two-week-old water hyacinth plants of approximately the same size (25-30 g) were selected to this study. The results show that the water hyacinth grew well in all EDTA sets, especially with the addition of 2 mg/L (ppm). In the DTPA set, the water hyacinth growth rate increased with DTPA concentration, but most especially with the addition of 0.5 mg/L (ppm) as shown in Figure 2. For the combination of EDTA and DTPA in a 1:1 ratio, the growth rate also increased with concentration, especially at 2 mg/L(ppm). From Figures 1, 2 and 3 we see that growth of water hyacinth tended to increase in accordance with time.

This finding is consistent with the work of Ariganon (2007) [12], who studied the effects of 5 kinds of chelating agent and organic acid (DTPA, ethylenediamine- N, N –disuccinic acid (EDDS), oxalic acid, citric acid and gallic acid) to increase the copper, zinc and nickel uptake capacity in *Ruelliatuberosa* (Burm.f.) Hochr. Ariganon reported that the chelating agent did not show phytotoxicity and did not effect plant growth rate. German et al. (2003) came to similar conclusions [13]; they studied the effects of EDTA and EDDS in increasing lead, zinc and cadmium uptake capacity in *Juncea* (L.) Czern. They found the application of EDTA at 3 and 5 ml/kg soil did not affect the dry weight of *Juncea* (L.) Czern.

2) Effect of Chelating agent on cadmium uptake by water hyacinth

2.1) Effect of EDTA

Figures 4 and 5 demonstrate that the cadmium accumulated by roots and shoots tended to increase with time. These results show that EDTA can indeed facilitate cadmium removal by water hyacinth. The cadmium accumulation in roots was higher than in shoots at 160.91 and 13.37 mg/kg dry weight of plant, respectively, at an EDTA concentration of 2 mg/L. The study found the cadmium accumulated in root and shoot tissue, as shown in Figure 4. The capacity of root uptake by water hyacinth was consistent with the findings of Jean et al. (2008) [14], who studied the effects of EDTA and citric acid on increasing chromium and nickel uptake capacity in *Datura innoxia* (Thorn apple) grown in contaminated soil with EDTA applied at 1 mM/kg soil, and citric acid at 1, 5 and 10 mM/kg soil. They found that the two chelating agents caused positive effects on chromium and nickel accumulation in the roots of *Datura innoxia*.

The uptake capacity of plant shoots (stem and leaf) was found to be similar to that found by Hernandez et al. (2006) [15], who studied the effects of EDTA on increasing uptake capacity of lead, zinc and cadmium in *Cynara carduncylus* (Globe artichoke). They found EDTA increased uptake of heavy metals in this plant species, with greater accumulation of heavy metals in stems and leaves compared with roots. This also corresponds with the findings of Wongtanet and Parkpain (2008) [16], who studied phytoremediation of lead from water with *Hydrocotyle umbellata* L., *Pityrogramma calomelanos* L. and *Pandanus amaryllifolius* Roxb. The result of EDTA additions showed that the accumulation of lead was increased in roots and stems of *Hydrocotyle umbellata*, *Pityrogramma calomelanos* and *Pandanus amaryllifolius* Roxb at 19, 31.7 and 3.2 µg/g, respectively. While the set without EDTA showed that the accumulation of lead in roots and stems of *Hydrocotyle umbellata, Pityrogramma calomelanos and Pandanus amaryllifolius* Roxb were 11.4, 17.4 and 2.4 µg/g, respectively.

2.2) Effect of DTPA

Figure 6 and 7 illustrate that cadmium removal by roots and shoots tended to increase with time. We found that DTPA promoted cadmium removal. At 100 days, cadmium accumulation in roots was higher than in shoots, at a DTPA concentration of 2 mg/L. Cadmium accumulation was highest in roots and shoots at 231.78 and 27.02 mg/kg dry weight of plant. However, the DTPA concentration of 1 mg/L and 80 days of growth time.

2.3) Effect of EDTA and DTPA in combination

Figures 8 and 9 illustrate that cadmium removal by roots and shoots tended to increase over time. We found that the combination of EDTA and DTPA in a 1:1 ratio increased cadmium removal. The cadmium accumulation in roots was higher than in shoots. Using the 1:1 combination of EDTA and DTPA and a concentration of 2 mg/L, at 100 days of growth time accumulation was 157.48 mg/kg dry weight of plant. In shoots the concentration of cadmium was highest at 23.61 mg/kg dry weight of plant, at the EDTA and DTPA mixture concentration of 2 mg/l (ppm) and 60 days of growth time.

We also found that DTPA promoted cadmium removal capacity. This result corresponds with the work of Hua-Yin Zhao et al. (2011) [17] who studied phytoremediation of lead and zinc from soil in a mining area by using the same two chelators (EDTA and DTPA) to enrich ryegrass. They found that EDTA and DTPA had great potential for this purpose.

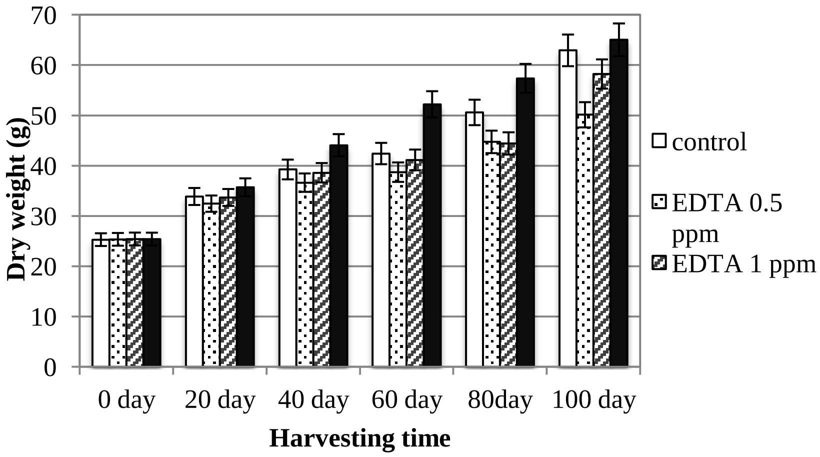

Figure 1 Growth rate (EDTA addition sets)

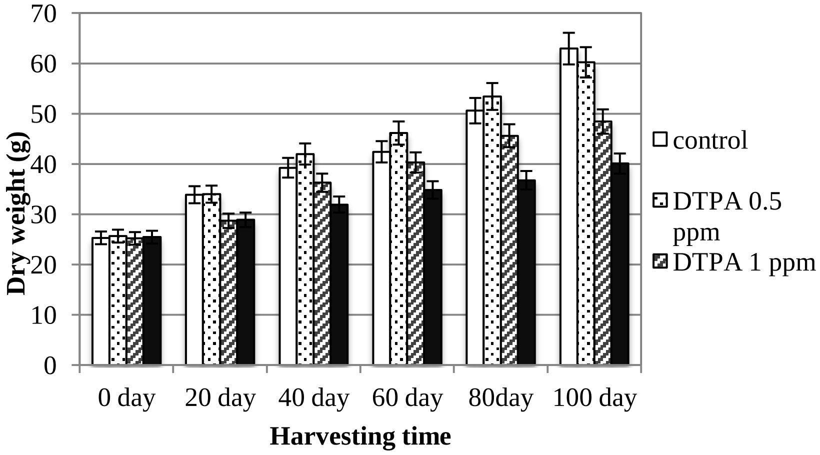

Figure 2 Growth rate (DTPA addition sets)

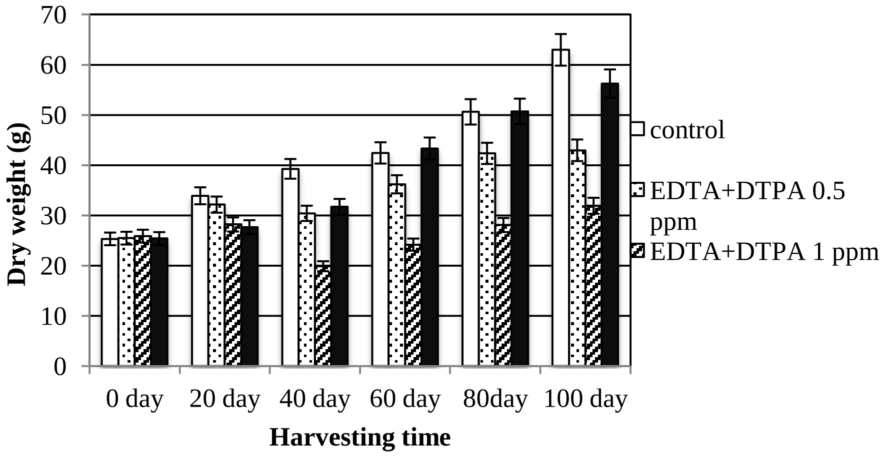

Figure 3 Growth rate of water hyacinth in mixed EDTA and DTPA addition sets at 1:1 ratio

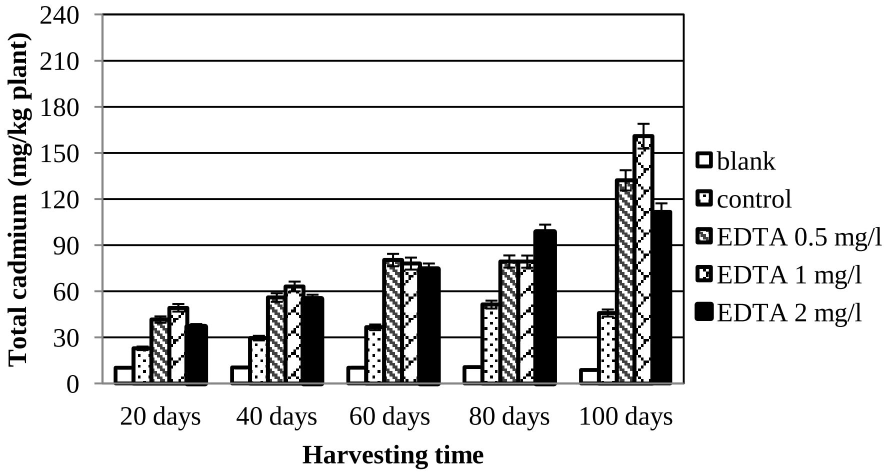

Figure 4 Effect of EDTA on cadmium uptake by roots of water hyacinth

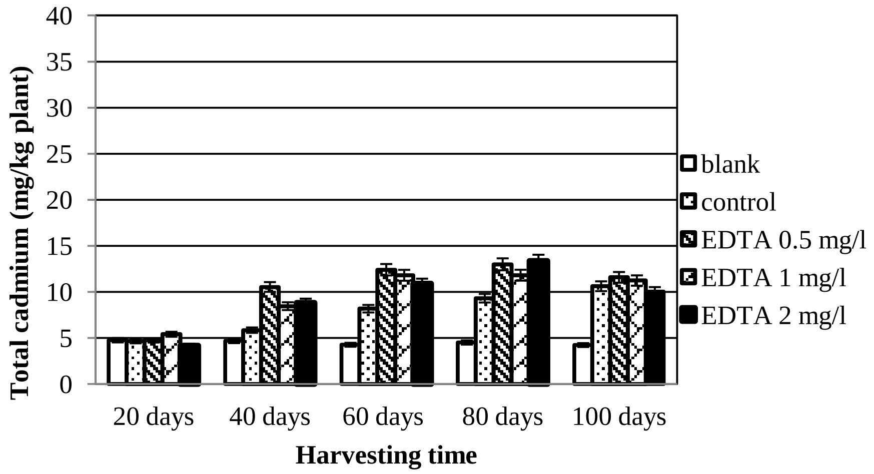

Figure 5 Effect of EDTA on cadmium uptake by shoots of water hyacinth

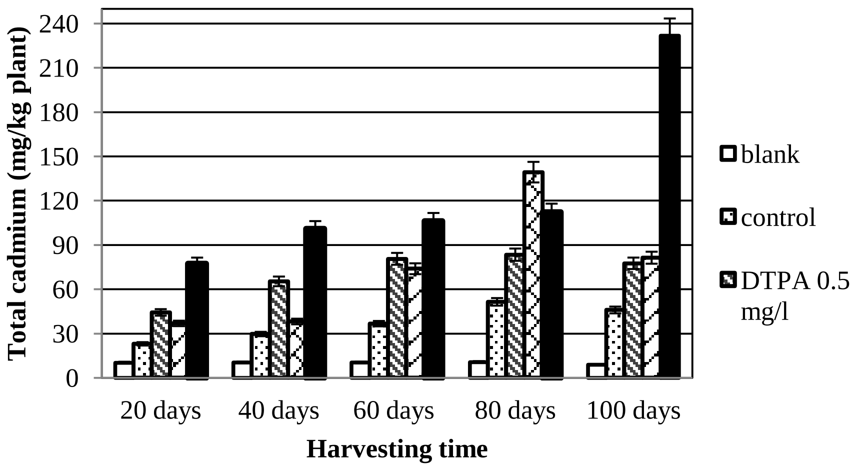

Figure 6 Effect of DTPA on cadmium uptake by roots of water hyacinth

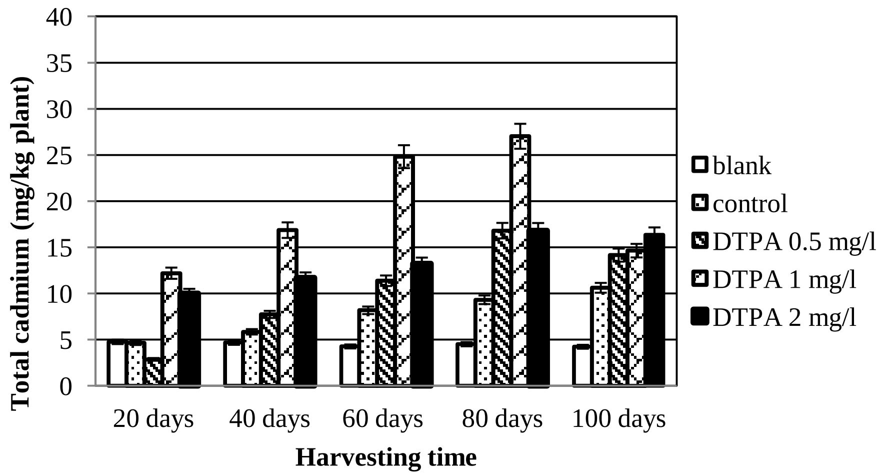

Figure 7 Effect of DTPA on cadmium uptake by shoots of water hyacinth

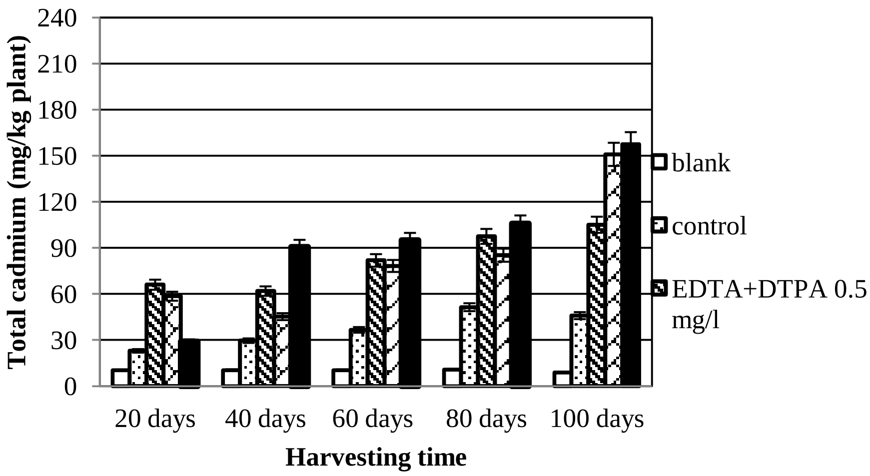

Figure 8 Effect of EDTA and DTPA mixture at 1:1 ratio on cadmium uptake by roots of water hyacinth

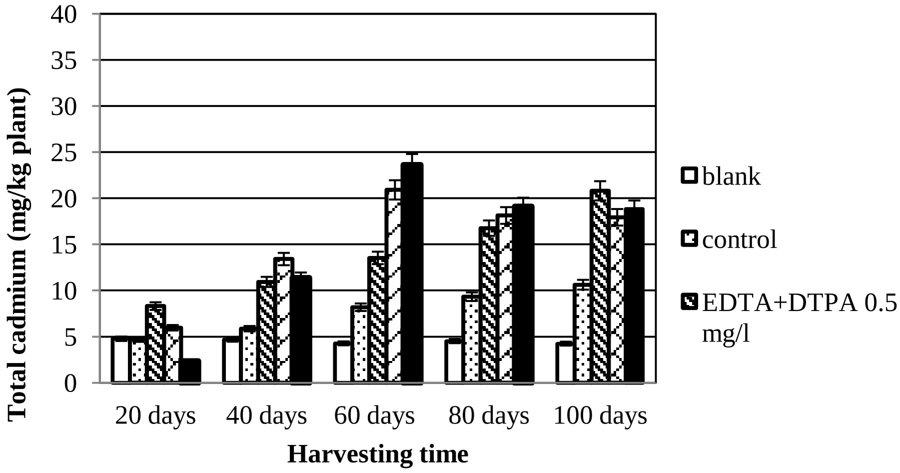

Figure 9 Effect of EDTA and DTPA mixture at 1:1 ratio on cadmium uptake by shoots of water hyacinth

2.4) Effect of EDTA and DTPA on cadmium

2.4.1) Total cadmium accumulation in soil

The concentration of total cadmium in the contaminated soil was determined after harvesting times of 20, 40, 60, 80 and 100 days of water hyacinth growth following USEPA method 3052 [11]. The addition of chelating agents; EDTA, DTPA and EDTA and DTPA combination (at 1:1 ratio) at 3 concentration levels; 0.5, 1 and 2 mg/L caused positive effects on removal of cadmium from the contaminated soil, with soil cadmium levels tending to decrease over time as shown in Table 2. The cumulative amounts of cadmium taken up by the plants in soil amended with EDTA at 0.5, 1 and 2 mg/L, were 97.82, 97.47 and 97.55 mg/kg, at 0 days, respectively. The total cadmium was 30.53, 26.41and 37.37 mg/kg, at 100 days, respectively. The amounts for DTPA at 0.5, 1 and 2 mg/L were 97.01, 96.14 and 97.91 mg/kg at 0 days and 48.48, 38.90 and 33.37 mg/kg, respectively at 100 days. The amounts for the combination of EDTA and DTPA at 0.5, 1 and 2 mg/L were 97.33, 97.49 and 97.61 mg/kg at 0 days and 67.25, 58.68 and 54.32 mg/kg, respectively, at 100 days.

2.4.2) Available cadmium in soil

After sample collection at 20, 40, 60, 80 and 100 days of water hyacinth growth, soil samples were tested for availability of cadmium by using the DTPA extraction method. Table 3 illustrates the concentration of available cadmium in the contaminated soils. The results show that available Cd decreased with time during the growth period. The highest availability of cadmium was at 20 days of growth. In general, the solubility and availability of metals in plants are related to the metal concentration in soil [18] as the amount of metal uptake by plants would relate to its availability in the soil. Therefore, these results confirm the expectation that the availability of cadmium in soil would decrease slightly with time during the growth period due to it uptake by the plants. The environment, nutrition, growth stage and some other factors controlling plant growth may also indirectly affect the metal level in the plant [19]

Table 2 Total cadmium accumulation in soil

Days	Concentration of chelating agent (mg/L)								
	EDTA			DTPA			EDTA and DTPA Mixture		
	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L
0	97.82	97.47	97.55	97.01	96.14	97.91	97.33	97.49	97.61
20	89.60	59.47	54.55	80.01	76.14	79.91	84.88	86.74	95.92
40	78.88	53.60	46.98	74.49	76.92	78.00	73.36	79.90	71.95
60	60.80	52.76	43.04	68.14	75.58	67.68	72.61	71.45	59.72
80	60.76	43.63	37.82	51.17	43.33	61.77	71.19	64.01	54.60
100	30.53	26.41	37.37	48.48	38.90	33.37	67.25	58.68	54.32

2.4.3) Total cadmium accumulation in water

Water samples collected after harvesting at 20, 40, 60, 80 and 100 days were examined for total cadmium following USEPA method 3051A [10]. As shown in Table 4, addition of chelating agent; EDTA, DTPA and combination of EDTA and DTPA (at a 1:1 ratio) at 3 concentration levels of 0.5, 1 and 2 mg/L increased the soluble form of cadmium in the water samples. The concentration of cadmium accumulated in the water decreased with time after planting of the water hyacinth. At 20 days, the amount of cadmium taken up by the water with EDTA at 0.5, 1 and 2 mg/L, were 12.11, 5.65 and 22.05 mg/L, respectively. At 100 days Cd concentrations of all treatments were lower than 0.05 mg/L. The amounts of cadmium for DTPA were 12.38, 11.58 and 11.22 mg/L at 20 days and lower than 0.05 mg/L, respectively, at 100 days. The amounts of Cd for the combination of EDTA and DTPA at 1:1 ratio were 9.62, 7.29 and 0.12 mg/L at 20 days and lower than 0.05 mg/L at 100 days.

Table 3 Available Cd accumulation in soil

Days	Concentration of chelating agent (mg/L)								
	EDTA			DTPA			EDTA and DTPA Mixture		
	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L
0	15.54	15.57	15.55	15.61	15.51	15.49	15.55	15.60	15.66
20	20.77	23.11	13.37	28.48	72.76	22.45	20.95	21.95	21.69
40	20.27	19.47	13.19	19.75	45.54	22.68	20.71	18.33	16.75
60	15.99	14.10	10.91	16.88	22.53	19.39	17.09	15.48	14.23
80	14.10	13.17	10.01	15.75	15.53	16.40	16.26	13.28	12.08
100	12.29	12.53	8.38	14.03	12.65	9.66	12.71	12.12	10.44

Table 4 Total Cadmium accumulation in water

Days	Concentration of chelating agent (mg/L)								
	EDTA			DTPA			EDTA and DTPA Mixture		
	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L	0.5 mg/L	1 mg/L	2 mg/L
0	0	0	0	0	0	0	0	0	0
20	12.11	5.65	22.05	12.38	11.58	11.22	9.62	7.29	0.12
40	0.12	0.11	0.11	0.11	0.11	0.08	0.07	0.08	0.09
60	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
80	0.07	0.06	0.07	0.07	0.08	<0.05	0.06	<0.05	0.06
100	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Conclusion

The effect of EDTA and DTPA on cadmium removal from contaminated soil with water hyacinth were investigated in this study. Both EDTA and DTPA increased the ability of water hyacinth to remove cadmium from contaminated soil. The amount of cadmium in the contaminated soil slightly decreased with elapsed time during the plant growth period (from 20 -100 days). The phytoavailability of cadmium in contaminated soil was proportional to the total cadmium in soil. In phytotoxicity studies of cadmium and chelating agent, no negative effects were observed on the water hyacinth in all experiments.

For all treatments, the highest cadmium concentrations were found in roots of water hyacinth. These concentrations, as well as total accumulation of cadmium, increased with plant age, and were highest at 100 days in all sets. In terms of management for cadmium removal, the water hyacinth biomass, including the roots, must be removed from the site after remediation.

The DTPA sets and the mixed EDTA and DTPA sets (ratio 1:1) were added at 3 concentrations; 0.5, 1.0 and 2 mg/L. Addition of DTPA at 2 mg/L showed the highest cadmium accumulation in water hyacinth, which correspondingly increased with time more than the other concentrations. EDTA was added at 3 concentrations; 0.5, 1 and 2 mg/L, At 1 mg/L cadmium accumulation in water hyacinth was found to be higher than in the other concentrations. Finally, among the 3 sets of experiments the DTPA at 1 mg/L resulted in an larger increase in plant cadmium accumulation than for other ratios.

The problem of soil cadmium contamination in the Mae Sot area has become a serious concern in recent years, with high concentrations detected in stream sediment and water. Thus, the results of our experiment can be applied to help manage this problem. DTPA is suitable and should be promoted for use in cadmium removal from soil and sediment using water hyacinth.

Acknowleddements

The author gratefully acknowledges financial support from the Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University. Additional gratitude goes to the Environmental Research Institute, Chulalongkorn University (ERIC) for assistance in providing academic facilities and research instruments throughout this research.

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