ผลของอีดีทีเอและดีทีพีเอต่อการกำจัดแคดเมียมในดินปนเปื้อนด้วยผักตบชวา

นายเอกชา ตนานนท์ชัย

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Effect of EDTA and DTPA on cadmium removal from contaminated soil with
Eichhornia crassipes (water hyacinth)

Mr. Akegacha Tananonchai

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เอกชา ตนานนท์ชัย : ผลของอีดีทีเอและดีทีพีเอต่อการดูดดึงแคดเมียมในดินปนเปื้อน ด้วยผักตบชวา. (EFFECT OF EDTA AND DTPA ON CADMIUM REMOVAL FROM CONTAMINATED SOIL BY WATER HYACINTH) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. พันธวัศ สัมพันธ์พานิช,103 หน้า.

การศึกษาผลของอีดีทีเอและดีทีพีเอต่อการดึงดูดแคดเมียมในดินปนเปื้อนด้วยผักตบชวา โดยแบ่งการ ทดลองออกเป็น 2 ส่วน โดยส่วนที่ 1 เป็นการศึกษาความเป็นพิษของสารอีดีทีเอ และสารดีทีพีเอที่มีต่อต้นผักตบชวา และผลของสารอีดีทีเอและสารดีทีพีเอต่อการเจริญเติบโตสัมพัทธ์ของต้นผักตบชวา ในการทดลองมีการมีการทดสอบ ความเป็นพิษโดยใช้สารอีดีทีเอและสารดีทีพีเอที่ความเข้มข้น 0, 0.5, 1, 2, 5, 10 และ 20 มิลลิกรัมต่อลิตร ทำการ สังเกตความเป็นพิษและวัดการเจริญเติบโตสัมพัทธ์ทุกๆ 15, 30, 45 และ 60 วัน ผลการทดลองพบว่าสารอีดีทีเอและ สารดีทีพีเอไม่ก่อให้เกิดความเป็นพิษในต้นผักตบชวาที่ทุกระดับความเข้มข้นแต่ความเข้มข้นของสารอีดีทีเอและสาร ดีทีพีเอที่เพิ่มขึ้นมีผลทำให้การเจริญเติบโตสัมพัทธ์มีค่าลดลง ส่วนที่ 2 เป็นการศึกษาผลของสารอีดีทีเอและ สารดีทีพีเอต่อการดูดดึงแคดเมียมในต้นผักตบชวา โดยแบ่งการทดลองออกเป็น 4 ชุดการทดลอง ได้แก่ 1) ชุดควบคุม ที่มีการเติมดินปนเปื้อนจำนวน 5 กิโลกรัมและไม่มีการเติมสารคีเลตทั้งสองชนิด 2) ชุดการทดลองที่มีการเติมดิน ปนเปื้อนจำนวน 5 กิโลกรัมและเติมสารอีดีทีเอที่ระดับความเข้มข้น 0.5, 1 และ 2 มิลลิกรัมต่อลิตร 3) ชุดการทดลองที่ มีการเติมดินปนเปื้อนจำนวน 5 กิโลกรัมและเติมสารดีทีพีเอที่ระดับความเข้มข้น 0.5, 1 และ 2 มิลลิกรัมต่อลิตร และ 4) ชุดการทดลองที่มีการเติมดินปนเปื้อนจำนวน 5 กิโลกรัมและเติมสารดีทีพีเอร่วมกับสารอีดีทีเอที่ระดับความเข้มข้น 0.5, 0.1 และ 2 มิลลิกรัมต่อลิตร ชนิดละเท่าๆ กัน ทำการเก็บตัวอย่างทุกๆ 20, 40, 60, 60 และ 100 วัน เพื่อหา ปริมาณแคดเมียมในส่วนเหนือน้ำ (ลำต้นและใบ) และส่วนใต้น้ำ (ราก) ของผักตบชวา ปริมาณแคดเมียมในดินที่ใช้ใน การทดลอง และปริมาณแคดเมียมในน้ำที่ใช้ในการทดลอง ผลการทดลองในทุกชุดการทดลอง พบว่า ผักตบชวามี ความสามารถในการสะสมแคดเมียมมากที่สุดในส่วนใต้น้ำ (ราก) รองลงมา คือ ส่วนเหนือน้ำ (ลำต้นและใบ) ซึ่งมี ความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P \leq 0.05$) เมื่อเปรียบเทียบกับชุดควบคุม แสดงให้เห็นว่าสารคีเลตทั้งสอง ชนิดมีส่วนช่วยในการดูดดึงแคดเมียมในผักตบชวา โดยในชุดที่เติมสารอีดีทีเอที่ระดับความเข้มข้น 1 มิลลิกรัมต่อลิตร มีปริมาณการสะสมแคดเมียมได้สูงที่สุดในส่วนใต้น้ำ (ราก) เท่ากับ 160.91 มิลลิกรัมต่อกิโลกรัมน้ำหนักแห้ง และ รองลงมาคือส่วนเหนือน้ำ (ลำต้นและใบ) เท่ากับ 13.37 มิลลิกรัมต่อกิโลกรัมน้ำหนักแห้ง ที่เวลา 100 วัน ในส่วนของ ชุดที่เติมสารดีทีพีเอและชุดที่มีการเติมสารดีทีพีเอร่วมกับสารอีดีทีเอที่ระดับความเข้มข้น 2 มิลลิกรัมต่อลิตร มีปริมาณการสะสมแคดเมียมได้สูงที่สุดในส่วนใต้น้ำ (ราก) เท่ากับ 231.78 และ 157.48 มิลลิกรัมต่อกิโลกรัมน้ำหนัก แห้ง ตามลำดับที่เวลา 100 วัน และรองลงมาคือส่วนเหนือน้ำ (ลำต้นและใบ) เท่ากับ 16.34 มิลลิกรัมต่อกิโลกรัม น้ำหนักแห้งที่เวลา 100 วัน และ 23.61 มิลลิกรัมต่อกิโลกรัมน้ำหนักแห้ง ที่เวลา 60 วัน ตามลำดับจึงสามารถสรุปได้ ว่าการเติมสารดีทีพีเอมีผลต่อการดูดดึงแคดเมียมของผักตบชวามากกว่าการเติมสารอีดีทีเอและการเติมสารดีทีพีเอ ร่วมกับสารอีดีทีเอ

สาขาวิชา การจัดการสิ่งแวดล้อม   ลายมือชื่อนิสิต..
ปีการศึกษา 2554   ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.......................

5387590020: MAJOR ENVIRONMENTAL MANAGEMENT
KEYWORDS: EDTA / DTPA / UPTAKE / CADMIUM / WATER HYACINTH

AKEGACHA TANANONCHAI: EFFECT OF EDTA AND DTPA ON CADMIUM REMOVAL FROM CONTAMINATED SOIL BY WATER HYACINTH. ADVISOR: ASST. PROF. PANTAWAT SAMPANPANISH, Ph.D., 103 pp.

The effects of ethylenediaminetetraacetic acid (EDTA) and diethylene triaminepentaacetic acid (DTPA) on cadmium (Cd) uptake by water hyacinth (*Eichhornia crassipes*) in Cd contaminated soil were studied. The experimental design was separated into 2 parts: preliminary study and experimental procedure. The preliminary study investigated the using EDTA and DTPA at doses of 0, 0.5, 1, 2, 5, 10 and 20 mg/L, for 15, 30, 45 and 60 days. The results showed that water hyacinth grew well and did not show phytotoxicity under the various applications of EDTA and DTPA. The addition of EDTA and DTPA in higher concentrations did causenegative effects on the relative growth rate of water hyacinth. For the experimental procedure samples were separated into 4 groups: 1) contaminated soil 5 kg without chelating agent (Control), 2) contaminated soil 5 kg with EDTA added at concentrations of 0.5, 1 and 2 mg/L, 3) contaminated soil 5 kg with DTPA added at concentration of 0.5, 1 and 2 mg/L, and 4) contaminated soil 5 kg with a mixture of EDTA and DTPA (1:1) at 3 doses of 0.25, 0.5 and 1 mg/L. Plants were harvested at 20, 40, 60, 80 and 100 days. Cd levels were measured in the soil samples, water samples and two parts of the plant: shoot (stem and leaves) and root. The results showed that Cd accumulation in the root in all groups were significantly ($P<0.05$) higher than that in the shoot. Cd accumulation in plants with added EDTA and DTPA were higher than the control set, which indicates that EDTA and DTPA addition increased Cd uptake by water hyacinth. In EDTA added sets, the Cd accumulation in root was higher than shoots and were measured at 160.91 and 13.37 mg/kg at 100 days, respectively. For DTPA sets, the Cd accumulation in roots was also higher than shoots. At the DTPA concentration of 2 mg/l (ppm) and after 100 days of growing time Cd accumulation was 231.78 in root and 16.34 for shoots mg/kg dry weight of plant. For the mixture of both EDTA and DTPA sets, the Cd accumulation in roots was again higher than shoots. The EDTA and DTPA concentration of 2 mg/l (ppm) after 100 days of growing time showed the highest accumulation in roots at 157.48 and after 60 days of growing time showed the highest accumulation in shoots at 23.61 mg/kg dry weight of plant, Our conclusion in this research is that DTPA alone positively effected Cd uptakeby water hyacinth more so than EDTA only and the mixture of EDTA and DTPA.

Field of student   Environmental Management   Student's signature ....

Academic year   2011 ....   Advisor's signature ....

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CHAPTER I
INTRODUCTION

1.1 General statement

Nowadays, increasing industrial pollution causes many negative effects on environmental quality. These effects are critically important due to the extent of the damage they impose on human health and the life cycle of plants and animals. Hazardous waste contamination is one of the increasing problems stemming from industrial activities such as mining. These activities result in hazardous waste that is released into the soil, water, sediment and groundwater.

Mae Sot District, Tak Province, Thailand was found to have high levels of cadmium contamination. The cadmium concentration in the stream sediment in Mae Tao creek measured 6.07 to 33.93 mg of Cd per kg of sediment and 11.66 to 65.22 mg of Cd per L of sediment (Karoonmakpol, 2009). The cadmium concentration in the water was 0.3 to 0.8 mg of Cd per L of water (Department of Primary Industries and Mines, 2006). Many researchers have studied and searched for methods to reduce the cadmium concentration in this area.

Remediation technology has many techniques to clean up heavy metal contamination in water, soil and sediment. These techniques include in situ physical and chemical processes (soil flushing, solidification and stabilization), thermal processes (verification), ex situ physical and chemical processes (soil washing, chemical reduction and oxidation), and other processes such as excavation and off-site disposal (Sampanpanish, 2005). However, most of these treatments are rather costly (Ensly, 2000). Thus, the removal of heavy metals by plants has been recommended due to its relatively low cost and high efficency. This method is called phytoremediation and uses plants to reduce, remove, degrade or immobilize contaminant toxins from soil, sediment, sludge and ground water (Schnoor, 1997; USEPA, 2000; Peer et al, 2007). Plants used can dispose of various contaminants for example, heavy metals, inorganic waste, pesticides, solution, explosives, petroleum oils, hydrocarbon compounds, polycyclic aromatic hydrocarbon compounds and

wastewater from garbage heap (USEPA, 2000). This technology is interesting and appropiate to the economic situation of Thailand. Phytoremediation is environmental friendly. The use of plants is a natural process and reduces the need to use additional chemical substances. Plants can uptake the metals from contaminated soil and accumulate them in roots and then translocate them to shoots and leaves. The plants have the metabolism to degrade and reduce the pollutant by their dehalogenase and oxygenase enzymes. . The pollutants are then removed by harvesting the above ground tissue of the plant which are incinerated and/or buried (Lai et al., 2004). Some metals can be reclaimed from the ash which further reduces hazardous waste and generates recycling revenues. Phytoremediation technology has been receiving attention lately as an innovative and cost effective alternative to the more established treatment methods used at hazardous waste sites (USEPA, 2000; Sampanpanish, 2005). Phytoremediation is a biological treatment technology, which uses selective plants for clean up of heavy metal contaminated soil and water.

In this research, *Eichhornia crassipes* (water hyacinth) was studied to determine its ability to reduce the heavy metal in water and synthetic sediment which was adjusted from cadmium contaminated soil. *Eichhornia crassipes* is not only generally found in the studied area but it also grows easily in every area of Thailand. It is a monocot weed plant species and contains high levels of xylem and phloem which may lead to increased uptake of heavy metals by the plant.

1.2 Objective

1.2.1 To investigate the possibility of using EDTA and DTPA to increase cadmium removal from contaminated soil and water by *Eichhornia crassipes* (Mart.) Solms.

1.2.2 To study the relationship between the cadmium removal capacities in plant and cadmium or available cadmium in soil.

1.2.3 To determine the cadmium accumulation in shoots and roots of the plants.

1.3 Hypotheses

   1.3.1 The introduction of EDTA and DTPA into soil may increase the cadmium removal capacity of certain plants.

   1.3.2 The cadmium accumulation in the plant increases with increasing harvest time.

   1.3.3 The cadmium in soil will be untaken and stored in the roots and shoots of plants.

1.4 Scope of the study

   This study investigated the possibility for removal of cadmium by using *Eichhornia crassipes* growing in contaminated water and soil. The chelating agents, EDTA and DTPA, were added to promote the plant's cadmium removal capacities. The scope of the work is as follows:

   1.4.1 Plants were selected from the Bangpakong River, Nahmeuang Sub-district, Muang District, Chachoengsao Province.

   1.4.2 Contaminated synthetic waste water and soil were used in this experiment.

   1.4.3 Chelating agents used in this experiment were Ethylenediamine tetraacetic acid (EDTA) and Diethylene triamine pentaacetic acid (DTPA).

   1.4.4 The experiment was separated into 2 parts.

  1) Preliminary study: This study investigated the possibility of using the EDTA and DTPA at doses of 0, 0.5, 1, 2, 5, 10 and 20 mg/per L. Recorded the growth rate and phytotoxicity level of *Eichhornia crassipes* on 15, 30, 45 and 60 days after planting.

  2) Experimental procedure: This research studied the effect of various doses of EDTA and DTPA on cadmium removal capacities from contaminated water and soil. Soil, water and plant samples were collected after 20, 40, 60, 80 and 100 days of the cultivation.

1.4.5 Sample collection; plants were separated into two parts; shoots (stems and leaves) and roots. Plant and soil samples were collected, dried in the open air and analyzed. Samples were analyzed for total cadmium with Atomic Absorption Spectrometer; AAS.

1.4.6 The experiment was done in a nursery. The research methodology is show in figure 1.1

Effect of EDTA and DTPA on cadmium removal with water hyacinth

Preliminary study: EDTA and DTPA dose of 0, 0.5, 1, 2, 5, 10 and 20 ppm, sample collection at 15, 30, 45 and 60 days

Prepare the contaminated Synthetic waste water and soil

Water hyacinth selection

Experimental procedure: Water, soil and plant collection at 20, 40, 60, 80 and 100 days

| Blank set uncontaminated soil/ without EDTA and DTPA | Control set contaminated soil/ without EDTA and DTPA | EDTA set EDTA at dose; 0.5, 1 and 2 mg/L | DTPA set DTPA atdose; 0.5, 1 and 2 mg/L | EDTA and DTPA set EDTA and DTPA mixing in separate dose; 0.25, 0.5 and 1 mg/L |

Water, soil and plant samples were analyzed for total cadmium with Atomic Absorption Spectrometer (AAs)

Data analyses to determine the relationship between the cadmium removal capacities in plant and cadmium or available cadmium in sediment

Figure 1.1 Diagram of scope of the study

1.5 Expected results

1.5.1 Understand the removal capacities and transport of cadmium from soil, water and plants for the phytoremediation of contaminated sediment.

1.5.2 Understand the relationship between available cadmium and total cadmium accumulation in soil and water hyacinth.

1.5.3 Use the information for the implementation of phytoremediation by water hyacinth of the cadmium contaminated sediment at site.

CHAPTER II
THEORETICAL BACKGROUND AND
LITTERATURE REVIEWS

2.1 Cadmium (Cd)

2.1.1 General properties of cadmium

Cadmium is a soft, ductile, silver-white, lustrous, electropositive metal. The atomic weight of Cd is 112.4; density is 8.64 g cm^{-3}; melting point is 321 °C; and atomic number is 48. Similar to Zn and Hg, Cd is a transition metal in Group II-B in the periodic table. In nature, Cd is usually found in CdS from. Moreover, it is also found in hydroxides and complex ions with ammonia and cyanide e.g., $Cd(NH_3)_6^{4-}$ and $Cd(CN)_4^{2-}$. Furthermore, Cd is at times found in a variety of complex organic amines, sulfur complexes and chelates. Cd ions are insoluble with carbonates, arsenates, phosphates, oxalates and ferrocyanides. Cadmium is easily soluble in nitric acid but reacts more slowly in hydrochloric and sulfuric acid (Adriano, 2001). Cadmium is produced commercially as a by-product of the Zn industry. The most important uses of Cd are as alloys, in electroplating (auto industry), in pigments (cadmium sulfide, cadmium selenide), as stabilizers for polyvinyl plastics, and in batteries (rechargeable Ni-Cd batteries). In addition, cadmium is also used in photography, lithography, process engraving, rubber curing and as a fungicide primarily for golf course greens (Adiano, 2001)

2.1.2 Cadmium in soil

Cadmium concentration in soil ranges from low in uncontaminated soil to high in soil receiving large quantities of cadmium through anthropogenic activities or in soil naturally rich in cadmium. In natural soils, cadmium concentration is largely influenced by the amout of cadmium in the parent rock. The average content of soil cadmium in soil is between 0.06 and 1.1 mg/kg (Kabata-Pendias, 2001). However, it

is expected to be much less than this in most soil (Alloway, 1995). In soil solution, dissolved cadmium may also form several complex ions ($CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $CdCl_4^{2-}$, $Cd(OH)_3^-$ and $Cd(OH)_4^{2-}$). However, the most prevalent factor valance state of cadmium in the natural environment is Cd^{2+} (Kabata-pendias, 2001).

Cadmium in uncontaminated soil may have been derived in situ from the weathering of minerals in underlying parent rock or from precipitation or accumulation of transported fragments or particles via hydraulic or atmospheric media. Varying amounts of cadmium in soil depend much upon the lithology and geography of the area where soil was formed. For example, the average cadmium concentrations in agricultural soils in remote locations in the USA (3054 samples) were found to be 0.27 mg/kg (Holmgren et al., 1993).

Present concentrations of cadmium in top soil are reported to be very high in the vicinity of lead and zinc mines. Adriano (2001) reported that areas affected by smelting operations showed cadmium concentrations ranging from 0.20 to 350 ppm in the surface soil. It has become apparent that cadmium from metallurgical activities (mining and smelting) is likely to be more bioavailable to organism than cadmium from unimpacted soils (Asami et al., 1988 and Chopecka et al., 1996).

2.1.3 Cadmium in plants

Cadmium is a non-essential element in plant nutrition. Under normal conditions, plants take up small quantities of Cd from soils. In a survey in the United States, samples of wheat and perennial grasses were collected. The levels of Cd found were below 0.30 ppm (wheat 0.20 ppm; grasses, 0.17 ppm) (Huffman and Hodgson, 1973). Cd uptake is related to soil factors. Species and genotype also influence the total uptake (Adriano, 2001).

Radish shoots can accumulate 5 ppm of Cd when grown on soils containing 0.6 ppm Cd (Lagerwerff, 1971). Leaf plants, such as lettuce, spinach and turnip greens accumulated 175 to 354 ppm Cd when grown on soils pretreated with sewage sludge enriched with Cd at up to 640 ppm (Bingham et al., 1975). Fruits and seeds of other plants tested usually accumulated no more than 10 to 15 ppm. Cadmium is rather readily translocated throughout the plant following its uptake by roots.

Distribution between roots and shoots differs with plant species, rooting medium, and time of treatment. In rice, about 99% of the total Cd taken up by the plants was found in the shoot in a wide range of redox potentials and pHs (Reddy and Patrick, 1977). Some environmental factors, such as Cd concentration in the medium and ambient temperature, can affect the distribution of the metal between the shoots and roots of the rice plant (Chino and Baha, 1981).

2.2 Chelating agent

A combination of metal and a chelating agent is called a chelation in which the metal is part of a ring. Chelator or chelating agent is an organic ligand, the chelate is a metal complex. If the size of ring in a metal atom is bigger, the compound is more stable. This phenomenon is called the chelate effect; it is generally attributed to an increase in the thermodynamic quality called entropy that accompanies chelation. The amout of atoms in the chelate ring is related to the stability of chelate. Monodentate ligands are easily broken out of other chemical processes because they have only one coordinating atom, while polydentate ligands can be more stable complexes because the polydentate ligands can be donated by multiple bonds to the metal ion. Chlorophyll is a green plant pigment, that consists of a central magnesium atom joined with complex chelating agents (pyrrole ring). The molecular structure of the chlorophyll is similar to that of the heme bound to proteins to form hemoglobin except that the latter contains iron (II) in the center of the porphyrine. Heme is an iron chelate. The application of chelating agents can be used in chemotherapeutic treatments for metal poisoning. Chelating agents offer a wide range of sequestrates to control metal ions in aqueous systems. By forming stable, water soluble complexes with multivalent metal ions, chelating agents prevent undesired interaction by blocking normal reactivity of metal ions. EDTA (ethylenediamine tetraacetate) is a good example of a common chelating agent which has nitrogen atoms and short chain carboxylic groups. The sodium salt of EDTA is used as an antidote for metal poisoning, an anticoagulant, and an ingredient in a variety of detergents. Chelating agents are important in the field of soap, detergents, textile dyeing, water softening,

metal finishing and plating, pulp and paper making, enzyme deactivation, photo chemistry and bactericides.

2.2.1 Ethylene Diamine Tetraacetic Acid; EDTA

1) Specification of EDTA

Ethylene diamine tetraacetic acid or EDTA is a synthetic chelating agent is a poly amino carboxylic acid and a colorless, water-soluble solid (Figure 2.1 and Table 2.1).
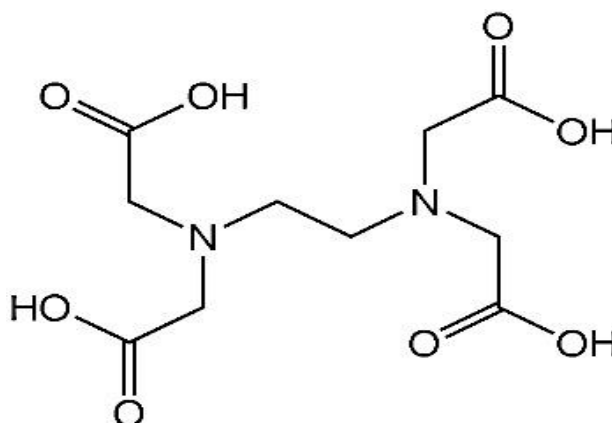
Figure 2.1 Structure of EDTA

Source: Maryadele et al. (2001)

Table 2.1 Specification of EDTA

Properties	Details
Chemical formula	$C_{10}H_{16}N_2O_8$
Molecular weight	292.25 g/mol
pH	2.5-3.0
Bulk Density	0.86 g/cm^3
Meiting points	240 ^{0}C
Chelation value	3.39 mmol/g
Solubillity at 20 ^{0}C	0.4 g/l (0.05 g/100 ml)

Source: Chemical (2003) and Maryadele et al. (2001)

2) Advantage of EDTA

EDTA is widely used in industrial activities such as paper production, photography and clothing manufacturing. Additionally EDTA are widely used in cleaning products and for metal removal and pharmaceutical production (Oviedo and Rodriguez, 2003).

- **Treat soil**: Metal contamination in soil can cause negative effects on human health and the life cycle of plants and animals. EDTA is used for sequestering such heavy metals. Even though the bonds created by EDTA will not last long since they are biodegradable, it can still offer huge benefits in soil treatment.

- **Preserves food:** The EDTA utilization were use to remove trace metals like copper, nickel and iron in food manufacturing that may have entered food during processing and harvesting. Thus metals may speed up food spoilage and breakdown through catalyzing fat oxidation. Even though the metals still persist in the foods, the EDTA forms a bond with them such that they cannot catalyze any oxidation. EDTA therefore works effectively in food preservation.

-**Aids emergency treatment**: The advantage of EDTA for medical, It is used for decreasing dangerously high levels of calcium in blood. In fact, in emergency instances of digitalis caused poisoning, the use of EDTA may prevent death. Digitalis is normally used for strengthening contractions and slowing heart rate. Overmedication may result in heart arrhythmia. However, EDTA intake may prevent such heart problems from developing.

- **Chelation**: Chelation refers to the process of enhancing the elimination of various trace metals from the body. EDTA is particularly efficient in dealing with lead poisoning. Those with iron, arsenic and mercury poisoning can also use EDTA to get relief. EDTA taken orally may cause gas, general discomfort and bloating. In rare instances, the stomach discomfort may result in constipation.

3) Decomposition of EDTA

Meers et al. (2005) studied the efficiency of EDDS and EDTA in several concentrations: 0.8, 1.6 and 4 mmol/kg soil. The results in this study showed that the initial concentration did not differ significantly from that at 40 days after adding EDTA solution. From this result Meers et al. (2005) estimate the half-life of EDTA equal to 36 days.

Ginkel et al. (1999) studied the decomposition of EDTA in water. In this study they added Na_2EDTA at 8 mg/L of water (from river and lake), at pH 6.5 and 8 in a closed system. The results did not find decomposition of EDTA at pH 6.5 in 28 days. After 49 days the decomposition of EDTA increased to 60-83%. For pH 8 the decomposition of EDTA was 53-72% and 75-89% at 28 and 35 days, respectively.

4) Toxicity of EDTA

In general, EDTA and its salts are mild skin irritants but considered severe eye irritants. A report by the Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE, 2003) concluded "both EDTA and tetrasodium EDTA are mild skin irritants, but comparatively potent eye irritants". Similarly, tetrasodium EDTA should not be applied to the eye unless first neutralized, because it forms a solution sufficiently alkaline to be injurious to the eye (Grant, 1986 as cited in TOXNET).

The greatest risk in the human body occurred when the EDTA attempts to scavenge the trace metals used and required by the body. The various toxicity studies, particularly Kimmel (1977) and Schardein et al. (1981) studies, indicate that developmental effects occurred if the body is not properly supplemented with necessary trace metals.

Several short term studies, reviewed by FAO/WHO in 1974, reported no adverse effects from administering doses up to 5% of EDTA and its salts to lab rodents daily for several weeks. Only diarrhea and lowered food consumption were reported in animals given 5% disodium EDTA. However, abnormal effects were seen in animals that were fed mineral deficient diets. Abnormal symptoms were observed in male and female rats fed a low mineral diet (0.54% Ca and 0.013% Fe) with the addition of 0%, 0.5%, or 1% disodium EDTA for 205 days. Rats fed a low percent of disodium EDTA in the diet for short term studies with adequate minerals showed no signs of toxicity. Rats fed 0.5% disodium EDTA for 44-52 weeks were without deleterious effects on weight gain, appetite, activity and appearance. Rats fed 1% disodium EDTA with adequate mineral diet for 220 days showed no evidence of dental erosion.

Disodium EDTA administered by different routes; 3% in diet, gastric intubation, or subcutaneously, produced different teratogenic rates in rats (Kimmel

1977). Disodium EDTA administered to pregnant rats on days 7 to 14 of gestation by dietary admixture (954 mg/kg/day) produced maternal toxicity and fetal death and malformations in 71% of the offspring. Rats given 1,250 mg/kg or 1,500 mg/kg by gavages exhibited more maternal toxicity than the diet group, but produced only 21% malformations in the offspring at the lower dose. The subcutaneous administration of 375 mg/kg was also maternally toxic, but did not result in malformations in the offspring. Differences in toxicity and teratogenicity are probably related to absorption differences and interaction with metals. Animals in the study by Kimmel (1977) were maintained on deionized water and possibly became zinc deficient, thus causing teratogenicity in the offspring. Similarly, EDTA and four of its salts (disodium, trisodium, calcium disodium, and tetrasodium) were administered to pregnant rats during Days 7 and 14 of gestation (Schardein et al., 1981). Equimolar doses based on 1,000 mg/kg (58.4 to 83.2 mg/ml) given by gastric intubation produced no teratogenic effects on the offspring, even at maternally toxic doses. Unlike the study by Kimmel (1977), the rats were given tap water of labium and probably did not suffer from zinc deficiency.

The Agency reviewed data from an early teratogenicity study submitted for disodium EDTA (USEPA, 1979). Female Sprague-Dawley rats were administered disodium EDTA in the diet ranging from 2% to 3%, or 3% EDTA plus 1,000 ppm zinc, during pregnancy. The conclusions in the memo reported that "disodium EDTA ingested during pregnancy is teratogenic in rats at 2% in the diet and greater." However, it was also concluded that the diet "supplemented with 1000 ppm zinc prevented the detrimental effects of EDTA during pregnancy in the rat". Effects from disodium EDTA in the young were likely due to "an induced deficiency of zinc...." and that "cells undergoing rapid growth and development are particularly sensitive to deficiency of zinc". Likewise, evaluation of EDTA and tetrasodium EDTA by the CSTEE (2003) concluded that "teratogenicity is most likely due to zinc depletion by the very high doses applied...."

The FAO and WHO Expert Committee on food additives (1974) reviewed acute toxicity data for calcium disodium EDTA and disodium EDTA. The Expert Panel commented that "the use of calcium disodium EDTA is preferable to that of disodium EDTA." In fact, the Expert Panel page 16 of 28 concluded that "because of

disodium EDTA's effect on calcium, the use of disodium EDTA as a food additive was not recommended." However, the Committee also concluded that "under certain circumstances, necessitating an accurate complexing of calcium, disodium EDTA may be used provided no excess of disodium EDTA remains and the only compound finally present is calcium disodium EDTA."

A 2002 safety assessment of EDTA, calcium disodium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA was performed by an expert panel of the Cosmetic Ingredient Review (CIR). This assessment considered numerous toxicological studies, including various acute, subchronic, and chronic/carcinogenicity toxicity studies, and mutagenicity studies. This report also details extensive use of these EDTA salts in numerous cosmetic products with EDTA salt formulations most commonly used at 2%, although a few formulations were reported using up to 10% and 25%. Based on the available information, the panel concluded that "EDTA, calcium disodium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA, are safe as used in cosmetic formulations."

Trisodium EDTA was tested in a bioassay for carcinogenicity by the National Cancer Institute. Trisodium EDTA administered to male and female rats at low (3,750 ppm) or high (7,500 ppm) concentrations for 103 weeks produced no compound-related signs of chemical toxicity, and tumor incidence was not related to treatment (NCI, 1977). The CSTEE (2003) also evaluated this study by the National Cancer Institute and concluded that "there is no concern for EDTA with regard to carcinogenicity."

EDTA has been demonstrated to affect inhibition of DNA synthesis in primary cultures of mammalian cells, which may be due to impairment of enzymes involved in DNA replication (Heindorff et al., 1983). EDTA has also been demonstrated to enhance mutagen-induced aberration frequencies in *Drosophila melanogaster*, *Chlamydomonas reinhardi*, *Neurospora crassa* and *Zea mays* by interfering with the DNA repair process that takes place after exposure to mutagens (Heindorff et al., 1983).

Mutagenicity studies such as mouse lymphoma were negative for EDTA and its salts except for a few positive tests when administered with sterile distilled water. Genotoxicity studies for EDTA and its salts were mixed positive and negative results, depending on assay type and cell type (CCRIS 2003 and Genetox 2003). The RTECS (2003) database for EDTA reported the following mutation data: DNA damage in mouse lymphocyte at 40,500 μmol/L; DNA inhibition in hamster fibroblast at 500 μg/L and in rat other cell types at 600 μmol/L; unscheduled DNA synthesis in hamster embryo at 100 μmol/L; mutation in mammalian somatic cells in mouse lymphocyte at 25, 200 μmol/L; and sister chromatid exchange in hamster embryo at 30 μmol/L.

2.2.2 Di-Ethylene Tri-Amine Penta Acetic Acid; DTPA

1) Specification of DTPA

Diethylenetriamine pentaacetic acid (DTPA) is a polyamino carboxylic acid, which consists of a diethylenetriamine backbone modified with five carboxymethyl groups (Figure 2.2 and Table 2.2). These acids are available in a wide range of compositions including DTPA (Diethylene Triamine Penta Acetic Acid), DTPA Na5 (Penta Sodium Salt Diethylene Triamine Penta Acetic Acid), DTPA potassium hydroxide (DTPA K5) andDTPA Fe DTPA pure acid. Its uses range from extensive application in photography, detergent manufacturing, chemical plating, electroplating without cyanide, cleaning agent formulas, and plastic additives.
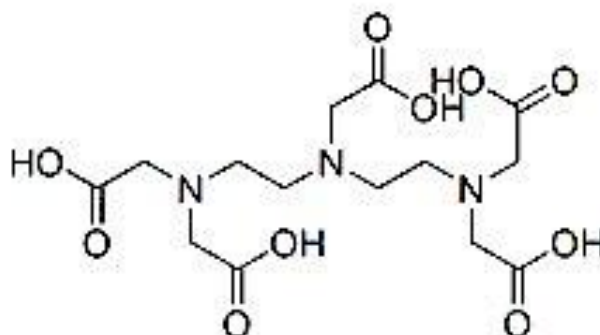
Figure 2.2 Structure of DTPA

http://chemistry.about.com/od/factsstructures/ig/Chemical-Stuctures---P/Penetic-Acid--DTPA.html

Table 2.2 Specification of DTPA

Properties	Details
Chemical formula	$C_{14}H_{23}N_3O_{10}$
Molecular weight	393.347 g/mol
pH	2.1- 2.5
Bulk Density	1300 g/cm^3
Meiting points	220 $°c$
Chelation value	255 mg CaCo^3 / g
Solubillity at 25 ^{0}C	4,800 mg·l (eau, 25 °C)

Source: http://www.avachemicals.net/dtpa.html

2) Decomposition of DTPA

Mika et.al., 2000 studied the chemical decomposition of β-alaninediacetic acid (ADA) and diethylenetriaminepentaacetic acid (DTPA) in a pilot-plant flow-through system simulating alkaline (pH 10−11) hydrogen peroxide bleaching environments. The study looked at the amount of hydrogen peroxide decomposition and a distribution calculation was performed. Under the conditions investigated, ADA was more degradable than DTPA (average residual 71% and 94%). The decomposition of hydrogen peroxide was not dependent on the chelate; the residual percent of hydrogen peroxide was 40 in both cases.

2.3 Phytoavailability

Phytoavilability is the potential of living organisms to take up chemicals from biotic and abiotic environment (i.e, external) to the extent that the chemicals may become involved in the metabolism of the organism. More specifically, it refers to biologically available chemicals that can be taken up by an organism. More specifically, it refers to biologically available chemicals that can be taken up by an organism and can react with its metabolic machinery (Campbell, 1995). It refers to the fraction of the total chemical that can interact with a biological target (Vangronsveld and Cuningham, 1998)

Since soil can be affected by industrial emission of metals, it is important to discuss some concepts pertinent to uptake and bioaccumulation. The sensitivity and tolerance of plant to excess metals is influenced by plant species and genotypes. Even among crops, sensitivity varies widely, with members of the Brassicaceae family generally considered as the most tolerant in terms of accumulation. In general, plants can be divided into three categories: excluders, indicators and accumulators (Figure 2.3). Excluders include members of the grass family (e.g., sudan grass, brome grass and fescue etc.) for their known insensitivity to metals over a wide range of soil concentrations; indicators include the grain and cereal crops (e.g., corn, soybean, wheat and oats etc.), and accumulators include the mustard and compositae families (e.g., lettue, spinach, chard and tobacco). There are extreme accumulators (know as hyperaccumulators) that seem to even thrive in heavily contaminated soils (or near ore deposits) and survive through a tolerance mechanism; in contrast, excluders survive through avoidance (or restriction) mechanism (Baker, 1987). Hyperaccumulators are plants and/or genotypes that accumulate metals above certain concentrations is leaves. Greger (1991) suggested hyperaccumulators should contain trace metals in leaves above the following levels (in ppm) : >100 Cd, >1000 Co, Cu, Ni and Pb, and >10000 Mn and Zn.
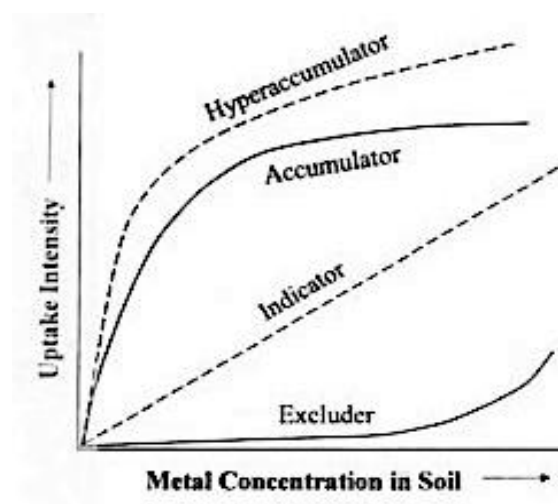
Figure 2.3 Relative uptake and bioaccumulation potential among plant species

Source: Adriano, 2001

In the leaves, metal ions may be incorporated into proteins or translocated around the plant in the phloem with photosynthetic. The relative distribution of heavy metals in plant tops, compared with their concentrations in nutrient or soil solutions is shown in Figure 2.4. Following root absorption, the extent to which elements decrease follows in the order Cd>B>Zn>Cu>Pb (Alloway, 1995).
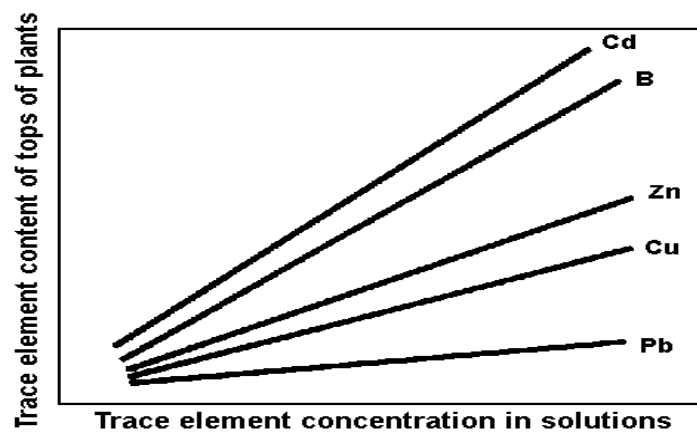
Figure 2.4 Trace element uptakes by plants as a function of their concentrations in nutrient solution

Source: Kabata-Pendias, 2000 and Alloway, 1995

2.3.1 Factor affecting mobility and bioavailability

1) pH

In general, the retention capacity of soils for trace metals increases when pH increases. Heavy metal, except As, Mo, Se, V and Cr are commonly more mobile under alkaline conditions. Accordingly, a decrease in plant uptake of B, Co, Cu, Mn and Zn was observed when pH value was around 5-8 (Hodgson, 1963).

The pH is the important factor because it can affect the surface charge of silicate layer clays, OM and oxides of Fe and Al. In addition, the effect on the sorption of cation could increase with an increase of pH value (Figure 2.5), and complexation with OM. It also influences the precipitation-dissolution reaction, redox

reactions, mobility and leaching, dispersion of colloids, and the eventual bioavailability of the metal ions.
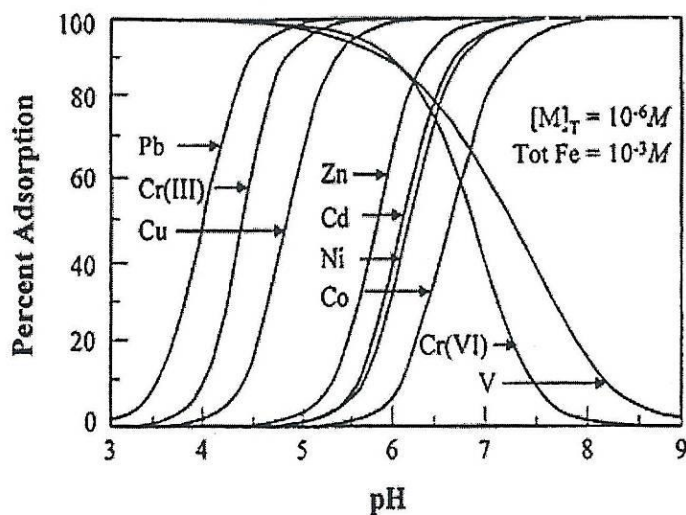
Figure 2.5 Modeled adsorptions of certain trace elements onto hydrous ferric oxide

Source: Adrianos, 2001

The optimal pH for crops is between 6 to 7. The number of plant species that may tolerate soil pH below 5.5 is limited to only a few agronomic (e.g., potatoes) and horticultural (e.g., azaleas and blueberries etc.) species. Above pH 7, the risk of micronutient denficiency including for Fe, Zn, Mn and B increased.

The effect of pH on chemical speciation of trace elements in soil and sediments is illustrated in Table 2.3.

Table 2.3 Expected dominant oxidation states and chemical species of trace elements in aqueous solution.

Element	Acid soils/sediments	Alkaline soils/sediments
Cd (II)	Cd^{2+}, $CdSO_4^0$, $CdCl^-$	Cd^{2+}, $CdCl^-$, $CdSO_4^0$, $CdHCO_3^+$
Cu (II)	Cu^{2+}, $CuCl^-$	$CuCO_3^0$, $CuHCO_3^+$
Pb (II)	Pb^{2+}, $PbSO_4^0$, $PbHCO_3^+$	$PbCO_3^0$, $PbHCO_3^+$, $Pb(Co_3)^{2-}$, $PbOH^+$
Zn (II)	Zn^{2+}, $ZnSO_4^0$	$ZnHCO_3^+$, $ZnCO_3^+$, Zn^{2+}, $ZnSO_4^0$

Source: Adriano, 2001

2) Cation exchange capacity

The cation exchange capacity (CEC) of soil is largely dependent on the amount and type of clay, OM and the oxidation of Fe, Al and Mn. Soil components have different cation exchange properties. In general, the higher the CEC of a soil, the larger the amount of metals a soil can retain without potential hazards. The CEC can be viewed as a general but imperfect indicator of soil components (i.e., clay, OM and oxide), which may limit the solubility and mobility of metals instead of being a specific factor in the bioavailability of these metals.

The mix of clay, silt and sand influences the CEC of soils. In general, high clay content causes high CEC. The CEC of soil is largely proportionate to the surface area of individual components. The degree of CEC by type of soil is clay > silt > sand.

3) Oxidation-reduction potential

The moisture content of soils influences their retention of trace metals through oxidation-reduction reactions. In oxidized soils, the range of redox potential was +400- +700 mV. In sediments and flooded soils, redox potential may range from around -400 (strongly reduced) to +700 mV (well oxidized) (Gambrell and Patrick, 1978). Under reducing conditions, heavy metals present in sulfide form. The metal-bearing sulfides are quite insoluble so metal mobility and bioavailability are considerably less than expected in oxidized soils Elemental concentrations in solution extracted from sludge treated soil indicate that under reducing conditions solubility of Cd, Cu and Zn were decreased but increased solubility was found for Mn and Fe (Bingham et al., 1976).

2.3.2 Interactions between metals and other elements

Figure 2.6 summarizes the known interactions between trace elements within plants and in the soil at the root surface where interactions can affect absorption. Antagonistic and synergistic interactions can also occur between heavy metals and major elements.
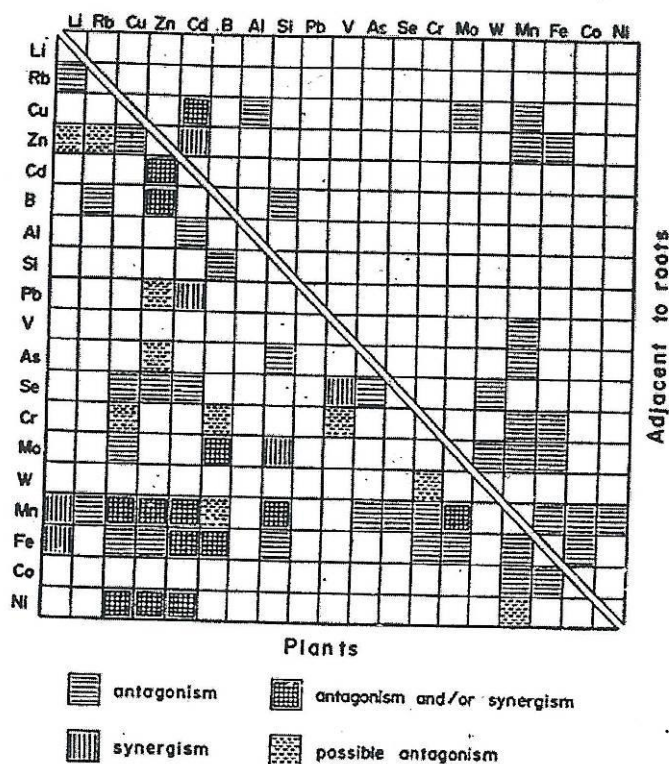
Figure 2.6 Interactions of trace elements within plant organisms and adjacent to plant root

Source: Kabata-Pendias, 2000 and Alloway, 1995

Antagonism occurs when the combined physiological effect of two or more elements is less than the sum of their independent effects, whereas synergism occurs when the combined effects of these elements is greater.

Cu-Zn interaction is commonly observed. These metals apparently are absorbed by the same mechanism and each may competitively inhibit root absorption of the other.

Cu-cd interaction is reported as both antagonistic and synergistic in the element uptake by root. Synergism may be a secondary effect of the damage to membrane due to the imbalanced proportion of the metals

Zn-Cd interactions appear to be somewhat controversial, since there are reports of both antagonism and synergism between the two elements in the uptake-transport process. Zn reduces the uptake of Cd by both root and foliar systems.

Zn-Cu antagonistic interactions have been observed in which the uptake of one element was competitively inhibited by the other.

The interference of Pb with trace elements has been reported only for Zn and Cd (Figure 2.4). The stimulating effect of Pb on Cd uptake by plant roots may be a secondary effect of the disturbance of the transmembrane transport of ions. The Zn-Pb antagonism adversely affects the translocation of each element from roots to tops.

2.4 Heavy metal toxicity in plant

Excessive concentrations of both essential and non-essential metals result in phytotoxicity. The possible causal mechanisms are as follow (Kabata-Pendias, 2001):

1) Changes in the permeability of the cell membrane: Ag, Au, Br, Cd, Cu, Hg, I, Pb and UO_2.

2) Reactions of sulphyldryl ($^-$SH) groups with cations: Ag, Hg and Pb.

3) Competition for sites with essential metabolites: As, Sb, Se, Te, W and F.

4) Affinity for reacting with phosphate groups and active groups of ADP or ATP: Al, Be, Y and Zr, lanthanides and, possibly, all heavy metal.

5) Replacement of essential ion (mainly major cations): Cs, Li, Rb, Se and Sr.

6) Occupation of sites for essential group such as phosphate and nitrate: arsenate, fluorate, borate, bromate, silenate, tellurate and tungstate.

Although the relative toxicity of different metals to plants can vary with plant genotype and experimental conditions, excessive Hg, Cu, Ni, Pb, Co, Cd and possibly also Ag, Be and Sn produce toxic effects in plants and microorganisms. This may result from:

1) Selective uptake of ions.

2) Decreased permeability of membranes of other differences in the structure and function of membranes.

3) Immobilization of ions in roots, foliage and seeds.

4) Removal of ions from metabolism by deposition (storage) in field and/or insoluble forms.

5) Alteration in metabolic patterns-increased enzyme system that is inhibited or increased by antagonistic metabolite, or reduced metabolic pathway by-passing an inhibited site.

6) Adaptation to toxic metal replacement of a physiological metal in an enzyme.

7) Release of ions from plants by leaching from foliage, guttation, leaf shedding and excretion from roots

The visible symptoms caused by the effect of heavy metal to the plants are shown in table 2.4

Table 2.4 General effects of trace element toxicity on common cultivars

Element	Symptoms	Sensitive Crop
Cd	Brown margin of leaves, chlorosis, reddish veins and petioles, curled leaves, and brown stunted roots. Severe reduction in growth of roots tops and number of tillers (in rice). Reduced conductivity of stem, caused by deterioration of xylem tissues. Reduction of chlorophyll and carotenoids	Legumes (bean, soybean), spinach, radish, carrots and oat
Cu	Dark green leaves followed by induced Fe chlorosis, thick, short or barbed-wire roots, depressed tillering. Changes in lipid content and losses of polypeptides involved in photochemical activities disease of rice	Cereals and legumes, spinach, citrus seedlings and gladiolus
Pb	Dark green leaves, wilting of older leaves, stunted foliage and brown short roots	-
Zn	Chlorotic and necrotic leaf tips, interveinal chlorosis in new leaves, retarded growth of entire plant and injured roots resemble barbed wire	Cereals and Spinach

Source: Kabata-Pendias, 2000

2.5 Phytoremediation

Phytoremediation is defined as the use of plants to remove pollutants from the environment (Baker et al. 1994; Cunningham et al., 1995; Chen et al., 2004; Salt et al., 1998) such as contaminated soil, contaminated sludge, contaminated sediment and waste water. The contaminant can be an organic t or inorganic pollutant. Inorganic pollutants, such as plant trace elements (e.g. Cr, Cu, Fe, Mn, Ni and Zn) and non-essential elements (e.g. Cd, Co and Pb); have been shown to be more difficult to remediate from contaminated soil as they cannot be degraded (Audet and Charest, 2007).

2.5.1 Types of phytoremediation

Several classification schemes were found relating to the types of phytoremediation, the most common of which is presented below.

1) Phytoextraction

Phytoextraction is the uptake of contaminants by plant roots and translocation within the plant (Kumar et al., 1995; Chaney et al., 1997; Wenzel et al., 1999; Lai and Chen, 2004). Contaminants are generally removed by harvesting the plants. This concentration technology leaves a much smaller mass to be disposed of than the excavation of the soil or other media. This technology is most often applied to metal contaminated soil as shown in Figure 2.7 and 2.8 (USEPA, 2000).
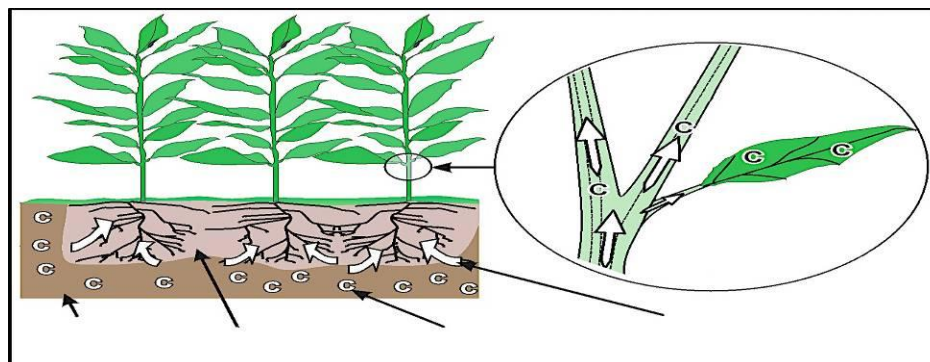
Figure 2.7 Phytoextraction process

Source: http://www.itrcweb.org/PHYTO02.pdf

2) Phytodegradation

Phytodegradation (also known as phytotransformation) is the breakdown of contaminants taken up by plants through metabolic process within the plant, or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants. As shown in Figure 2.8, the main mechanism is plant uptake and metabolism. Additionally, degradation may occur outside the plant, due to the release of compounds that cause transformation. Any degradation caused by microorganisms associated with or affected by the root of plant is considered biodegradation.

3) Phytovolatilization

Phytovolatillization (Figure 2.8) is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified from of the contaminant to the atmosphere from the plant through contaminant uptake, plant metabolism and plant transpiration. Phytoremediation is a related phytoremediation process that can occur along with Phytovolatilization.

4) Rhyzodegradation

Rhyzodegradation is the breakdown of an organic contaminant in soil through microbial activity that is enhanced by the presence of the root zone (Figure 2.8). Rhizodegradation is also known as plant-assisted degradation, plant-assisted bioremediation, plant-aided in situ biodegradation and enhanced rhizosphere biodegradation.

5) Rhyzofiltration

Rhyzofiltration is the adsorption or precipitation onto root of plant or absorption into the roots of contaminants that are in solution surrounding the root zone due to biotic or antibiotic processes (Figure 2.8). Plant uptake, concentration and translocation might occur, depending on the contaminant. Exudates from the root of plant might cause precipitation of some metals. Rhizofiltration first results in contaminant containment, in which the contaminants are immobilized or accumulated on or within the plant. Contaminants are then removed by physically removing the plant (Peer et al., 2007).

6) Phytostabilization

Phytostabilization (Figure 2.8) is defined as immobilization of contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants, and the use of plants and plant roots to present contaminant migration via wind and water erosion, leaching and soil dispersion.
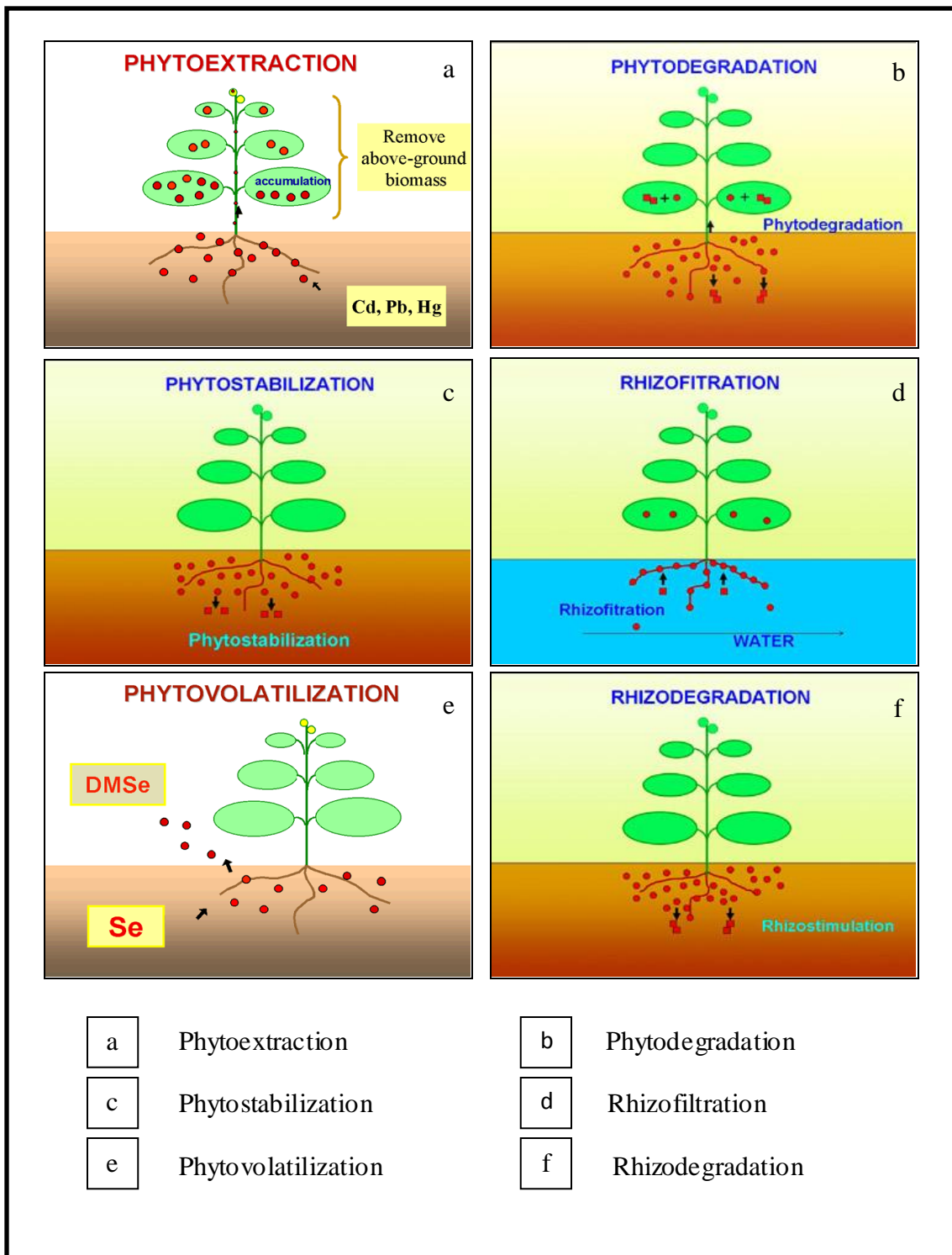
Figure 2.8 Phytoremediation technology

Source: Sampanpanish, 2006

Phytostabilization occurs through root-zone microbiology and chemistry, and/or alteration of the soil environment or contaminant chemistry. Soil pH may be changed by plant root exudates or through the production of CO_2. Phytostabilization can change metal solubility and mobility or impact the dissociation of organic compounds. The plant affected soil environment can convert metals from a soluble to an insoluble oxidation state (Salt et al. 1995). Phytostabilization can occur through sorption, precipitation, complexation, or metal valence reduction (USEPA, 2000). Plants can also be used to reduce the erosion of metal contaminated soil.

The term of phytolignification has been used to refer to a form of phytostabilization in which organic compounds are incorporated into plant lignin (Cunningham et al., 1995). Compounds can also be incorporated into humid material in soils in a process likely related to phytostabilization in its use of plant material.

2.5.2 The process of metal accumulation in plant

1) Solubilization of the metal from the soil matrix

Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential metals from the soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution. To our knowledge, there are no reports of plants with the ability to solubilize Pb from the soil matrix where most soil Pb exists in an insoluble form (Blaylock and Huang 2000). Experiments demonstrating Pb hyperaccumulation have used $Pb(NO_3)_2$, a soluble from of Pb, though it must be questioned whether this is the most appropriate from of Pb for analysis. Aside from Pb, the solubilization mechanisms for hyperaccumulators are similar for metals discussed and therefore will not be addressed independently for each metal. While no hyperaccumulators have evolved to handle high concentrations of toxic metal if they are present in solution, phytoremediator plants could be modified to solubilize contaminants that are bound to the soil.

2) Uptake into the root

Soluble metal can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells (Figure 2.9). While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called the xylem. To enter the xylem, solutes must cross the casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis (Figure 2.9). Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channels intended to transport essential elements or pumping the toxic metal back out of the plant (Hall 2002; Mecharg and Macnair 1992).

3) Transport to the leaves

Once loaded into xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane (Figure 2.9). The cell types where the metals are deposited vary between hyper accumulator species. For example, *T. caerulescens* was found to have more Zn in its epidermis than in its mesophyll (Kupper et al. 1999), while *A. halleri* preferentially accumulates its Zn in its mesophyll cells instead of its epidermal cells (Kupper et al. 2000).

4) Detoxification/Chelation

At any point along the pathway, the metal could be converted to a less toxic form through chemical conversion or by complexation. Various oxidation states of toxic elements have very different uptake, transport and sequestration or toxicity characteristics in plants. Chelation of toxins by endogenous plant compounds can have similar effects on all of these properties as well. As many chelators use the group as ligands, the sulfur (S) biosynthetic pathways have been shown to be critical for hyperaccumulator function (Ng and Anderson 1979; Pickering et al. 2003; Van huysen et al. 2004) and for possible phytoremediation strategies. Oxidative stress is one of the most common effects of heavy metal accumulation in plants, and the increased anti-oxidant capabilities of hyper accumulators allow tolerance of higher concentration of metals (Freeman et al. 2004).

5) Sequestration/Volatilization

The sequestration of the metal is the last step for the accumulation of most metals away from any cellular process it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal/metal-ligand must be transported across the vacuolar membrane. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge site on the cell walls may interact with polyvalent cations (Wang and Evangelou, 1994). Selenium can be volatized through the stomata.
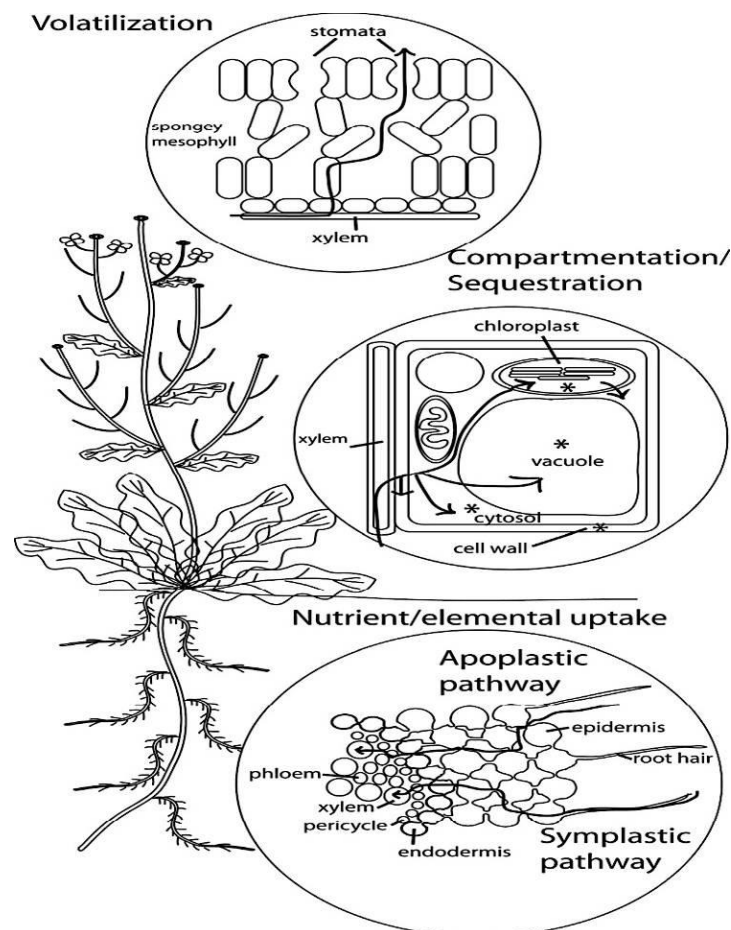
Figure 2.9 Pathway of metal a nutrient uptake in plant

Source: http://www.hort.purdue.edu/hort/research/murphy/pdfs/metals11.pdf

2.5.3 Advantages and disadvantages of phytoremediation

When using phytoremediation there are many positive and negative aspects to consider. The advantage and disadvantages are listed below.

1) Advantages

- Can be use on organic and inorganic compounds
- Can be apply for In Situ/Ex Situ
- Easy to implementation and maintainance
- Lower cost than other treatment methods
- Environmentally friendly and aesthetically pleasing to the public
- Diminishs the amount of wastes to be added to land fills

2) Disadvantages

- May depend on climatic conditions
- Harvested biomass from phytoextraction may be classified as a RCRA hazardous waste
- Limitted to sites with superficial contamination within rooting zone
- May use several timess to remediate
- Consumption of contaminated plant tissue is also a concern
- Causes negative effect on the food chain

2.6 Water hyacinth (*Eichhornia crassipes*)

Eichhornia crassipes (Water hyacinth) is an aquatic, perennial and herbaceous plant. It is usually free floating on the rivers, canals or ponds. The native land of Water hyacinth is Amazons river in Brazil, South-Africa. The flower of the water hyacinths are violet like orchids, they can growth well and quickly. Water hyacinth can cause negative effect in transportation and the water quality.

In Thailand, water hyacinth was introduced from Indonesia in 1896 and is considered to be among the most important water weeds in Thailand. Three species of grasshopper (Atractomorpha crenulata, Gesonula punctifrons and Oxyaminuta), two cutworms (Spodoptera litura and S.mauritia) and sphingid moth (Hippotion echeclus) were found to attack monochoria hastate and M. vaginalis. The glasshopper G.

punctifrons is widely distributed in China, India, Myanmar and Taiwan (Sankaran et al., 1966) and in Thailand it is widespread on water hyacinth, causing obvious leaf damage when populations are high. Although the other insects mentioned are polyphagous and some are known crop pests, G. punctifrons was found to feed to only a limited extent on Colocasia spp., Caladium spp. and Ipomoea aquatic and was not known to be an important pest of crops in fields (Burikam and Napompeth, 1980). Amoung plant pathogens were the fungi Alternaria eichhorniae, Myrothecium roridum and Rhizoctonia solani, but only A. eichhorniae was specific to water hyacinth. However it mainly attacked older plants and did not act as a useful control agent (Napompeth 1982, 1984, Napompeth et al. 1977, Ponnappa, 1976). Release of N. eichhorniae started in 1978 and after initial failures, the weevil is now established widely (Napompeth 1984) and significant reduction of water hyacinth has been observed in all major bodies of water. Those utilizing water hyacinth for handicrafts are now complaining of poor quality plants. Where water hyacinth is now under a significant measure of control other aquatic weeds are becoming important.

2.6.1 Taxonomy of *Eichhornia crassipes*

Taxonomy of *Eichhornia crassipes* (Cronquist, 1988; Thorne, 1992; Takhtajan, 1997) was studied following water hyacinth taxonomic placement (Center et al., 2002) as follow.

Division	: Magnoliophyta
Class	: Liliopsida
Subclass	: Commeinidae
Superorder	: Commelinanae
Order	: Pontederiales
Family	: Pontederiaceae
Genus	: Eichhornia
Specific epithet	: *crassipes* (Martius) *Solms-Laubach*.

2.6.2 Morphology of *Eichhornia crassipes*

Water hyacinth is the most investigated species of the family Pontederiaceae. A comparative account of Pontederiaceae and also summarized the important features of different species. Since then, numerous studies have been made on one or more organs of the plant body.

The free-floating plant body is comprised of a shoot with a rosette of petiolate leaves, a terminal inflorescence and numerous roots hanging in water (Figure 2.10). The structure and development of these organs is only briefly described below.

1) Shoot

The water hyacinth is a plant with bunched leaves; each shoot has more than 2 leaves. It has sheaths at the base of the bunch. The sheath is mingled white and soft-green when it is young and changes to brown when it is older. The sheath joins with the stolon and lays along the surface of water. This produces a new generation. There is more than one stolon in each plant (Water Quality management Bureau, Pollution Controll Department, 2002).

Figure 2.10 Water hyacinth- a flowering plant

Source: Brij Gopal, 1987

2) Leaf

Water hyacinth have simple leaves. They consist of blade and petiole, the blade is reniform or cordateand usually wider than long or nearly the same when it is young. The end of the leaf is nearly round but changes to a sharp point when it is older and has developed a dark color. The renation which transports water and food is a parallel style. The petiole is smooth and well rounded. If the water hyacinth float far away from each other, the shoots are small and the petoile usually bulge out to act as a pontoon which is called a "buoyancy leaf". If the water hyacinth lives in crowned groups (figure 2.11) the petiole will not bulge out and it gets very long. (Water Quality management Bureau, Pollution Controll Department, 2002).
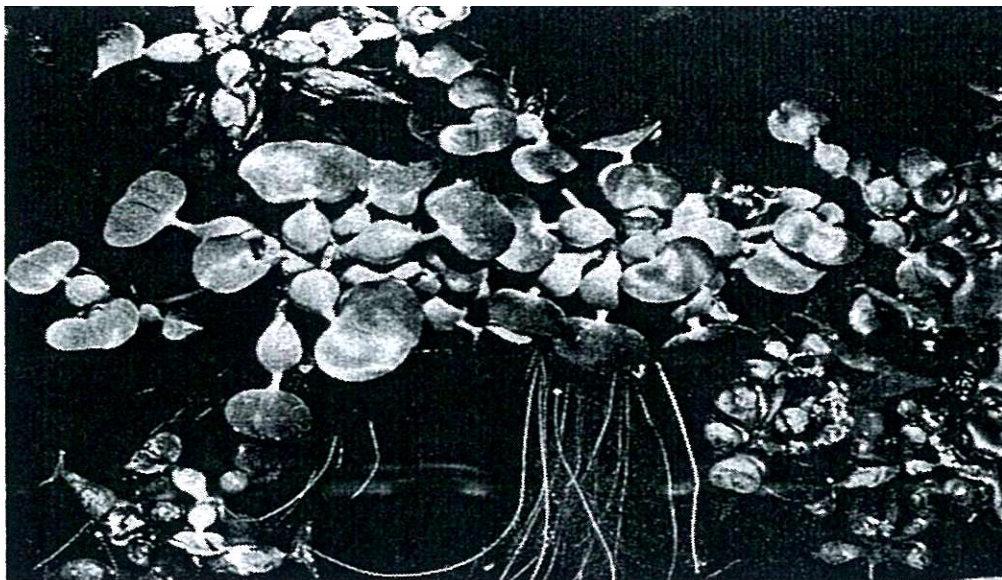
Figure 2.11 Crownded group of plants leaves with bulbous floats

Source: Brij Gopal, 1987
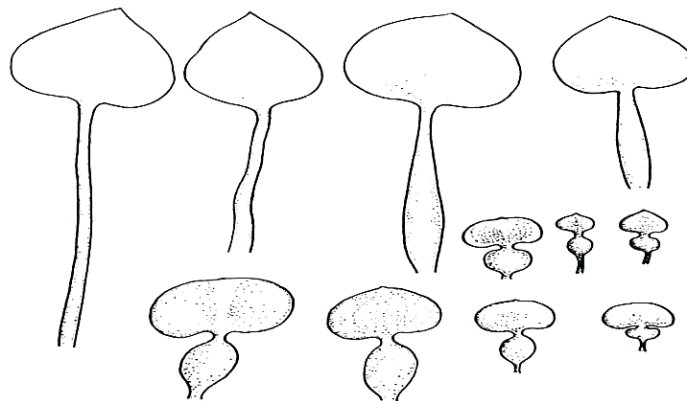
Figure 2.12 Range of variation the shape and size of the leaves of water hyacinth

Source: Brij Gopal, 1987

3) Root

Water hyacinth's roots are fibrous. The rootlets are grouped, well rounded and white. When the roots get older they are soft brown with brown and black root hairs. The length of each root is related to the age of the water hyacinth. Some roots have been measured at 60-90 cms. (Water Quality management Bureau, Pollution Controll Department, 2002).

4) Flowers and seeds

The water hyacinth has blue or purple flowers, inflorescence and no spike. The smallest influorescence have about 4 - 5 flowers. The big ones have about 60 flowers. There is a small leaf at the end of petiole which will become a bract of the peduncle. Each flower has a perianth which stick together using a green tube. This green tube joins with the peduncle. The organ perianth is soft purple. It is in the middle and bigger than the rest. There is a yellow spot on the purple surface which makes the flowers more beautiful (Water Quality management Bureau, Pollution Controll Department, 2002).

There are 6 stamen at the botom of the petal. The anther is yellow and the pistil has purple stigma at the end. Strick with the style from the ovary will develop into seed if the plant has been pollinated. The seed is very small and brown but it is hardly found in Thailand because of the environment.

2.6.3 Breeding and Seedling of *Eichhornia crassipes*

Water hyacinth has 2 ways of reproducing; sexual system and asexual system. The sexual system will happen 48 hours after pollination by insects. After 3 weeks the seeds fall into the water and may produce a new plant. Normally water hyacinth will not grow from seeds except in very dry conditions where most of the water hyacinths have died. Later, when the rains return water levels to normal, seeds in the soil may grow into new plants. Normally the most efficient and prolific way of breeding is for the stolons to change into new generations. This allows the plant to increase very quickly.

2.6.4 Chemical compositions of *Eichhornia crassipes*

The largest component of waterhyacinth is water. It makes up 90 – 95% of the weight of the plant. Plant fiber makes up the remaining 5 - 10% as shown in table 2.5.

Table 2.5 Chemical compositions of water hyacinth

Chemical compositions	Shoots (%)	Root (%)
Crude protein	11.1	15.2
Nitrogen (N)	16.8	25.8
Phosphorus (P)	4.3	6.8
Calcium (Ca)	2.1	2.8
Potassium (K)	3.8	4.9
Magnesium (Mg)	0.9	1.6
Iron (Fe)	0.09	0.57
Copper (Cu)	0.05	0.44
Zinc (Zn)	0.2	0.27
Nicle (Ni)	0.07	0.16

From: Mishra et al. (2008)

2.7 Literature reviews

Pramoon (2003) studied cadmium accumulation by *Ipomoea aquatica* in water contaminated for a long term in several concentrations. The results in this study showed that the *Ipomoea aquatica* had cadmium accumulation in high concentration from water contaminated for a short term. The phytotoxicity of *Ipomoea aquatica* leaves showed cadmium concentration from 7.6 ml/L at 30 days to 59.1 ml/L at 130 days. At a concentration of cadmium at 0.446 ml/L, the plants would begin to die after 40 days of growth. This research showed the highest levels of cadmium was stored in the roots, stems and leaves, respectively.

Chen et al. (2004) studied the use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. In pot experiments, the uptake and transport of Pb by vetiver grass from Pb contaminated soils with EDTA at various application levels was investigated. The results showed that vetiver grass has the capacity to tolerate high Pb concentrations in soils. The translocation ratio of Pb from vetiver grass roots to shoots was significantly increased by EDTA addition. The shoot Pb concentration reached 42, 160 and 243 mg kg^{-1} DW and the root Pb concentrations were 266, 951 and 2280 mg kg^{-1} DW in the 500, 2500 and 500 mg Pb kg^{-1} soil, respectively. In the short soil leaching experiment, about 3.7%, 15.6%, 14.3% and 22.2% of the soil Pb, Cu, Zn and Cd were leached from the artificially contaminated soil profile after 5.0 mmol EDTA kg^{-1} of soil application. In the long soil leaching experiment, soil columns were packed with uncontaminated soils and planted with vetiver grass. Heavy metal leachate from the short column experiment was applied to the surface of the long soil column, artificial rainwater was percolated through the soil, and the final leachate was collected at the bottom of the soil columns. The results showed that soil matrix with planted vetiver grass, could reabsorb 98%, 54%, 41% and 88% of the initially applied Pb, Cu, Zn and Cd, respectively, which may reduce the risk of heavy metals flowing downwards and entering the groundwater.

Lai et al. (2004) studied the effects of EDTA on solubility of cadmium, zinc and lead and their uptake by rainbow pink and vetiver grass. Soil was moderately artificially contaminated by cadmium (20 mg kg^{-1}), zinc (500 mg kg^{-1}) and lead (1000 mg kg^{-1}) in pot experiments. Three concentrations of Na_2-EDTA solution (0, 5 and 10 mmol kg^{-1} soil) were added to the contaminated soils to study the influence of EDTA solution on phytoextraction by rainbow or phytostabilization by vetiver grass. The results showed that the concentrations of Cd, Zn and Pb in a soil solution of rainbow pink significantly increased following the addition of EDTA ($p < 0.05$). The concentrations of Cd and Pb in the shoots of rainbow pink also significantly increased after EDTA solution was applied ($p < 0.05$), but the increase for Zn was insignificant. EDTA treatment significantly increased the total uptake of Pb in the shoot over that obtained with the control treatment ($p < 0.001$), but it did not significantly increase the total uptake of Cd and Zn. The concentrations of Zn and Pb in the shoots of rainbow pink are significantly correlated with those in the soil solution, but no relationship exists with concentrations in vetiver grass. The toxicity of highly contaminating metals did not affect the growth of vetiver grass, which was found to grow very well in this study

Hasan et al. (2006) studied the removal of cadmium and zinc in synthetic waste water by using *Eichhornia crassipes*. The solution of cadmium in this experiment was added at 1.0, 2.0, 2.5, 4.0 and 6.0 ml/L. This result shows that the *Eichhornia crassipes* has a good ability to remove both heavy metals. The percentage of cadmium and zinc removed from synthetic waste water was shown to be more than 70% in each level. In addition, *Eichhornia crassipes* accumulated cadmium and zinc more in its roots than stems. Zinc accumulation in plants was found to be higher than cadmium. Especially at the end of the experiment the *Eichhornia crassipes* died at concentration level 4.0 and 6.0 ml/L of heavy metal. This research showed *Eichhornia crassipes* has an enduring level, which depends on the heavy metal concentration level in the solution.

Santos et al. (2006) studied the chelating of EDTA and EDDS on cadmium, zinc and lead contaminated soil of 10 mg/kg soil. The *Brachiaria decumbent* was used in this study on the uptake or translocation of heavy metals. This study found that EDTA reduced phytotoxicity better than EDDS. However, heavy metal removal

efficiency by EDDS was better than EDTA. The results showed that EDDS has more efficiency than EDTA in the removal of the positive charge of heavy metals in roots to stems. This study also found that *Brachiaria decumbent* grew well in contaminated soil and without suffering phytotoxicity and had a high biomass.

Wadeesirisak (2007) studied chromium removal from contaminated soil at concentrations of 0 and 100 mg hexavalent chromium/kg soil as compared with hydroponic techniques from synthetic waste water at 0, 5 and 10 ml hexavalent chromium/L water by *Phyllanthus reticulates*. The results showed that the highest hexavalent chromium uptake was by *Phyllanthus reticulates* This was reported in roots, stems and leaves at 390.57, 61.47 and 58.67 mg/kg in dry weight basis, respectively, at 90 days. Hydroponic results showed hexavalent chromium accumulation efficiency in *Phyllanthus reticulates* by roots, stems and leaves was 6,616.12, 14.46 and 0 ml/kg in dry weight basis, respectively, at 60 days. The resulting hexavalent chromium concentration and the removal capacities of the plant by phytoextraction show it as a plant which can remove Cr well. It may be especially usefull for treatment of hexavalent chromium in water because the roots in water can remove hexavalent chromium directly.

Sampanpanish and Tippayasak (2007) studied chromium removal by using hydroponic plantings. The plants studied were *Eichhornia crassipes* and *Hydrocotyle umbellate*. The synthetic waste water added was at 0, 5, 10 and 15 mg hexavalent chromium/L water. Their results found that with hexavalent chromium of 15 mg/L, after 7, 15, 21 and 30 days, all parts of *Eichhornia crassipes* and *Hydrocotyle umbellate* had accumulated chromium However, *Eichhornia crassipes* had the highest efficiency of chromium accumulate.

Mishra et al. (2008) studied the heavy metals removal of waste water from mining industry areas. This research compared the 3 plant species; *Eichhornia crassipes*, *Lemna minor* and *Spirodela polyrihiza*. The result found that the 3 species of plants have different levels of heavy metals removal capacities. *Eichhornia crassipes* had the highest efficiency at 70.5%, 69.1%, 76.9%, 66.4%, 65.3% and 55.4%, respectively, of the various heavy metals. These plants grow well and are suitable to be used in heavy metal removal when compared with the other plants in the experiment.

Mishra and Tripathi (2009) studied the capacities of the *Eichhornia crassipes* for chromium and zinc removal from synthetic wastewater. The concentrations of both heavy metals were 1, 5, 10 and 20 ml/L. Their tests found that *Eichhornia crassipes* had the ability to remove zinc and chromium at 95% and 84%, respectively. In addition, this research found that the accumulation of both heavy metals were highest in the roots followed by stems and leaves. The harvest time of 21 days found *Eichhornia crassipes* had reached the phytotoxicity level for chromium accumulation but the level was not determined for phytotoxicity from zinc.

CHAPTER III
METHODOLOGY

3.1 Apparatus, Instruments and Chemicals

3.1.1 Apparatus, Instruments for Planttation

1) *Eichhornia crassipes*
2) Green house (4 m. x 6 m. x 3 m.)
3) Pots (daimeter of 35 cm. and height of 30 cm.)
4) Plastic bag (16 inches x 20 inches)
5) Tap water
6) Deionized water
7) Cylinder 2 L
8) Homogenizer
9) Permanent pen

3.1.2 Apparatus, Instruments for collecting plants, Sediment and Water

1) Plastic bag for storing the sediment
2) Plastic bottle (200 ml)
3) Knife and Scissors
4) Siphon
5) Deionized water
6) Balance for approximate weighing
7) Basket
10) Permanent pen

3.1.3 Apparatus, Instruments for laboratory

1) Laboratory Glassware

    (1) Beaker

    (2) Cylinder

    (3) Pipet

    (4) Funnel

    (5) Glass rod

    (6) Volumetric flask

    (7) Watch grass

    (8) Erlenmeyer flask

2) Whatman filter paper No.40 (Diameter 110 ml)

3) Whatman Glass micro filter paper (Diameter 70 ml,)

4) Parafilm

5) Flask Buchner filtration

6) Plastic bottle (60 ml)

7) Atomic absorption spectrometer; AAS (AAnalyst 800, Perkin Elmer)

8) Microwave digestor (ETHOS SEL, MILESTONE)

9) Hot air oven (ULE 500, MEMMERT)

10) Hot plate (Cimarec 2, Thermolyne)

11) pH Meter

12) EC Meter

13) ORP Meter

14) Weighing machine 4 rank (BP 221S, Sartorius)

15) Pump (N035AN.18-IP20)

16) Blender (RT04A)

17) Hood (Wiwatsan)

3.1.4 Chemicals

1) Ethylenedinitrilotetraacetic acid ($C_6H_{16}N_2O_8$)

2) Diethylenetriaminepentaacetic acid ($C_{14}H_{23}N_3O_{10}$)

3) Nitric acid (65% HNO_3)

4) Hydrogenperoxide (30% H_2O_2)

5) Potassiumnitrate (KNO_3)

6) Potassium Hydrogen Phosphate (KH_2PO_4)

7) Magnisium Sulphate Heptahydrate ($MgSO_4.7H_2O$)

8) Sodium chloride (NaCl)

9) Boric acid (H_3BO_3 85%)

10) Manganese Sulphate ($MnSO_4.H_2O$)

11) Zinc Sulphate heptahydrate ($ZnSO_4.7H_2O$)

12) Copper Sulphate tetrahydrate ($CuSO_4.5H_2O$)

13) Molibdic acid ($MoO_3.2H_2O$ 85%)

14) Feric chloride ($FeCl_3$)

15) Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$)

3.2 Research locations

This research took place in the nursery on the 2nd floor with samples taken for analysis to the 3rd floor of building 2, Environmental Research Institute, Chulalongkorn University (ERIC), Chulalongkorn University.

3.3 Research time

The research ran from February 2011 to December 2011 and included literature review, planning and designing the experiment, preparing plant samples, sample collection, sample analysis, result analysis, conclusion and discussion.

1) A preliminary study determined the phytotoxicity for EDTA and DTPA at dose levels of 0, 0.5, 1, 2, 5, 10, 20 ppm mv by recording the growth rate and observing the plants for symptoms at 15, 30, 45 and 60 days after cultivation.

2) The experimental procedure investigated the effect of EDTA and DTPA on cadmium removal capacities from contaminated synthetic water and soil. Plant

samples were harvested at 20, 40, 60, 80 and 100 days and analysis made of water, soil and plants. The experiment design is shown in detail in Table 3.1.

Table 3.1 Experimental design

Experiment design	Time
1. Preliminary study	
1.1 Preparation of nursery, contaminated sediment, water and plants	1–31 March 2011
1.2 Sample collection	
1st Harvesting time	10 March 2011
2nd Harvesting time	25 March 2011
3th Harvesting time	10 April 2011
4th Harvesting time	25 April 2011
2. Experimental procedure	
2.1 Preparation of nursery, contaminated sediment, water and plants	1– 20 July 2011
2.2 Sample collection	
1st Harvesting time	10 August 2011
2nd Harvesting time	30 August 2011
3th Harvesting time	20 September 2011
4th Harvesting time	10 October 2011
5th Harvesting time	30 October 2011

3.4 Experimental procedure

3.4.1 Preliminary study

1) Pots preparation: 14 pots (30 cm dimeter x 35 cm high) were washed with 10% nitric acid solution twice, then rinsed with distilled water one more time.

2) Chelating agent preparation: Ethylenedinitrilotetraacetic acid $(C_6H_{16}N_2O_8)$; EDTA and Diethylenetriaminepentaacetic acid $(C_{14}H_{23}N_3O_{10})$; DTPA were diluted in deionized water to obtain concentrations of 0, 0.5, 1, 2, 5, 10 and 20 mg/L (ppm) in 10 L pots.

3) *Eichhornia crassipes* preparation: Picked up three plants *Eichhornia crassipes* from an uncontaminated area and analyzed the cadmium accumulate by using the United States Environmental Protection Agency (USEPA) Method 3052 (USEPA, 1996) utilizing Atomic Absorption Spectrometer (AAs).

4) Planting and caring: selected plants (3 plants per 1 pot) were planted in pots and chelating agent applied. Observed water level in each pot and maintained at 10 L until the end of the experiment.

5) Sample collection: This process studied the growth rate and the phytotoxicity level every 15, 30, 45 and 60 days.

6) Analysis of phytotoxicity from use of EDTA and DTPA with *Eichhornia crassipes* as follows: Growth rate of *Eichhornia crassipes* comparsion and Phytotoxicity of *Eichhornia crassipes* comparsion.

3.4.2 Experimental procedure

1) Soil properties: Soil samples were collected at a depth of 0-30 cm at a site in Mae Sot district, Tak province, Thailand. The samples were air-dried, crushed to pass through a 2 mm diameter sieve, and mixed thoroughly. They were analyzed for pH, moisture content, conductivity, soil texture, organic matter, cation exchange capacity (CEC), nitrogen (N), phosphorus (P), potassium (K) and total cadmium (TCd) (Table 3.2). Uncontaminated soil from the site, which exhibits similar properties as the contaminated soil found within the site, were excavated and used for the experiments.

Table 3.2 Methods for analyzed physical and chemical properties

of soil used in the experiment

Soil properties	Methods
pH	1:1 soil/water ratio
Conductivity (mS/cm)	1:1 soil/water ratio
Organic matter (%)	$K_2Cr_2O_7$ digestion
Nitrogen (%)	Kjedahl
Phosphorus (mg/kg)	molybdenum blue
Potassium (mg/kg)	digested with Na_2CO_3
CEC (meq/100g)	Ammonium acetate
Soil texture	Hydrometer method
Cd (mg/kg)	US EPA-3052

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

3) Chelating agent and soil preparation:

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 in 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 celating agents were applied in 3 levels of concentration as follows:

3.1) Experiment set 1; blank pot without added chelating agent and using uncontaminated soil.

3.2) Experiment set 2; control pot without added chelating agent and using contaminated soil.

3.3) Experiment set 3; the addition of EDTA at 3 doses of 0.5, 1 and 2 mg/L and using contaminated soil.

3.4) Experiment set 4; the addition of DTPA at 3 doses of 0.5, 1 and 2 mg/L and using contaminated soil.

3.5) Experiment set 5; the addition of either EDTA or DTPA (1:1) at 3 doses of 0.25, 0.5 and 1 mg/L and using contaminated soil.

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

5) Planting and maintenance: A rotater 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. Two bottles per sample were collected at 20, 40, 60, 80 and 100 days of the experiment. First bottle was analyzed for pH, conductivity and oxidation reduction potential (ORP). The second bottle 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 was 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 pH, conductivity and available cadmium in the sediment.

6.3) Plant samples were collected at 20, 40, 60, 80 and 100 days. The plant samples were washed with tap water twice and rinsed with distilled water before being separated into 2 parts: roots and shoots. These samples were open air dried at room temperature for 2-3 hours then placed in a scale to obtain wet weight. After that, the plant samples were oven-dried at $70^{o}C$ for 2-3 days to get a constant weight and to determine dry matter yield. Then plant samples were ground with electric mill until homogenized to determine the total cadmium concentration. To ensure that heavy metal from the root were not contaminated with metals from the soil for the metals analysis; the roots were washed until no heavy metal is detected in the washing water. Conditions and symptoms of phytotoxicity (eg. discoloration, pigmentation, yellowing, and stunting) displayed by plants were recorded (Tanhan et al., 2007; Lai et al., 2004).

7) Sample analysis:

7.1) Water sample were analyzed for total cadmium using USEPA method 3051A (USEPA, 1998). All samples were made up to 50 ml with deionized water and preserved at 4^oC until analysis. The digested solution was analyzed by Atomic absorption spectrometer (AAS).

7.2) Soil sample were analyzed for total cadmium as determined by USEPA method 3052 (USEPA, 1996). All samples were made up to 50 ml with deionized water and preserved at 4^oC until analysis. Available cadmium in soil was estimated by DTPA extraction method (Lindsay and Norvel, 1978). Total cadmium and available cadmium in solution were analyzed by Atomic absorption spectrometer (AAS).

7.3) Plant sample were analyzed for total cadmium in roots and shoots of *Eeichhornia crassipes* determined by USEPA method 3052 (USEPA, 1996). After digestion, all samples were made up to 25 ml by the addition of deionized water and stored at 4^oC until analysis. The digested solutions were analyzed by Atomic absorption spectrometer (AAS).

3.4.3 Data and statistic analysis

1) The relative growth rate (RGR) was calculated by this formula:

$$RGR = [Ln(W_2) - Ln(W_1)] / (t_2 - t_1)$$

2) Analysis of variance (ANOVA) were performed by using the Statistical Package for the Social Sciences (SPSS) progam and analyzing cadmium accumulation in water, sediment, whole plant and separate parts of plant with significant level at $p < 0.05$. Another analysis was done using Duncan's new Multiple Range Test (DMRT), to analyse the different concentrations of cadmium in plants, water and soil at the differrent harvesting times. Also, this test was used to obtain the mean values of heavy metal uptake that may not be significantly different among themselves. These statistical analyses were conducted through the Statistical Analysis System (SAS) and/or Statistical Package for the Social Sciences (SPSS).

CHAPTER IV

RESULTS AND DISCUSSION

The effects of EDTA and DTPA on cadmium removal from contaminated soil with water hyacinth were investigated in this study. The study was classified into 2 parts; 1)Preliminary study; studied the growth rate and the phytotoxicity level of EDTA and DTPA with water hyacinth and 2)Experimental procedure; studied the effect of EDTA and DTPA to increase the cadmium removal capacity ofwater hyacinth. These results are as follow.

4.1 Soil properties and plant growth

4.1.1 Soil properties and water solution

Soil samples were collected at a depth of 0-30 cm at a site from Mae Sot district, Tak province, Thailand. The soil properties are shown in Table 4.1. Soil pH is the most important single soil property that controls the soil reaction (Kabata-Pendias and Pendias, 2001). The pH value was 7.5 and soil texture was loam.

Table 4.1 Physical and chemical properties of soil used in experiment

Soil properties	Methods	Value	Unit
pH	1:1 soil/water ratio	7.5	
Conductivity	1:1 soil/water ratio	0.18	dS/m
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	94.94	mg/kg

4.1.2 Plant growth and relative growth rate (RGR)

1) The effect of EDTA

In this study we observed the growth rate and phytotoxicity of water hyacinth with EDTA and DTPA at 6 concentration levels: 0, 0.5, 1, 2, 5, 10 and 20 mg/l (ppm) for 15, 30, 45 and 60 days. At the end of the experiment, the results show that the addition of EDTA concentration 0, 0.5, 1, 2, 5, 10 and 20 mg/l (ppm) produced wet weights of 152.55, 137.53, 137.79, 146.39, 123.61, 115.99 and 113.16 g, respectively. The wet weight of water hyacinth in the EDTA set after 30 days tended to decrease over time as shown in Figure 4.1 and Table 4.2. At the end of the experiment with EDTA sets of 0.5, 1 and 2 mg/l it was found that the water hyacinth grew better than the others sets.

From wet weight value we calculated the relative growth rate (RGR) of water hyacinth. The relative growth rate values were 8.90, 7.98, 8.10, 8.32, 7.14, 6.63 and 6.19, respectively. The water hyacinth grew well and did not show phytotoxicity from the EDTA compound, but increasing EDTA concentration had a negative effect on the relative growth rate of water hyacinth.

2) The effect of DTPA

The results of DTPA sets showed that the water hyacinth can grow well and did not show phytotoxicity from DTPA solution. The growth rates of water hyacinth increased over time in the various concentrations of DTPA (as shown in Figure 4.2).Especially,in DTPA sets at 0.5, 1 and 2 mg/l, we found that the water hyacinth grew better than in the other concentrations. This research related well with the effects of 5 types of chelating agent and organic acid (DTPA, EDDS, oxalic acid, citric acid and garlic acid) to increase uptake of copper, zinc and nickel with *Ruelliatuberosa* (Burm.f.) *Hochr.*(Ariganon, 2007).That research reported that the chelating agents did not show phytotoxicity in the plants.

Table 4.3 illustrates that the relative growth rate (RGR) values were 7.98, 7.61, 7.59, 7.40, 6.52, 6.52 and 5.86 in DTPA concentrations of 0, 0.5, 1, 2, 5, 10 and 20 mg/l , respectively. The water hyacinth grew well and no phytotoxicity was noted. We found that EDTA added at high concentration has a negative effect of the relative growth rate of plants.
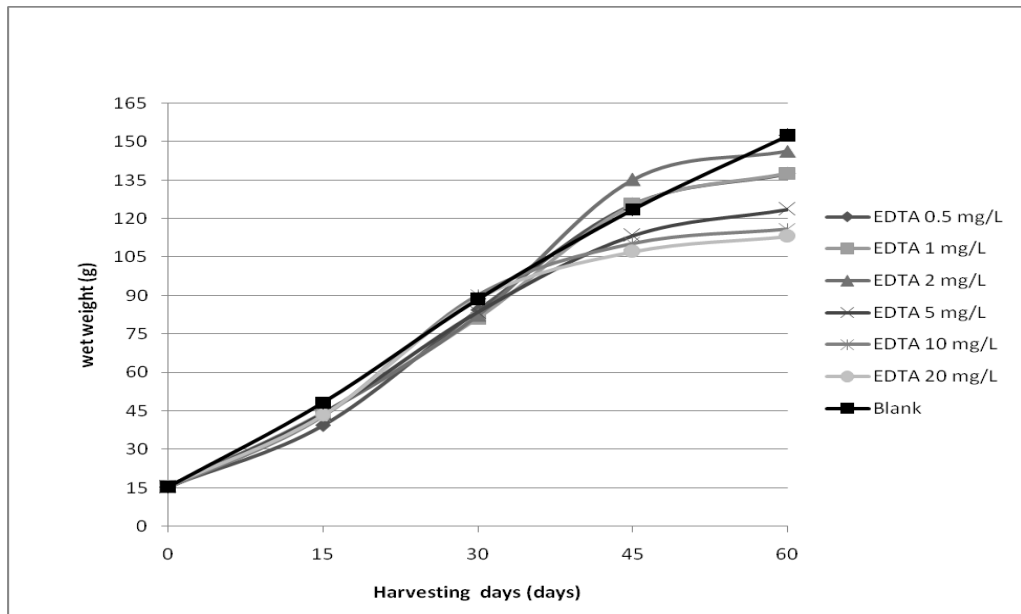
Figure 4.1 Effect of EDTA on growth rate of water hyacinth
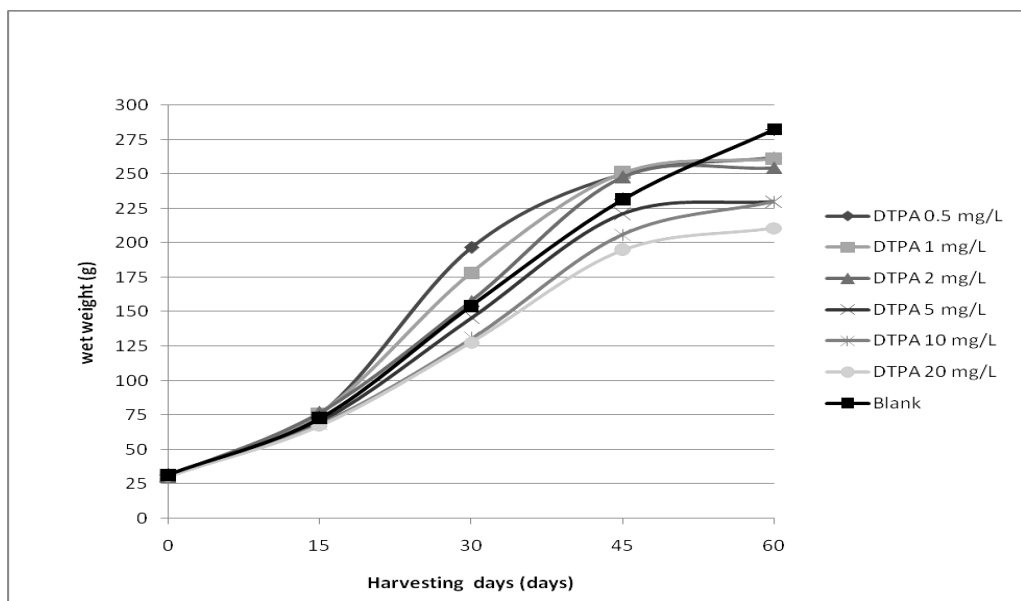
Figure 4.2 Effect of DTPA on growth rate of water hyacinth

Table 4.2 Effect of EDTA on wet weight, relative growth rate (RGR)

and % growth rate of water hyacinth.

The concentration of EDTA (mg/L)		Harvesting times (days)				
		0	15	30	45	60
0	Wet weight (g)	15.41	48.14	88.64	123.56	152.55
	% growth rate	-	212.39	475.33	701.77	889.95
	relative growth rate	-	2.12	4.75	7.02	8.90
0.5	Wet weight (g)	15.32	39.36	84.26	125.64	137.53
	% growth rate	-	156.61	449.35	720.23	797.80
	relative growth rate	-	1.57	4.49	7.20	7.98
1	Wet weight (g)	15.15	44.25	81.23	125.34	137.79
	% growth rate	-	191.66	434.75	727.93	810.26
	relative growth rate	-	1.92	4.35	7.28	8.10
2	Wet weight (g)	15.67	44.01	82.37	135.34	146.39
	% growth rate	-	180.54	424.10	761.47	831.91
	relative growth rate	-	1.81	4.24	7.61	8.32
5	Wet weight (g)	15.21	43.97	83.48	113.43	123.61
	% growth rate	-	189.65	449.74	646.36	713.58
	relative growth rate	-	1.90	4.50	6.46	7.14
10	Wet weight (g)	15.20	42.91	90.20	110.23	115.99
	% growth rate	-	181.46	492.34	625.39	663.44
	relative growth rate	-	1.81	4.92	6.25	6.63
20	Wet weight (g)	15.73	43.30	88.81	107.15	113.16
	% growth rate	-	175.24	464.38	581.26	619.43
	relative growth rate	-	1.75	4.64	5.81	6.19

Table 4.3 Effect of DTPA on wet weight, relative growth rate (RGR)

and % growth rate of water hyacinth.

The concentration of DTPA (mg/L)		Harvesting times (days)				
		0	15	30	45	60
0	Wet weight (g)	31.49	72.91	154.23	231.50	282.41
	% growth rate	-	131.76	390.09	635.86	797.54
	relative growth rate	-	1.32	3.90	6.36	7.98
0.5	Wet weight (g)	30.43	75.47	196.77	249.85	261.93
	% growth rate	-	148.02	546.70	721.14	760.83
	relative growth rate	-	1.48	5.47	7.21	7.61
1	Wet weight (g)	30.41	76.14	178.21	250.75	261.35
	% growth rate	-	150.37	486.06	724.62	759.45
	relative growth rate	-	1.50	4.86	7.25	7.59
2	Wet weight (g)	30.31	76.95	157.91	247.74	254.62
	% growth rate	-	153.93	421.14	717.58	740.28
	relative growth rate	-	1.54	4.21	7.18	7.40
5	Wet weight (g)	30.58	70.44	145.54	220.86	230.07
	% growth rate	-	130.34	375.93	622.26	652.36
	relative growth rate	-	1.30	3.76	6.22	6.52
10	Wet weight (g)	30.62	68.52	130.39	206.08	230.16
	% growth rate	-	123.80	325.88	573.04	651.77
	relative growth rate	-	1.24	3.26	5.73	6.52
20	Wet weight (g)	30.73	67.32	127.85	194.73	210.90
	% growth rate	-	119.05	316.02	533.64	586.25
	relative growth rate	-	1.19	3.16	5.34	5.86

4.2 Effect of EDTA and DTPA on soil in experimental procedure

4.2.1 pH in soil

Soil samples werecollected from the pot experiment and their pH values were measured at 20, 40, 60, 80 and 100 days. The results are illustrated in Table 4.4. This table shows that soil pH for all of the soil samples in the experiment were non-significant ($P \leq 0.05$) for the addition of chelating agent sets and control sets. Because of this exeeriment were added the chelating agent in the lowest doses. Also found that the pH tened to increase over times as show in Table 4.4. In EDTA sets at the concentration of 0.5, 1 and 2 mg/L, we found that pH at experiment end were 7.0, 7.2 and 6.8 respectively. For the DTPA sets at the concentration of 0.5, 1 and 2 mg/L, at the end of the experiment pH levels were 6.9, 6.8 and 6.4 respectively. For EDTA and DTPA mixed sets at the 1:1 ratio of 0.5, 1 and 2 mg/L, we found that the levels of pH were 6.6, 6.7 and 6.7 respectively at the end of the experiment. Because the pH of soil in water tends to decrease over times, the soil pH can cause a direct effect on the cadmium uptake capacity. This effect was shown by Jinadasa et al. (1997). They studied the effects of cadmium levels in vegetables and soils of greater Sydney, Australia. Radish were planted in soil with pH 6.8 and the concentration of cadmium at 6.3 mg/kg soil. They found that the accumulation of cadmium was 0.001 mg/kg in Radish. Cabbages were planted in soil with pH 4.4 and the concentration of cadmium at 1.12 mg/kg soil. They found that the accumulation of cadmium was 0.56 mg/kg.

The results show that pH in all of the experimental sets tend to increase over time. However, the concentration of chelating agents decreased over time because the chelating agent has a short half -life span.

Table 4.4 pH value in soil

Concentration of chelating agent	pH value				
	20 days	40 days	60 days	80 days	100 days
Control	7.1±0.05	7.2±0.05	7.3±0.01	7.3±0.05	7.3±0.02
EDTA0.5 mg/L	6.1±0.04	6.2±0.05	6.3±0.03	6.8±0.05	7.0±0.02
EDTA 1 mg/L	6.1±0.06	6.4±0.03	6.5±0.05	6.6±0.05	7.2±0.05
EDTA 2 mg/L	6.1±0.06	6.3±0.05	6.4±0.03	6.5±0.05	6.8±0.07
DTPA 0.5 mg/L	6.2±0.05	6.3±0.06	6.4±0.03	6.7±0.07	6.9±0.03
DTPA 1 mg/L	6.2±0.06	6.2±0.05	6.5±0.07	6.7±0.11	6.8±0.01
DTPA 2 mg/L	6.1±0.05	6.1±0.04	6.5±0.06	6.4±0.05	6.4±0.06
EDTA+DTPA 0.5 mg/L	6.1±0.06	6.4±0.06	6.5±0.03	6.5±0.06	6.6±0.07
EDTA+DTPA 1 mg/L	6.2±0.07	6.3±0.05	6.5±0.05	6.5±0.07	6.7±0.04
EDTA+DTPA 2 mg/L	6.1±0.04	6.2±0.06	6.4±0.05	6.6±0.08	6.7±0.03

Table 4.5 Electro conductivity value in soil

Concentration of chelating agent	Electro conductivity value (dS/cm)				
	20 days	40 days	60 days	80 days	100 days
Control	0.19±0.02	0.21±0.01	0.23±0.01	0.24±0.01	0.26±0.01
EDTA0.5 mg/L	0.20±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.25±0.01
EDTA 1 mg/L	0.19±0.02	0.21±0.01	0.22±0.01	0.24±0.01	0.24±0.01
EDTA 2 mg/L	0.18±0.02	0.21±0.01	0.22±0.01	0.22±0.01	0.23±0.01
DTPA 0.5 mg/L	0.22±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.26±0.01
DTPA 1 mg/L	0.21±0.02	0.22±0.01	0.23±0.01	0.25±0.01	0.26±0.01
DTPA 2 mg/L	0.21±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.25±0.01
EDTA+DTPA 0.5 mg/L	0.20±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.25±0.01
EDTA+DTPA 1 mg/L	0.19±0.02	0.21±0.01	0.22±0.01	0.23±0.01	0.25±0.01
EDTA+DTPA 2 mg/L	0.20±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.24±0.01

4.2.2 Electrical conductivity in soil

Soil electrical conductivity (EC) is a measurement that correlates with soil properties that affect crop productivity, including soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil characteristics. In this study we analyzed electrical conductivity in the soil at 20, 40, 60,80 and 100 days. We found that the electrical conductivity in all samples with the addition of chelating agent tended to decrease over time as show in Table 4.5. The concentration of electrolytes was high at the initial phase and increased over time because of the plant was uptake some kind of electrolyte. (Suksawat, 2002)

4.2.3 Oxidation-reduction potential in soil

Oxidation-reduction potential (ORP) is a measurement of the relative capacity of a solution to oxidize or reduce in soil. The decomposition of organic matter in soil oxidation reactions is most important to produce electrons and then oxygen in soil works as an electron accepter (Wiwatwongwana, 2003). Table 4.6 illustrates that the oxidation-reduction potential value in the control set increased over time. The oxidation-reduction potential values at 20, 40, 60, 80 and 100 days of harvesting times were 230, 231, 232, 235 and 240 mV, respectively.

For the addition of EDTA sets the oxidation-reduction potential values increased over time. For the additions of EDTA 0.5, 1 and 2 mg/l at 20 days of harvesting time the oxidation-reduction potential values were 229, 231 and 232 mV, respectively. At the end of the experiment (100 days of harvesting) the oxidation-reduction potential values were 237, 239 and 240 mV, respectively.In the additions of DTPA sets the oxidation-reduction potential value increased over time. At EDTA 0.5, 1 and 2 mg/l at 20 days of harvesting times the oxidation-reduction potential values were 232, 234 and 234 mV, respectively. At the end of the experiment (100 days of harvesting time) the oxidation-reduction potential values were 241, 242 and 243 mV, respectively.In the additions of mixed EDTA and DTPA, we found that the oxidation-reduction potential values increased over time. The additions of EDTA and

DTPA mixat a ratioof 1:1of 0.5, 1 and 2 mg/l at 20 days of harvesting times showed the oxidation-reduction potential values were 231, 232 and 231 mV, respectively. At the end of the experiment at 100 days of harvesting time, the oxidation-reduction potential values were 239, 239 and 238 mV, respectively. This result shows that the oxidation-reduction potential values were not different between the concentrations of chelating agent.

Table 4.6 Oxidation-reduction potential in soil

Concentration of chelating agent	Oxidation reduction potential value (mV)				
	20 days	40 days	60 days	80 days	100 days
Control	230±0.01	231±0.01	232±0.03	235±0.05	240±0.02
EDTA0.5 mg/L	229±0.03	231±0.02	233±0.01	234±0.07	237±0.02
EDTA 1 mg/L	231±0.05	233±0.02	233±0.02	235±0.04	239±0.05
EDTA 2 mg/L	232±0.04	233±0.01	235±0.02	237±0.02	240±0.07
DTPA 0.5 mg/L	232±0.05	234±0.05	234±0.01	237±0.03	241±0.02
DTPA 1 mg/L	234±0.04	235±0.02	235±0.03	238±0.11	242±0.02
DTPA 2 mg/L	234±0.05	236±0.03	236±0.04	240±0.01	243±0.06
EDTA+DTPA 0.5 mg/L	231±0.06	234±0.04	236±0.02	236±0.06	239±0.06
EDTA+DTPA 1 mg/L	232±0.03	233±0.03	235±0.01	237±0.07	239±0.04
EDTA+DTPA 2 mg/L	231±0.01	232±0.02	233±0.01	236±0.08	238±0.02

4.3 Effect of EDTA and DTPA on water in experimental procedure

4.3.1 pH of water

For this study of the effect of EDTA and DTPA on cadmium removal from contaminated soil with water hyacinth we analyzed the pH of water solution samples at all harvest times. From statistical analysis, the pH in the experimental procedure shows no significantly different results ($P \leq 0.05$) between the additions of chelating agent sets because in this exeperiment we added the lowest volume of chelating agent.

However we found that the pH tended to increase over time as show in Table 4.7. In EDTA sets; 0.5, 1 and 2 mg/L, the final pH values were 6.9, 7.0 and 6.6 respectively. For the DTPA sets at 0.5, 1 and 2 mg/L, at the end of the experiment pH levels were 6.8, 6.6 and 6.4 respectively. For the mixed EDTA and DTPA ratio of 1:1 at 0.5, 1 and 2 mg/L, at the end of the experiment pH levels were 6.4, 6.5 and 6.7 respectively.

These results shown that pH levels in all experimental sets increased over time because pH in water is controlled by carbondioxide and carbornate (Sengsay, 2001Also the concentration of chelating agent was decreased over time because chelating agents have a short half life span.

4.3.2 Electro conductivity in water

Our study on effect of EDTA and DTPA on cadmium removal from contaminated soil with water hyacinth also analyzed with electro conductivity in water samples in all harvest times. The statistical analysis was shown non-significantly different for electro conductivity in the experimental procedure between the additions of chelating sets. Table 4.8 illustrates that the electro conductivity in all sets decreased from 20 days to 40 days and then rebounded as time increased.. High initial rates of electrolytes decreased up to the 40 day mark and then the plants apparently found and took up new sources of electrolytes, but this source was not persued in our work. Electrolyte presence caused a positive effect on the electro conductivity for control pots at 20, 40, 60, 80 and 100 days and were 177.7, 137.8, 210.1, 270.0 and 325.3 $\mu S/cm$ respectively.

Table 4.7 pH value in water

Concentration of chelating agent	pH value				
	20 days	40 days	60 days	80 days	100 days
Control	7.0±0.04	7.1±0.05	7.2±0.03	7.0±0.05	7.3±0.02
EDTA0.5 mg/L	6.1±0.03	6.2±0.02	6.3±0.01	6.2±0.07	6.9±0.02
EDTA 1 mg/L	6.1±0.06	6.0±0.05	6.2±0.08	6.6±0.04	7.0±0.05
EDTA 2 mg/L	6.1±0.06	6.3±0.02	6.7±0.07	6.5±0.02	6.6±0.07
DTPA 0.5 mg/L	6.2±0.04	6.5±0.07	6.4±0.01	6.2±0.03	6.8±0.02
DTPA 1 mg/L	6.2±0.05	6.2±0.02	6.5±0.07	6.2±0.11	6.6±0.02
DTPA 2 mg/L	6.1±0.05	6.1±0.03	6.5±0.06	6.4±0.01	6.4±0.06
EDTA+DTPA 0.5 mg/L	6.1±0.06	6.4±0.08	6.5±0.02	6.5±0.06	6.4±0.06
EDTA+DTPA 1 mg/L	6.2±0.07	6.3±0.06	6.5±0.04	6.5±0.07	6.5±0.04
EDTA+DTPA 2 mg/L	6.1±0.01	6.2±0.02	6.4±0.04	6.6±0.08	6.7±0.02

Table 4.8 Electro conductivity value in water

Concentration of chelating agent	Electro conductivity value ($\mu S/cm$)				
	20 days	40 days	60 days	80 days	100 days
Control	177.7±0.04	137.8±0.05	210.1±0.03	270.0±0.05	325.3±0.02
EDTA0.5 mg/L	265.1±0.03	186.2±0.02	230.3±0.01	253.2±0.07	299.6±0.02
EDTA 1 mg/L	278.2±0.06	167.0±0.05	196.2±0.08	201.6±0.04	204.0±0.05
EDTA 2 mg/L	327.8±0.06	174.3±0.02	206.7±0.07	217.5±0.02	226.6±0.07
DTPA 0.5 mg/L	247.5±0.04	206.5±0.07	245.4±0.01	277.2±0.03	296.8±0.02
DTPA 1 mg/L	255.1±0.05	206.2±0.02	259.5±0.07	282.2±0.11	306.6±0.02
DTPA 2 mg/L	279.3±0.05	217.1±0.03	236.5±0.06	287.4±0.01	327.4±0.06
EDTA+DTPA0.5 mg/L	284.0±0.06	257.4±0.08	286.3±0.02	314.2±0.06	324.4±0.06
EDTA+DTPA 1 mg/L	307.1±0.07	255.3±0.06	287.5±0.04	334.5±0.07	356.5±0.04
EDTA+DTPA 2 mg/L	350.9±0.01	272.2±0.02	294.4±0.04	353.6±0.08	386.7±0.02

4.3.3 Oxidation-reduction potential in water

Oxidation-reduction potential (ORP) (Table 4.9) in the initial phase were not significantly different . In control sets, the oxidation-reduction potential was 239.3, 217.9, 232.7, 232.9 and 254.7 mV at the harvesting time of 20, 40, 60, 80 and 100 days, respectively. The results show that after planting with water hyacinth oxidation-reduction potential increased overtime. For the DTPA additions sets, the oxidation-reduction potential in water at 0.5, 1 and 2 mg/L at 20 days were 230.4, 224.5 and 214.4 mV, respectively. At the end of the experiment (100 days) oxidation-reduction potential was 274.9, 257.8 and 282.7 mV, respectively.

The result of this was related with the mixed EDTA and DTPA addition sets. The mixed EDTA and DTPA additions sets, at 0.5, 1 and 2 mg/L at 20 days were 235.3, 225.2 and 228.4 respectively. At the end of the experiment (100 days) oxidation-reduction potential (Table 4.9) was 258.4, 283.7 and 269.9 mV, respectively. The result from DTPA additions sets and mixed of EDTA and DTPA additions sets show that the oxidation-reduction potential in water tends to increase over time.

The oxidation-reduction potential in water with EDTA at 0.5, 1 and 2 mg/L at 20 days was 258.7, 255.7 and 248.4 mV, respectively. At the end of the experiment, oxidation-reduction potential was 250.1, 252.5 and 247.8 mV, respectively. This result was not replicated in any of the other samples. This research showed the oxidation-reduction potential decreased over time.

The results show a positive for oxidation-reduction potential (ORP) in water is very high in all sets, so that the water can accept electrons from the soluble form of cadmium. Cd^{2+} is an oxidant, it can accept electron. It resembles chromic acid (H_2CrO_4) or dichromate salts, for example $Na_2Cr_2O_7$, have Cr^{6+}, It cause effect on oxidations reduction potential value. The values of oxidations reduction were highly positive value. (Tuntullveat M, 2002)

Table 4.9 Oxidation-reduction potential in water

Concentration of	Oxidation reduction potentialvalue (mV)				
chelating agent	20 days	40 days	60 days	80 days	100 days
Control	239.3±0.01	217.2±0.01	232.3±0.03	232.2±0.02	254.1±0.02
EDTA0.5 mg/L	258.7±0.01	257.1±0.02	256.4±0.01	253.2±0.01	250.1±0.02
EDTA 1 mg/L	255.7±0.02	253.4±0.02	253.1±0.02	252.9±0.04	252.5±0.02
EDTA 2 mg/L	248.4±0.01	252.4±0.01	251.6±0.02	248.9±0.02	247.8±0.03
DTPA 0.5 mg/L	230.4±0.03	244.5±0.03	244.9±0.01	267.2±0.03	274.9±0.02
DTPA 1 mg/L	224.5±0.02	235.4±0.02	243.7±0.03	248.9±0.01	257.8±0.02
DTPA 2 mg/L	214.4±0.01	236.4±0.03	256.8±0.01	270.4±0.01	282.7±0.01
EDTA+DTPA 0.5 mg/L	235.3±0.01	244.6±0.01	246.8±0.02	256.3±0.03	258.4±0.01
EDTA+DTPA 1 mg/L	225.2±0.03	233.6±0.03	245.3±0.01	267.5±0.04	283.7±0.04
EDTA+DTPA 2 mg/L	228.4±0.01	237.5±0.02	243.1±0.01	256.3±0.02	269.9±0.02

4.4 Effect of EDTA and DTPA on the growth rate and phytotoxicity of plant

In our study, we were looked at the effect on growth rate of water hyacinth in the cadmium contaminated soil caused by the addition of chelating agent EDTA, DTPA and mixed EDTA and DTPA at ratio of 1:1 in 3 concentration levels; 0.5, 1 and 2 mg/L (ppm) and compared the effects of using EDTA and DTPA to increase cadmium removal from contaminated soil in Mae Sot District, Tak Province, Thailand.

4.4.1 Effect of EDTA on growth rate and phytotoxicity

From Figure 4.3 illustrates that the water hyacinth grew well in all EDTA sets, but most especially with the addition of 2 mg/L (ppm). This result relates to the findings of German et al. (2003). They studied the effects of EDTA and EDDS in increasing lead, zinc and cadmium uptake capacity in *Juncea* (L.) Czern. They found that the application of EDTA at 3 and 5 ml/kg soil did not affect dry weight of *Juncea* (L.) Czern. This research also relates to Wu et al. (2004). They studied the

phytoremediation of heavy metal contaminated soil with Indian mastard and associated potential leaching risk. They found that the additions of EDTA 3 m mol/kg Indian mastard did not show phytotoxicity.
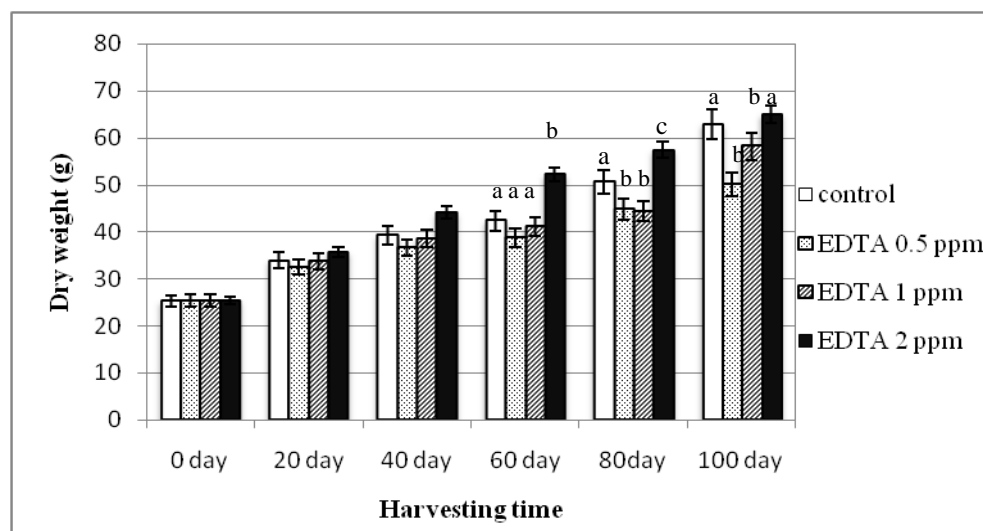
4.4.2 Effect of DTPA on growth rate and phytotoxicity

Figure 4.4 illustrates that the growth rate of water hyacinth increased with the various DTPA concentrations, but most especially with the addition of 0.5 mg/L (ppm).This research is related with Ariganon (2007). Ariganon studied the effects of 5 types of chelating agent and organic acid (DTPA, EDDS, oxalic acid, citric acid and garlic 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 affect the growth rate in plants. This research is related also to Delgado et al. (1993). They studied the uptake of Zn, Cr and Cd by water hyacinth. They reported that the concentration of Zn, Cr and Cd can cause negative effects on growth rate of water hyacinth.

4.4.3 Effect of EDTA and DTPA mixing on growth rate and phytotoxicity

Figure 4.5 illustrates that the growth rate of water hyacinth increased with the various concentrations where EDTA and DTPA were mixed at a ratio of 1:1, and especially the addition at 2 mg/L(ppm).This research finding relates with Ariganon (2007). She studied the effects of 5 types of chelating agent and organic acid on copper, zinc and nickel with *Ruelliatuberosa* (Burm.f.) Hochr. She reported that the chelating agent did not show phytotoxicity and did not affect the growth rate in plants. German et al. (2003) studied the effects of EDTA and EDDS in increasing lead, zinc and cadmium uptake capacity with *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. This also relates to Lombi et al. (2001), they studied the phytoremediation of heavy metal contaminated soil. They found that the additions of EDTA can cause positive effects increasing the cadmium accumulation in *Thaaspicaeruscens* and *Zea mays* L.
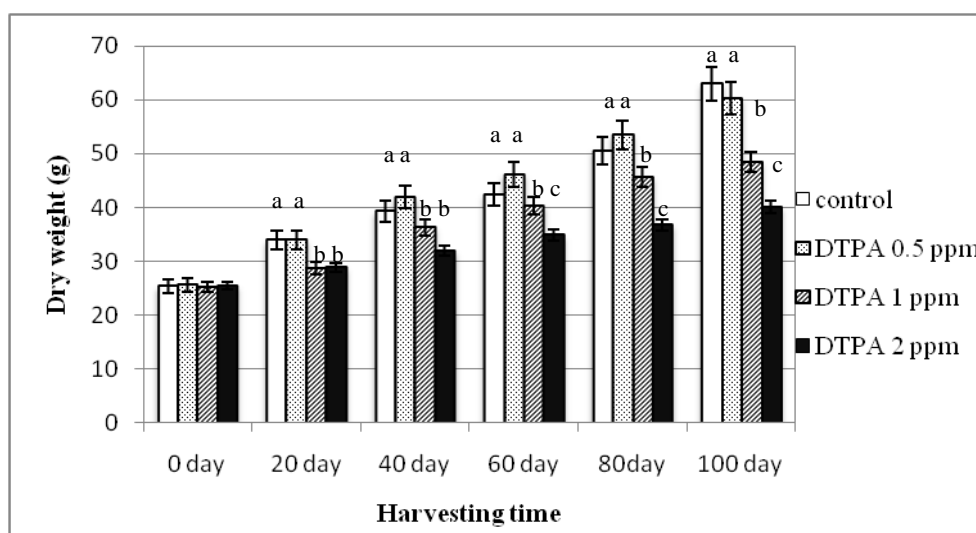
Figure 4.4 Growth rate of water hyacinth in DTPA addition sets
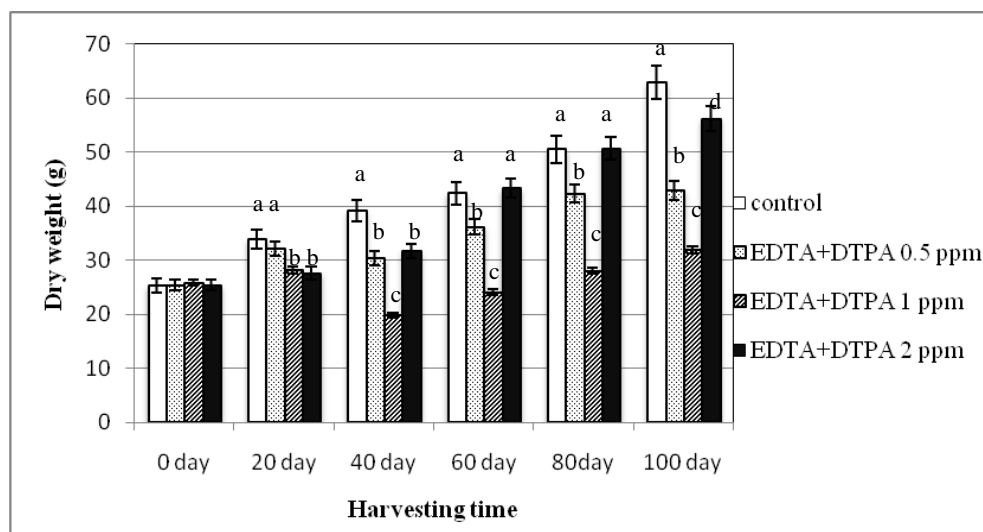
Figure 4.5 Growth rate of water hyacinth

in mixed EDTA and DTPA at ratio of 1:1 addition sets

4.5 Effect of EDTA and DTPA on Cd removal by plants

Cadmium removal by water hyacinth was investigated and separated into 2 parts; shoots (stem and leaf) and roots. The results are expressed in milligram per kilogram (mg/kg) of plant on a dry weight basis.

4.5.1 Effect of EDTA on Cd uptake

Figure 4.6 and 4.7 demonstrate that the cadmium accumulated by roots and shoots tended to increase over time. These results show the EDTA can increase cadmium removal by water hyacinth. The cadmium accumulation in roots was higher than shoots at 160.91 and 13.37 mg/kg dry weight of plant, respectively, at the EDTA concentration of 2 mg/L(ppm). We found the cadmium accumulated in root and shoot as show in Figure 4.6 and 4.7. The capacity of roots uptake by water hyacinth related with Jean et al. (2008). They studied the effects of EDTA and Citric acid to increase chromium and nickel uptake capacity in *DaruraInno*xia and *DaruraInnoxia*was 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 the chromium and nickel accumulation in the root of *DaruraInnoxia.*
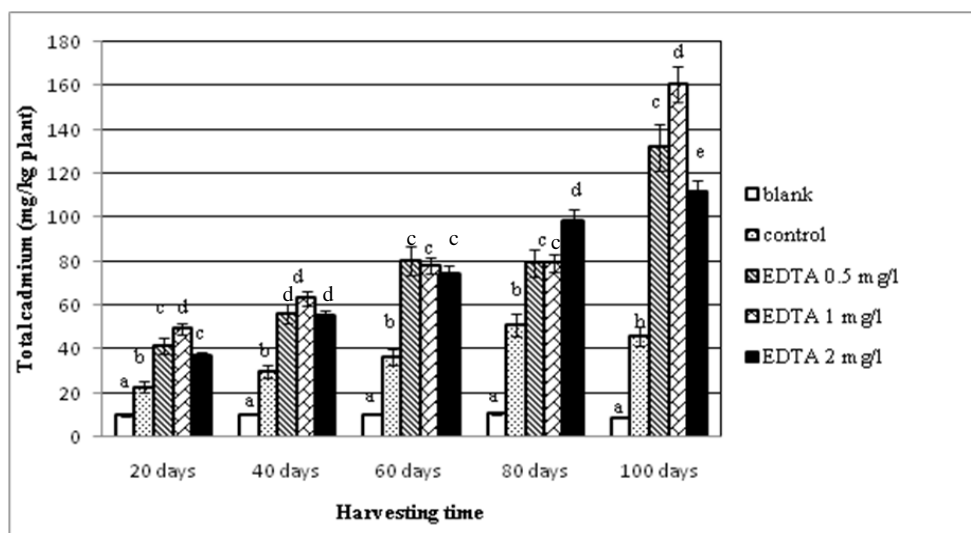
Figure 4.6 The effect of EDTA on Cd uptake by roots of water hyacinth
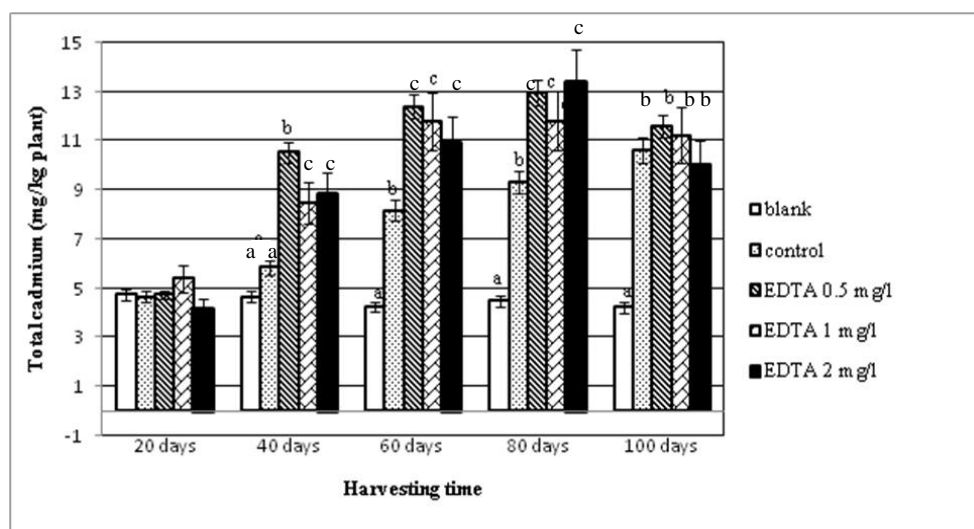
Figure 4.7 The effect of EDTA on Cd uptake by shoots of water hyacinth

The uptake capacity of plant shoots (stem and leaf) related with Hernandez et al. (2006). They studied the effects of EDTA on increasing uptake capacity of lead, zinc and cadmium in *Cynaracarduncylus*. They found that EDTA can increase the uptake of heavy metals in plants. The heavy metal accumulation in stems and leaves was greater than in roots. This also relates to Wongtanet and Parkpain (2008), who studied the phytoremediation of lead from water with *Hydrocotyleumbella*,

Pityrogrammacalomelanos and *Pandanusamaryllifolius* Roxb.. The result of EDTA additions showed that the accumulation of lead was increased in roots and stems of *Hydrocotyleumbella*, *Pityrogrammacalomelanos* and *Pandanusamaryllifolius* 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 *Hydrocotyleumbella*, *Pityrogrammacalomelanos* and *Pandanusamaryllifolius* Roxb were 11.4, 17.4 and 2.4 µg/g, respectively.

4.5.2 Effect of DTPA on Cd uptake

Figure 4.8 and 4.9 illustrate that the cadmium removal by roots and shoots tended to increase over time. We found that DTPA promoted cadmium removal. The cadmium accumulation in roots was higher than shoots, at the DTPA concentration of 2 mg/l (ppm), at 100 days of growth time. The cadmium accumulation was highest in root and shoot at 231.78 and 27.02 mg/kg dry weight of plant at the DTPA concentration of 1 mg/l (ppm) and 80 days of growth time. This result is shown in Figure 4.8 and 4.9. That DTPA can promote cadmium removal capacity,relates with Hua-Yin Zhao et al. (2011). They studied phytoremediation of lead and zinc mining area soil by using EDTA and DTPA to enrich ryegrass. They found that the EDTA and DTPA were effective for this purpose.
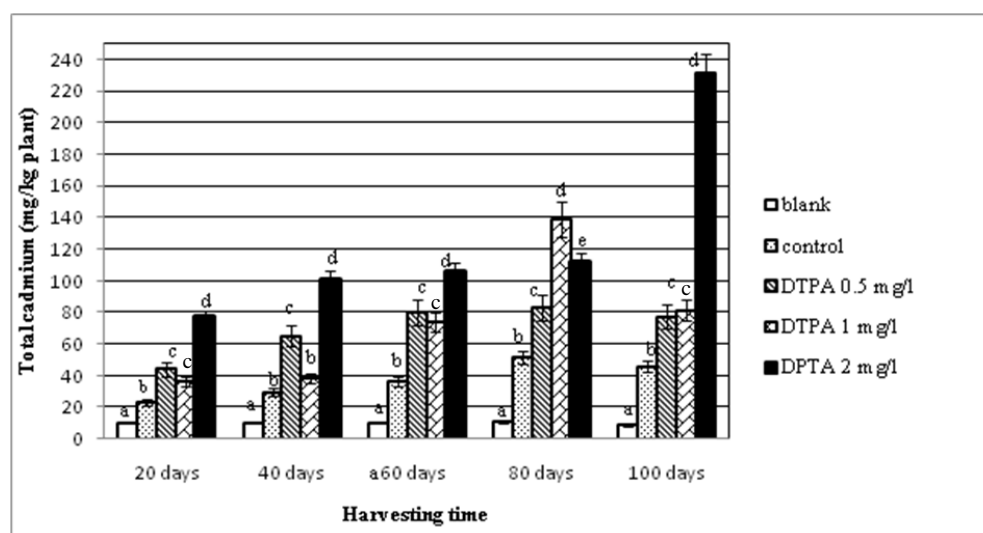
Figure 4.8 The effect of DTPA on Cd uptake by roots of water hyacinth
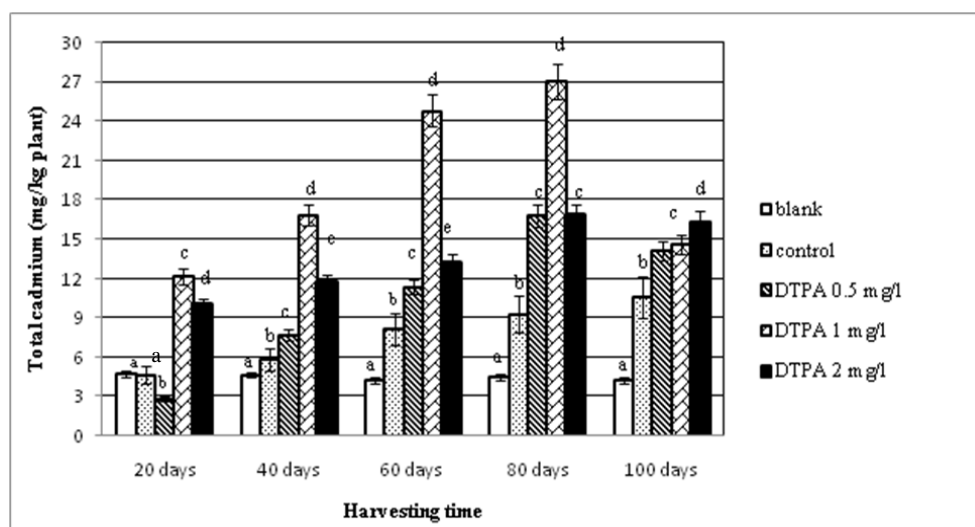
Figure 4.9 The effect of DTPA on Cd uptake by shoots of water hyacinth

4.5.3 Effect of mixed EDTA and DTPA solution on Cd uptake

Figure 4.10 and 4.11 illustrate that the cadmium removal by roots and shoots tended to increase over time. We found that the mixing of EDTA and DTPA in a ratio of 1:1 increased cadmium removal. The cadmium accumulation in roots was higher than shoots. Using the mixture of EDTA and DTPA (ratio 1:1) and a concentration of 2 mg/l (ppm), at 100 days of growth time accumulation was 157.48 mg/kg dry weight of plant. In shoot the concentration of cadmium was highest at 23.61 mg/kg dry weight of plant, at the DTPA concentration of 2 mg/l (ppm) and 60 days of growth time as shown in Figure 4.10 and 4.11.

We found that DTPA promoted cadmium removal capacity. This result is related to Hua-Yin Zhao et al. (2011)who studied phytoremediation of lead and zinc from soil in a mining area by using two chelators (EDTA and DTPA) to enrich ryegrass. They found that EDTA and DTPA had grate potential for this purpose.
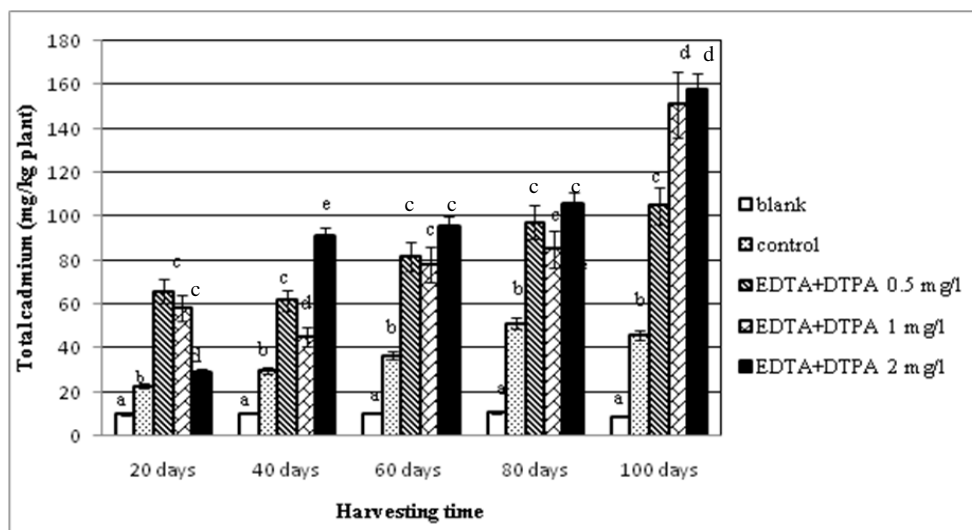
Figure 4.10 The effect of EDTA and DTPA mixture at the ratio of 1:1 on Cd uptake by roots of water hyacinth
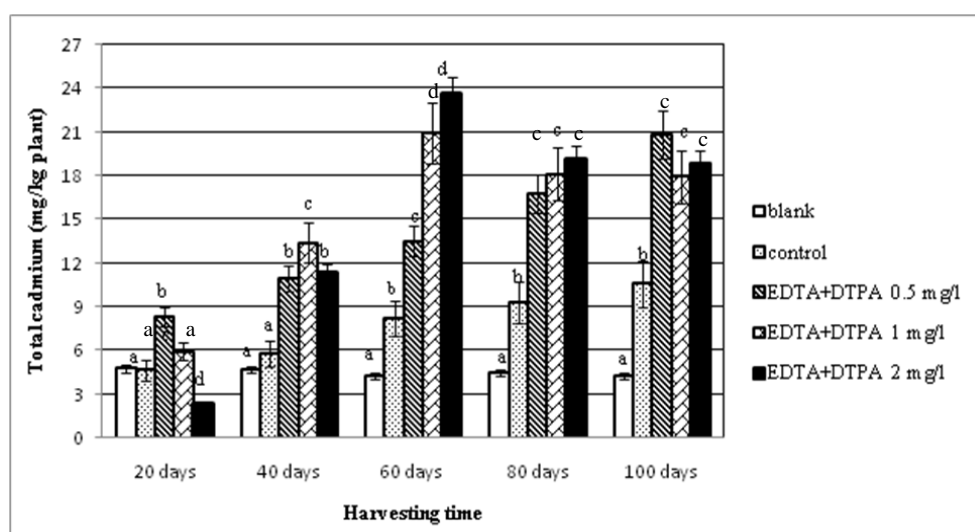
Figure 4.11 The effect of EDTA and DTPA mixture at the ratio of 1:1 on Cd uptake by shoots of water hyacinth

4.6 Effect of EDTA and DTPA on Cadmium in soil

4.6.1 Total Cadmium accumulation in soil

The concentration of total cadmium (TC) in the contaminated soil was determined after harvesting times of 20, 40, 60, 80 and 100 days of water hyacinth growth by USEPA method 3052 (USEPA, 1996). The addition of chelating agents;

EDTA, DTPA and EDTA and DTPA mixture (at ratio of 1:1) in 3 concentration levels; 0.5, 1 and 2 mg/L (ppm) caused positive effects on the removal of the cadmium contamination from the contaminated soil. The results show that the accumulated cadmium in soil tended to decrease over time as show in Table 4.10. The cumulative amounts of cadmium taken up by the 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, 26.41 and 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 mixture 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.

Table 4.10 The Total Cadmium accumulation in soil

Times	Chelating agent								
	EDTA			DTPA			Mixture EDTA and DTPA		
	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

4.6.2 The 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 4.11 illustrates the concentration of available cadmium in the contaminated soil. The results show that heavy metal availability decreased by time

during the growth period. The highest availability of heavy metals was at 20 days of growth. In general, the solubility and availability of metal in plants are related to the metal concentration in soil (Adriano, 2001) as the amount of metal uptake by plant would relate to the metal available in soil. Therefore, as in the results, it may be expected that the availability of cadmium in soil would decrease slightly over time during the growth period due to cadmium being taken up by plants. The environment, nutrition, growth stage and some other factors controlling plant growth may also indirectly affect the metal level in plant (Xian, 1989).

Table 4.11 Available Cd accumulation in soil

Times	Chelating agent								
	EDTA			DTPA			Mixture EDTA and DTPA		
	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

4.6.3 Total Cadmium accumulation in water

Water samples collected after the harvesting times of 20, 40, 60, 80 and 100 days were examined for total cadmium by USEPA method 3051A (USEPA, 1998). As shown in Table 4.12, the additions of chelating agent; EDTA, DTPA and mixture of EDTA and DTPA (at ratio of 1:1) in 3 concentration levels of 0.5, 1 and 2 mg/L (ppm) increased the soluble form of cadmium in the water samples. The concentration of cadmium accumulated in the water decreased over 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 all of them were lower than 0.05 mg/L. The amounts 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 for mixture of EDTA and DTPA at ratio of 1:1 were 9.62, 7.29 and 0.12 mg/L at 20 days and lower than 0.05 mg/L at 100 days.

Table 4.12 Total Cadmium accumulation in water

Times	Chelating agent								
	EDTA			DTPA			Mixing EDTA and DTPA		
	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

4.7 The Comparison of EDTA, DTPA and mixture of EDTA and DTPA (at ratio of 1:1) on cadmium uptake capacities by water hyacinth

In this study of the effect of EDTA, DTPA and mixed set on cadmium removal capacities by water hyacinth, the results show that the addition of EDTA 1mg/L, DTPA 2 mg/L and mixture of EDTA and DTPA at 2 mg/l were more efective than the other tested compositions. Statistical analysis showed that cadmium capacity uptake % for the additions of EDTA 1mg/L, DTPA 2 mg/L and mixing of EDTA and DTPA 2 mg/L were not significantly different. The results show that the addition of chelating agents can cause a positive effect on % cadmium uptake capacities by water hyacinth and is illustrated in Figure 4.12. Per centage of cadmium uptake capacity by

water hyacinth with chelating agents added differed significantly (P<0.05) from control sets.

This result relates with Aussawaphokee S. (2007 who studied the effects of EDTA, EDDS and CA for increasing the cadmium uptake capacity by *Heliantus annus* Linn. In that study, 0.5 mg/kg soil of EDTA, EDDS and CA were added to soil samples. Then, *35* days after planting, the results showed that the 3 kinds of chelating agent had a positive effect on cadmium uptake capacity of the water hyacinth. The addition of EDTA had a more positive effect than EDDS and CA.
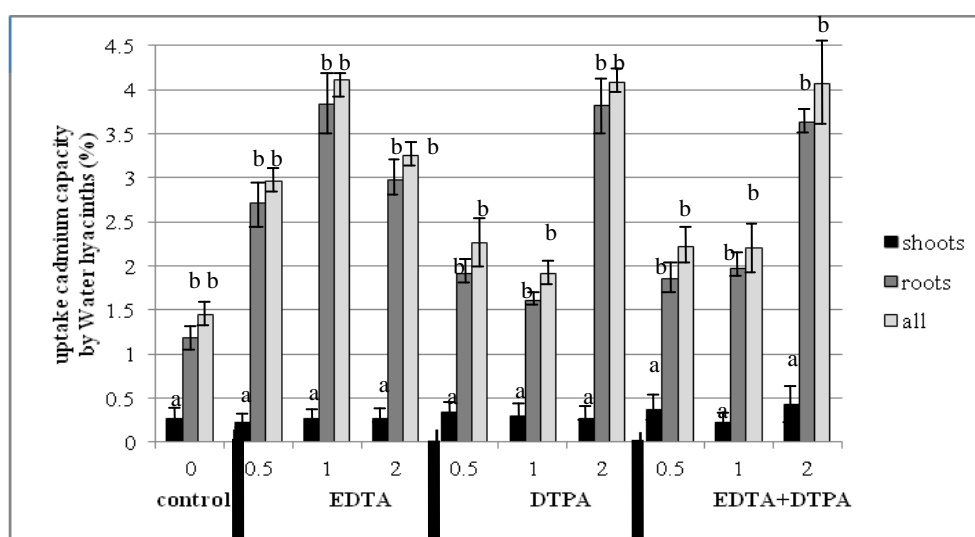
Figure 4.12 The Comparison of EDTA, DTPA and DTPA mixture at
the ratio of 1:1 on Cadmium uptake capacity by water hyacinth.

4.8 Percentage of cadmium removal from contaminated soil by water hyacinth

Table 4.13 illustrates the percentage of cadmium removed from contaminated soil by water hyacinth. The results showed that the additions of EDTA , DTPA and Mixing of EDTA and DTPA ratio 1:1 can have a positive effect on cadmium removal from contaminated soil by water hyacinth. In control sets the percentage of cadmium removal was 0.96%. The EDTA additions of 0.5, 1 and 2 mg/L resulted in cadmium removal percentages of 1.96, 2.74 and 2.17% respectively. The DTPA additions of 0.5, 1 and 2 mg/L found percentage levels of 1.52, 1.29 and 2.71 %, respectively. For

the mixture of EDTA and DTPA we found 0.5, 1 and 2 mg/L removed cadmium at 1.48, 1.47 and 2.70 %, respectively.

Table 4.13 Percentage of cadmium removal from contaminated soil to water hyacinth

Concentration of chelating agent (mg/L)	Concentration of cadmium at the start time (mg)	Concentration of cadmium in water hyacinth (mg)			Percentage of cadmium removal from contaminated soil by water hyacinth
		roots	Shoots	all	
Control	488.10	3.84	0.88	4.72	0.96
EDTA 0.5	489.10	8.84	0.76	9.60	1.96
EDTA 1	487.35	12.48	0.88	13.36	2.74
EDTA 2	487.75	9.68	0.88	10.56	2.17
DTPA 0.5	485.05	6.24	1.12	7.36	1.52
DTPA 1	480.70	5.24	0.96	6.20	1.29
DTPA 2	489.55	12.40	0.88	13.28	2.71
EDTA+DTPA 0.5	486.65	6.00	1.20	7.20	1.48
EDTA+DTPA 1	487.45	6.40	0.76	7.16	1.47
EDTA+DTPA 2	488.05	11.80	1.40	13.20	2.70

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The effect of EDTA and DTPA on cadmium removal from contaminated soil with water hyacinth were investigated in this study. The study was classified into 2 parts; 1) Preliminary study; studied the growth rate of and the phytotoxicity level of EDTA and DTPA on water hyacinth. 2) Experimental procedure; studied the ability of EDTA and DTPA to increase the cadmium removal capacity of water hyacinth.

Our preliminary study showed that with EDTA and DTPA concentrations at 0.5, 1 and 2 mg/l plants grew well. Even higher concentrations of 5, 10 and 20 mg/l were not cause phytotoxicity in water hyacinth. We selected concentrations 0.5, 1 and 2 mg/L to study if EDTA and DTPA could increase the cadmium removal capacity of the plants.

In the experimental procedure, the EDTA and DTPA caused an increased ability to remove cadmium from contaminated soil using water hyacinths. The amount of cadmium in the contaminated soil slightly decreased over time during the growth period (from 20 - 100 days) using water hyacinth. The phytoavailability of cadmium in contaminated soil was proportional with the total cadmium in soil. In phytotoxicity studies of cadmium and chelating agent no negative effects were observed on the water hyacinth in all of the experiment. The EDTA and DTPA did cause negative effects on growth rate of water hyacinth.

The concentration of cadmium was found the highest in roots of water hyacinth for all experimental conditions. These concentrations as well as total accumulation of cadmium increased in proportion to age of plants or harvesting time. At 100 days of harvesting the concentrations of cadmium was the highest in all sets. Thus, in terms of management for cadmium removal, the water hyacinth should be removed after remediation to eliminate cadmium from reentering the site.

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. The adding of DTPA at 2 mg/L showed cadmium accumulation in water hyacinth increased over time more than the other concentrations.. EDTA was added at 3 concentrations; 0.5, 1 and 2 mg/L, and we found that at 1 mg/L cadmium accumulation in water hyacinth was higher than in the other concentrations. Finally, the 3 sets comparison showed that DTPA at 1 mg/l increased cadmium accumulation in plant more than the other ratios.

The problem of cadmium contaminated soil in Mae Sot area has become a concern in recent years. The cadmium concentration is high in the 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

5.2 Recommendation

Water hyacinth is a native plant found commonly growing in Thailand. Moreover, the result from this study indicate that DTPA addition at 1 mg/l to water hyacinth could increase its ability to accumulate cadmium more than the other studied additives.

An additional studies to investigate the equilibrium point of the concentration of DTPA and the effects of increased growth time of water hyacinth is recommended.

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APPENDIX

APPENDIX A

1. Calculation of chelating agent for adding to the plant in the experimental procedure

The chelating agent was calculated by this formula:

$$\frac{A \times MW \times S}{MC}$$

When: A   =   Concentration of chelating agent (mg/L)

S   =   Volume of water in pot experiment.(L)

MC   =   Atomic Weight (g)

MW   =   Molecular weight (g)

2. Calculating Relative Growth Rate and Percentage of Growth Rate

3.1) The Relative Growth Rate was calculated by this formula:

$$\frac{\text{Dry weight of plant at the end of the experiment}}{\text{Dry weight of plant at the start of experiment}}$$

3.2) The Percentage of Growth Rate was calculated by this formula:

$$\frac{\text{Wet weight of plant at harvest times - Wet weight of plant at start}}{\text{Wet weight of plant at start}} \times 100$$

APPENDIXS B

1. USEPA 3052 method

1.1 Analysis of total Cadmium in soil sample

A representative sample of 0.5 g was digested in 9 ml HCl (37%) and 3ml HNO_3 (65%). The sample and acid were placed into inert polymeric microwave vessels that were sealed and heated in the microwave digestion System. After cooling, the samples were filtered using Whatman filter No. 40 (Ø 110 mm.). All samples were made up to 50 ml by adding deionized water and maintained at 4°C until analysis. Process is outlined in Table B. 1.

Table B.1 Temperature and time used for soil digestion

Step	Time (min)	Temperature (°C)
1	10	200
2	15	200

1.2 Analysis of total Cadmium in water hyacinth sample

A representative 0.5 g of roots and shoots was digested in 9 ml HNO_3 (65%). The sample and acid were placed into inert polymeric microwave vessels then the vessels were sealed and heated in the microwave Digestion System. The steps of temperature and time in the microwave Digestion System are presented in Table B.2. After cooling, the samples were filtered using Whatman filter No. 40 (Ø 110 mm.). All samples were made up to 50 ml by adding deionized water and preserved at 4°C until analysis.

Table B.2 Temperature and time used for water hyacinth digestion

Step	Time (min)	Temperature (°C)
1	5	180
2	10	180

2. USEPA 3051A method

2.1 Analysis of total Cadmium in water sample

A representative sample of 45 ml of water was digested in 5 ml HNO_3 (65%). The sample and acid were placed into inert polymeric microwave vessels then the vessels were sealed and heated in the microwave Digestion System. Temperature and time for each stepare presented in Table B.3. After cooling, each sample was filted using Whatman filter No. 40 (Ø 110 mm.). All samples were made up to 50 ml by adding deionized water and preserved at 4°C until analysis.

Table B.3 Temperature and time used for water digestion

Step	Time (min)	Temperature (°C)
1	10	160
2	10	165

3. DTPA extraction method

Ten gram sub-samples of air-dried soil were placed in Erlenmeyer flasks with DTPA extracted solution(0.005 M DTPA, 0.01 M $Cacl_2$ and 0.1 M TEA) and sealed with parafilm. Each flask was sharken for 2 hour at 120 rpm. After that, sample was filtrated using Buchner's funnel and vacuum pump with GF/C (Glass Micro Filters) filter paper (Ø 70 mm). The sample was stored in polyethylene containers stored at 4 °C until analysis.

3.1 Preparation for DTPA extractant: 0.005 mol/L DTPA

The DTPA extracting solution was prepared containing 0.005 mol/l diethylenetriamine-pentaacetic acid (DTPA) [$C_{14}H_{23}N_3O_{10}$], 0.01 mol/l triethanolamine (TEA) [$(HOCH_2CH_2)_3N$] and adjusted to pH 7.3. To prepare 10 L of this solution required 149.2 g reagent grade TEA, 19.75 g DTPA and 14.7 g calcium chloride [$CaCl_2.2H_2O$] in approximately 200 ml distilled water. Sufficient time was provide for the DTPA to dissolve and dilute to approximately 9 L. The pH was adjusted to 7.3+0.5 with HCl while stirring and diluted to 10 L. This solution was stable for several months.

APPENDIXS C

1. Heavy metals removed from contaminated soil using water hyacinth

1.1 Concentration of cadmium in soil

Table C.1 Concentration of cadmium in contaminated soil

Chelating agent	Concentration of cadmium in contaminated soil (mg/kg soil)									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	91.80		82.36		72.87		64.60		22.21	
	93.22	91.33	84.00	82.26	76.67	74.67	63.06	64.80	61.98	50.10
	88.96		80.43		74.46		66.74		66.13	
EDTA 0.5 mg/l	82.72		87.36		68.44		56.71		30.17	
	92.81	89.60	81.94	78.88	56.92	60.80	52.51	54.09	30.64	30.53
	93.28		67.34		57.06		53.06		30.78	
EDTA 1 mg/l	61.86		54.45		50.29		42.11		27.07	
	55.32	59.47	49.79	53.60	56.40	52.76	42.16	43.63	26.75	26.41
	61.23		56.56		51.59		46.62		25.41	
EDTA 2 mg/l	55.76		48.23		42.97		36.68		39.24	
	54.12	54.55	48.76	46.98	41.93	43.04	37.77	37.82	35.18	37.37
	53.76		43.95		44.23		39.02		37.68	
DTPA 0.5 mg/l	78.09		66.07		40.88		70.39		52.62	
	83.03	80.01	82.75	74.49	83.62	68.14	28.78	51.17	45.52	48.48
	78.90		74.65		79.93		54.34		47.32	
DTPA 1 mg/l	72.07		89.55		73.23		47.10		37.11	
	76.59	76.14	73.45	76.92	78.49	75.58	44.79	43.33	42.76	38.90
	79.75		67.75		75.01		38.10		36.84	
DTPA 2 mg/l	77.29		61.83		64.93		60.77		32.49	
	81.63	79.91	64.99	64.67	67.69	67.68	62.09	61.77	33.80	33.37
	80.82		67.19		70.41		62.45		33.83	
EDTA +DTPA 0.5 mg/l	76.45		71.51		76.87		72.04		69.80	
	95.16	84.88	68.81	73.36	79.31	72.61	64.94	71.19	63.68	67.25
	83.04		79.77		61.66		76.58		68.26	
EDTA +DTPA 1 mg/l	90.06		75.15		76.02		59.20		56.26	
	86.85	86.74	83.37	79.90	75.35	71.45	68.10	64.01	63.27	58.68
	83.32		81.18		62.98		64.72		56.53	
EDTA +DTPA 2 mg/l	100.62		72.70		47.55		54.47		52.73	
	101.12	95.92	71.55	71.95	68.55	59.72	54.34	54.60	53.13	54.32
	86.01		71.59		63.07		54.97		57.09	

1.2 Phytoavailablility of cadmium in soil

Table C.2 Phytoavailablility of cadmium in contaminated soil

Chelating agent	Phytoavailablility of cadmium in contaminated soil (mg/kg soil)									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	12.71		8.54		12.31		9.20		8.18	
	13.76	13.50	7.75	12.06	13.34	12.75	10.11	9.95	8.85	8.65
	14.04		19.90		12.61		10.55		8.93	
EDTA 0.5 mg/l	18.30		19.83		15.78		14.77		12.62	
	20.99	20.77	19.44	20.27	16.10	15.99	14.30	14.10	12.29	12.29
	23.03		21.55		16.08		13.24		11.96	
EDTA 1 mg/l	21.69		19.20		13.81		15.20		12.25	
	24.92	23.11	19.05	19.47	14.54	14.10	15.39	13.17	12.83	12.53
	22.72		20.15		13.94		8.93		12.51	
EDTA 2 mg/l	14.17		13.83		11.16		10.05		8.79	
	13.03	13.37	12.94	13.19	10.86	10.91	10.02	10.01	8.14	8.38
	12.91		12.79		10.71		9.96		8.20	
DTPA 0.5 mg/l	27.12		19.54		19.64		15.45		14.55	
	31.32	28.48	20.00	19.75	20.45	16.88	15.84	15.75	13.99	14.03
	26.99		19.72		10.56		15.95		13.56	
DTPA 1 mg/l	77.77		48.72		22.69		15.16		13.00	
	78.79	72.76	40.36	45.54	22.05	22.53	16.14	15.53	12.64	12.65
	61.73		47.52		22.87		15.28		12.31	
DTPA 2 mg/l	21.35		24.55		20.26		16.39		9.33	
	22.70	22.45	21.05	22.68	18.37	19.39	16.50	16.40	9.57	9.66
	23.29		22.45		19.53		16.32		10.06	
EDTA +DTPA 0.5 mg/l	20.84		20.52		16.76		15.81		12.27	
	21.23	20.95	20.41	20.71	17.29	17.09	16.23	16.26	12.45	12.71
	20.79		21.21		17.21		16.75		13.42	
EDTA +DTPA 1 mg/l	22.47		17.73		17.01		12.90		12.30	
	21.82	21.95	19.06	18.33	14.25	15.48	13.40	13.28	12.38	12.12
	21.56		18.19		15.17		13.55		11.70	
EDTA +DTPA 2 mg/l	21.36		16.52		15.47		11.77		10.28	
	23.91	21.69	17.37	16.75	14.14	14.23	12.51	12.08	10.58	10.44
	19.81		16.37		13.06		11.97		10.46	

1.3 Concentration of cadmium in water

Table C.3 Concentration of cadmium in water

Chelating agent	Concentration of cadmium in water (mg/L of water)									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	11.92		0.11		0.07		0.03		0.05	
	9.92	11.00	0.11	0.11	0.08	0.07	0.04	0.04	0.05	0.05
	11.17		0.11		0.08		0.04		0.05	
EDTA 0.5 mg/l	10.85		0.12		0.09		0.07		0.06	
	13.60	12.11	0.11	0.12	0.08	0.08	0.05	0.07	0.07	0.06
	11.89		0.14		0.08		0.07		0.06	
EDTA 1 mg/l	5.75		0.11		0.07		0.06		0.05	
	5.80	5.65	0.11	0.11	0.09	0.08	0.06	0.06	0.05	0.05
	5.41		0.11		0.09		0.05		0.06	
EDTA 2 mg/l	10.52		0.11		0.08		0.06		0.05	
	10.29	22.05	0.13	0.11	0.08	0.08	0.06	0.07	0.06	0.06
	45.33		0.10		0.09		0.08		0.05	
DTPA 0.5 mg/l	14.41		0.11		0.06		0.08		0.05	
	11.43	12.38	0.11	0.11	0.10	0.08	0.06	0.07	0.05	0.06
	11.30		0.11		0.09		0.07		0.07	
DTPA 1 mg/l	12.36		0.11		0.07		0.08		0.05	
	10.69	11.58	0.11	0.11	0.08	0.08	0.08	0.08	0.05	0.05
	11.68		0.12		0.10		0.09		0.05	
DTPA 2 mg/l	10.51		0.08		0.07		0.05		0.05	
	12.15	11.22	0.07	0.08	0.09	0.08	0.05	0.05	0.05	0.05
	10.99		0.09		0.07		0.05		0.05	
EDTA +DTPA 0.5 mg/l	9.62		0.05		0.08		0.06		0.05	
	9.76	9.62	0.07	0.07	0.08	0.08	0.05	0.06	0.05	0.05
	11.86		0.09		0.08		0.06		0.05	
EDTA +DTPA 1 mg/l	8.18		0.09		0.08		0.05		0.05	
	7.22	7.29	0.09	0.08	0.08	0.08	0.06	0.05	0.05	0.05
	6.45		0.08		0.08		0.05		0.05	
EDTA +DTPA 2 mg/l	0.12		0.08		0.08		0.07		0.05	
	0.12	0.12	0.09	0.09	0.08	0.08	0.06	0.06	0.05	0.05
	0.12		0.09		0.09		0.05		0.05	

1.4 Cadmium in various parts of water hyacinth

1.4.1Cadmium in roots part of water hyacinth

Table C.4 Concentration of cadmium in roots part of water hyacinth

Chelating agent	Concentration of cadmium in roots part of water hyacinth (mg/kg dry weight plant)									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	17.65		29.39		35.07		41.54		46.98	
	25.35	22.93	29.34	29.57	53.72	36.60	74.47	51.37	35.28	45.88
	25.80		29.98		21.00		38.10		55.38	
EDTA 0.5 mg/l	62.04		56.13		110.40		23.80		157.81	
	24.32	41.61	55.92	55.97	15.44	80.34	53.94	79.38	72.07	132.17
	38.46		55.86		115.18		160.40		166.63	
EDTA 1 mg/l	49.45		53.76		104.16		82.73		116.52	
	48.78	49.28	56.96	63.16	57.25	78.03	77.92	79.30	150.89	160.91
	49.61		78.76		72.69		77.26		215.31	
EDTA 2 mg/l	55.66		39.67		137.64		36.96		101.82	
	24.72	36.88	102.10	55.03	53.24	74.39	65.01	98.45	123.97	111.66
	30.25		23.32		32.28		193.38		109.18	
DTPA 0.5 mg/l	111.76		30.42		44.14		104.89		45.92	
	8.99	44.22	19.58	65.26	165.97	80.52	80.34	83.33	98.43	77.48
	11.90		145.76		31.44		64.75		88.09	
DTPA 1 mg/l	52.28		39.55		28.97		97.57		42.10	
	8.30	36.76	41.42	38.06	65.09	73.81	145.87	139.26	163.87	81.27
	49.72		33.21		127.38		174.34		37.85	
DTPA 2 mg/l	132.14		101.19		232.40		194.14		395.91	
	1.94	77.48	98.61	101.03	43.79	106.28	75.45	112.29	216.37	231.78
	98.35		103.31		42.64		67.27		83.05	
EDTA +DTPA 0.5 mg/l	64.82		28.02		96.63		76.65		63.27	
	63.73	66.00	56.10	61.84	28.66	81.83	57.09	97.46	152.93	105.04
	69.47		101.40		120.21		158.64		98.92	
EDTA +DTPA 1 mg/l	59.87		48.42		77.92		83.39		76.60	
	57.49	58.52	45.49	45.23	76.77	78.22	86.10	85.23	122.51	150.89
	58.20		41.78		79.96		86.20		253.57	
EDTA +DTPA 2 mg/l	23.58		88.81		97.38		105.86		135.88	
	10.49	28.98	139.52	90.68	95.13	95.00	104.77	105.82	161.99	157.48
	52.87		43.70		92.49		106.82		174.56	

1.4.2 Cadmium in shoots part of water hyacinth

Table C.5 Concentration of cadmium in shoots part of water hyacinth

Chelating agent	Concentration of cadmium in shoots part of water hyacinth (mg/kg dry weight plant)									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	4.62		5.56		9.45		8.16		10.45	
	4.42	4.65	4.81	5.85	7.52	8.18	10.35	9.32	12.90	10.62
	4.92		7.19		7.57		9.45		8.50	
EDTA 0.5 mg/l	4.90		8.19		12.04		10.99		10.67	
	4.69	4.75	12.62	10.54	8.17	12.41	11.21	12.99	9.86	11.58
	4.65		10.80		17.02		16.78		14.21	
EDTA 1 mg/l	6.88		9.33		12.50		11.35		9.18	
	4.60	5.41	9.96	8.45	9.19	11.80	11.20	11.82	12.92	11.23
	4.74		6.07		13.73		12.90		11.60	
EDTA 2 mg/l	3.68		6.02		9.78		18.10		10.63	
	6.99	4.17	6.24	8.83	14.19	10.90	13.38	13.37	10.11	10.02
	1.85		14.24		8.72		8.63		9.33	
DTPA 0.5 mg/l	6.89		5.29		7.89		21.53		8.06	
	0.85	2.87	12.82	7.73	9.66	11.38	14.08	16.81	11.10	14.16
	0.87		5.09		16.60		14.82		23.31	
DTPA 1 mg/l	5.96		10.38		13.99		3.26		7.74	
	8.10	12.20	11.99	16.86	49.70	24.82	70.97	27.03	20.27	14.64
	22.53		28.21		10.77		6.84		15.91	
DTPA 2 mg/l	14.63		9.67		21.54		22.30		26.36	
	1.92	10.00	11.94	11.70	7.82	13.23	12.68	16.79	13.86	16.34
	13.47		13.48		10.32		15.41		8.80	
EDTA +DTPA 0.5 mg/l	4.35		12.18		12.29		13.89		15.26	
	2.22	8.30	8.51	10.93	11.41	13.52	12.40	16.75	17.76	20.80
	18.33		12.09		16.88		23.97		29.39	
EDTA +DTPA 1 mg/l	2.97		11.13		20.55		16.62		20.04	
	9.85	5.95	11.79	13.40	20.69	20.90	17.41	18.12	17.05	17.93
	5.03		17.29		21.46		20.34		16.69	
EDTA +DTPA 2 mg/l	4.98		10.51		20.29		12.11		16.61	
	1.00	2.35	11.83	11.37	27.54	23.61	19.84	19.10	21.23	18.81
	1.08		11.78		23.00		25.35		18.59	

2. Percentage Growth rate and Relative Growth Rate (RGR) of water hyacinth

2.1 Percentage Growth rate of water hyacinth

Table C.6 Percentage Growth rate of water hyacinth

Chelating agent	Percentage Growth rate of water hyacinth									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	30.66	73.08	74.73	115.98	144.50	146.07	237.47	199.04	424.26	419.74
	166.69		74.38		182.48		303.40		297.29	
	104.51		190.05		110.17		101.64		571.62	
EDTA 0.5 mg/l	173.88	94.83	99.28	99.99	249.91	274.47	90.87	98.17	83.03	140.47
	53.13		89.07		314.24		159.32		178.50	
	55.34		112.27		267.03		61.32		150.73	
EDTA 1 mg/l	109.86	112.98	118.11	135.38	76.49	101.96	185.33	221.29	286.86	297.06
	187.54		135.82		105.78		174.45		295.49	
	69.78		157.86		130.94		331.90		308.30	
EDTA 2 mg/l	122.45	66.75	330.76	210.53	112.53	132.80	255.56	257.62	428.64	358.86
	26.47		175.97		122.44		288.16		360.29	
	62.24		187.90		168.03		213.63		303.44	
DTPA 0.5 mg/l	81.46	62.44	275.41	178.14	163.92	192.85	467.21	212.90	188.05	231.91
	4.03		254.40		157.25		190.57		462.08	
	85.59		68.36		346.26		125.80		140.78	
DTPA 1 mg/l	48.33	58.44	82.69	160.23	224.09	187.79	204.81	159.77	61.08	127.07
	12.65		107.64		130.52		226.89		166.66	
	93.60		350.81		277.21		97.95		191.86	
DTPA 2 mg/l	40.70	54.62	94.79	160.53	162.50	200.78	56.09	94.14	53.14	99.86
	75.61		161.00		226.16		77.34		179.17	
	59.01		232.51		235.39		198.62		93.14	
EDTA +DTPA 0.5 mg/l	21.95	65.08	42.40	129.50	222.41	149.11	62.15	69.94	16.59	13.34
	69.27		142.59		118.24		43.90		122.20	
	97.42		204.47		114.64		107.68		8.78	
EDTA +DTPA 1 mg/l	64.06	72.06	234.85	194.93	218.78	261.88	81.77	62.84	141.36	74.10
	56.10		195.65		314.30		53.08		69.07	
	99.21		158.00		287.90		54.19		39.50	
EDTA +DTPA 2 mg/l	82.33	121.54	313.82	252.31	208.81	119.07	61.08	98.25	51.93	40.56
	153.82		310.64		125.99		163.14		13.11	
	126.95		160.43		43.62		89.49		60.31	

2.2 Relative Growth Rate (RGR) of water hyacinth

Table C.7 Relative Growth Rate of water hyacinth

Chelating agent	Relative Growth Rate of water hyacinth									
	20 day	AVG	40 day	AVG	60 day	AVG	80 day	AVG	100day	AVG
Control	1.31	1.73	2.45	2.46	3.37	2.99	3.68	4.06	5.24	5.20
	2.67		2.82		4.03		2.62		3.97	
	2.05		2.10		2.02		6.24		6.72	
EDTA 0.5 mg/l	2.74	1.95	1.99	2.00	3.50	3.74	2.44	2.10	1.83	2.40
	1.53		1.89		4.14		2.02		2.79	
	1.55		2.12		3.67		1.88		2.51	
EDTA 1 mg/l	2.18	2.35	2.10	2.13	1.76	2.02	3.42	3.37	3.87	3.97
	2.36		2.88		2.06		3.08		3.95	
	2.58		1.70		2.31		3.59		4.08	
EDTA 2 mg/l	2.22	1.67	4.31	3.11	2.13	2.33	3.41	3.28	5.29	4.59
	1.26		2.76		2.22		3.87		4.60	
	1.62		2.88		2.68		2.48		4.03	
DTPA 0.5 mg/l	1.81	1.62	2.88	3.32	3.75	2.78	5.52	2.91	5.67	3.13
	1.04		5.62		3.54		2.75		2.91	
	1.86		2.41		1.68		1.95		2.26	
DTPA 1 mg/l	1.48	1.58	1.83	2.60	3.24	2.88	3.29	2.86	3.05	2.60
	1.13		2.08		2.31		3.13		3.27	
	1.94		4.51		3.77		2.47		1.98	
DTPA 2 mg/l	1.41	1.55	1.95	2.61	2.62	3.01	1.34	1.94	1.53	2.00
	1.76		2.61		3.26		3.41		2.79	
	1.59		3.33		3.35		1.55		1.93	
EDTA +DTPA 0.5 mg/l	1.22	1.65	1.42	2.30	3.22	2.49	1.00	1.23	0.83	1.13
	1.69		2.43		2.18		1.84		2.22	
	1.97		3.04		2.15		1.16		0.91	
EDTA +DTPA 1 mg/l	1.64	1.72	3.35	2.95	3.19	3.62	2.63	1.81	2.41	1.74
	1.56		2.96		4.14		1.57		1.69	
	1.99		2.58		3.88		1.52		1.39	
EDTA +DTPA 2 mg/l	1.82	2.22	4.14	3.52	3.09	2.19	2.03	1.88	1.61	1.98
	2.54		4.11		2.26		2.17		2.63	
	2.27		2.60		1.44		1.59		1.89	
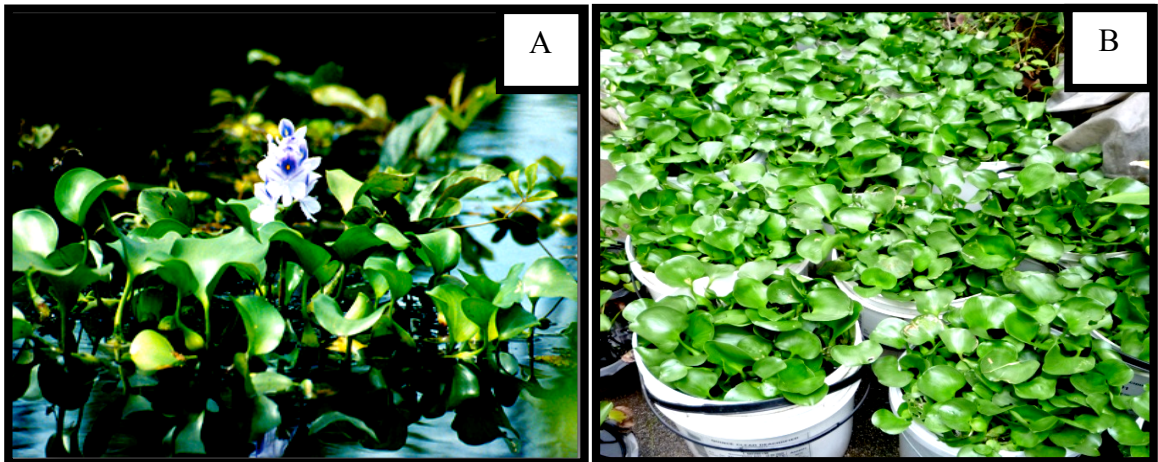
Figure D-1 A: Uncontaminated area and B: Water hyacinth preparations

Figure D-2 A: Water hyacinth collections and B: The size of water hyacinth

Figure D-3: Chelating agent preparation and Pot preparation

Figure D-4: Green house

Figure D-5 A: Water hyacinth sample and B: Water sample and Soil sample

Figure D-6 A: Microwave digester and B: Atomic absorption spectrometer

APPENDIXS E

STATISTIC ANALYSIS

1. Cadmium in various parts of water hyacinth

Table E.1 Concentration of total cadmium in roots part of water hyacinth

EDTA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05								
		1	2	3	4	5	6	7	8	9
Blank : 20 day	3	10.26								
Blank : 40 day	3	10.41								
Blank : 60 day	3	10.34								
Blank : 80 day	3	10.66								
Blank : 100 day	3	8.81								
Control : 20 day	3		22.93							
Control : 40 day	3		29.57							
Control : 60 day	3		36.60							
Control : 80 day	3						51.37			
Control : 100 day	3						45.88			
EDTA 0.5 mg/L : 20 day	3			41.61						
EDTA 0.5 mg/L : 40 day	3				55.97					
EDTA 0.5 mg/L : 60 day	3					80.34				
EDTA 0.5 mg/L : 80 day	3					79.38				
EDTA 0.5 mg/L : 100 day	3								132.17	
EDTA 1 mg/L : 20 day	3				49.28					
EDTA 1 mg/L : 40 day	3				63.16					
EDTA 1 mg/L : 60 day	3					78.03				
EDTA 1 mg/L : 80 day	3					79.30				
EDTA 1 mg/L : 100 day	3									160.91
EDTA 2 mg/L : 20 day	3		36.88							
EDTA 2 mg/L : 40 day	3				55.03					
EDTA 2 mg/L : 60 day	3					74.39				
EDTA 2 mg/L : 80 day	3							98.45		
EDTA 2 mg/L : 100 day	3							111.66		

DTPA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05							
		1	2	3	4	5	6	7	8
Blank : 20 day	3	10.26							
Blank : 40 day	3	10.41							
Blank : 60 day	3	10.34							
Blank : 80 day	3	10.66							
Blank : 100 day	3	8.81							
Control : 20 day	3		22.93						
Control : 40 day	3		29.57						
Control : 60 day	3			36.60					
Control : 80 day	3			51.37					
Control : 100 day	3			45.88					
DTPA 0.5 mg/L : 20 day	3			44.22					
DTPA 0.5 mg/L : 40 day	3				65.26				
DTPA 0.5 mg/L : 60 day	3						80.52		
DTPA 0.5 mg/L : 80 day	3						83.33		
DTPA 0.5 mg/L : 100 day	3						77.48		
DTPA 1 mg/L : 20 day	3			36.76					
DTPA 1 mg/L : 40 day	3			38.06					
DTPA 1 mg/L : 60 day	3						73.81		
DTPA 1 mg/L : 80 day	3							139.26	
DTPA 1 mg/L : 100 day	3						81.27		
DTPA 2 mg/L : 20 day	3				77.48				
DTPA 2 mg/L : 40 day	3					101.03			
DTPA 2 mg/L : 60 day	3					106.28			
DTPA 2 mg/L : 80 day	3					112.29			
DTPA 2 mg/L : 100 day	3								231.78

EDTA and DTPA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
Blank : 20 day	3	10.26						
Blank : 40 day	3	10.41						
Blank : 60 day	3	10.34						
Blank : 80 day	3	10.66						
Blank : 100 day	3	8.81						
Control : 20 day	3				22.93			
Control : 40 day	3					29.57		
Control : 60 day	3					36.60		
Control : 80 day	3			51.37				
Control : 100 day	3			45.88				
EDTA +DTPA 0.5 mg/L : 20 day	3			66.00				
EDTA +DTPA 0.5 mg/L : 40 day	3			61.84				
EDTA +DTPA 0.5 mg/L : 60 day	3						81.83	
EDTA+DTPA 0.5 mg/L : 80 day	3						97.46	
EDTA +DTPA 0.5 mg/L : 100 day	3						105.04	
EDTA +DTPA 1 mg/L : 20 day	3			58.52				
EDTA +DTPA 1 mg/L : 40 day	3			45.23				
EDTA +DTPA 1 mg/L : 60 day	3						78.22	
EDTA +DTPA 1 mg/L : 80 day	3						85.23	
EDTA +DTPA 1 mg/L : 100 day	3							150.89
EDTA +DTPA 2 mg/L : 20 day	3				28.98			
EDTA +DTPA 2 mg/L : 40 day	3						90.68	
EDTA+DTPA 2 mg/L : 60 day	3						95.00	
EDTA+DTPA 2 mg/L : 80 day	3						105.82	
EDTA+DTPA 2 mg/L : 100 day	3							157.48

Table E.2 Concentration of total cadmium in shoots part of water hyacinth
EDTA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05		
		1	2	3
Blank : 20 day	3	4.76		
Blank : 40 day	3	4.68		
Blank : 60 day	3	4.27		
Blank : 80 day	3	4.51		
Blank : 100 day	3	4.23		
Control : 20 day	3	4.65		
Control : 40 day	3	5.85		
Control : 60 day	3		8.18	
Control : 80 day	3		9.32	
Control : 100 day	3			10.62
EDTA 0.5 mg/L : 20 day	3	4.75		
EDTA 0.5 mg/L : 40 day	3			10.54
EDTA 0.5 mg/L : 60 day	3			12.41
EDTA 0.5 mg/L : 80 day	3			12.99
EDTA 0.5 mg/L : 100 day	3			11.58
EDTA 1 mg/L : 20 day	3	5.41		
EDTA 1 mg/L : 40 day	3		8.45	
EDTA 1 mg/L : 60 day	3			11.80
EDTA 1 mg/L : 80 day	3			11.82
EDTA 1 mg/L : 100 day	3			11.23
EDTA 2 mg/L : 20 day	3	4.17		
EDTA 2 mg/L : 40 day	3		8.83	
EDTA 2 mg/L : 60 day	3			10.90
EDTA 2 mg/L : 80 day	3			13.37
EDTA 2 mg/L : 100 day	3			10.02

DTPA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05							
		1	2	3	4	5	6	7	8
Blank : 20 day	3	4.76							
Blank : 40 day	3	4.68							
Blank : 60 day	3	4.27							
Blank : 80 day	3	4.51							
Blank : 100 day	3	4.23							
Control : 20 day	3	4.65							
Control : 40 day	3	5.85							
Control : 60 day	3					8.18			
Control : 80 day	3					9.32			
Control : 100 day	3					10.62			
DTPA 0.5 mg/L : 20 day	3		2.87						
DTPA 0.5 mg/L : 40 day	3					7.73			
DTPA 0.5 mg/L : 60 day	3			11.38					
DTPA 0.5 mg/L : 80 day	3						16.81		
DTPA 0.5 mg/L : 100 day	3								14.16
DTPA 1 mg/L : 20 day	3								14.64
DTPA 1 mg/L : 40 day	3							27.03	
DTPA 1 mg/L : 60 day	3							24.82	
DTPA 1 mg/L : 80 day	3						16.86		
DTPA 1 mg/L : 100 day	3			12.20					
DTPA 2 mg/L : 20 day	3				10.00				
DTPA 2 mg/L : 40 day	3			11.70					
DTPA 2 mg/L : 60 day	3			13.23					
DTPA 2 mg/L : 80 day	3						16.79		
DTPA 2 mg/L : 100 day	3						16.34		

EDTA and DTPA set

Ducan

Time and concentration of EDTA	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
Blank : 20 day	3	4.76						
Blank : 40 day	3	4.68						
Blank : 60 day	3	4.27						
Blank : 80 day	3	4.51						
Blank : 100 day	3	4.23						
Control : 20 day	3	4.65						
Control : 40 day	3	5.85		5.85				
Control : 60 day	3		8.18					
Control : 80 day	3		9.32					
Control : 100 day	3		10.62					
EDTA +DTPA 0.5 mg/L : 20 day	3		8.30					
EDTA +DTPA 0.5 mg/L : 40 day	3					10.93		
EDTA +DTPA 0.5 mg/L : 60 day	3						13.52	
EDTA+DTPA 0.5 mg/L : 80 day	3							16.75
EDTA +DTPA 0.5 mg/L : 100 day	3							20.80
EDTA +DTPA 1 mg/L : 20 day	3			5.95				
EDTA +DTPA 1 mg/L : 40 day	3						13.40	
EDTA +DTPA 1 mg/L : 60 day	3							20.90
EDTA +DTPA 1 mg/L : 80 day	3							18.12
EDTA +DTPA 1 mg/L : 100 day	3							17.93
EDTA +DTPA 2 mg/L : 20 day	3	2.35						
EDTA +DTPA 2 mg/L : 40 day	3					11.37		
EDTA+DTPA 2 mg/L : 60 day	3							23.61
EDTA+DTPA 2 mg/L : 80 day	3							19.10
EDTA+DTPA 2 mg/L : 100 day	3							18.81

BIOGRAPHY

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