## **CHAPTER II**

## THEORETICAL BACKGROUND AND

## LITERATURE REVIEWS

#### 2.1 Heavy metals

## 2.1.1 Cadmium (Cd)

## 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<sup>-3</sup>; 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 form. 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, chloro-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 (Adriano, 2001).

#### 2) Cadmium in soil

In the earth's crust, the highest average concentration of Cd is in a range between 0.15 to 0.20 ppm. Cd is closely related geochemical to Zn, and mainly found in Zn, Pb-Zn, and Pb-Cu-Zn ores. Cadmium is also found in wurtzite, another ZnS, and in trace amounts in galena, tetrahedrite, and a variety of other sulfosalts (Adriano, 2001). Most soil has Cd levels of about 0.30 ppm. In agricultural soils, Cd level is about 1 ppm (or less). From the worldwide, the average value of Cd in soil is around 0.40 ppm (Berrow and Reaves, 1984).

Cadmium is strongly associated with Zn in its geochemistry, but seems to have a stronger affinity for S than Zn, and also exhibits a higher mobility than Zn in acid environment. During weathering, Cd goes readily into solution and, although known to occur as  $Cd^{2+}$ , it may also form several complex ions (CdCl<sup>+,</sup> CdOH<sup>+</sup>, CdHCO<sub>3</sub><sup>+</sup>, CdCl<sub>3</sub><sup>-</sup>, CdCl<sub>4</sub><sup>2-</sup>, Cd(OH)<sub>3</sub>, and CdCO<sub>3</sub>) and organic chelates. However, the most important valence state of Cd in the natural environment is +2, and the most important factors which control Cd ion mobility are pH and oxidation potential. Under conditions of strong oxidation, Cd is likely to form minerals (CdO, CdCO<sub>3</sub>) and is also likely to be accumulated in phosphate and in biolith deposits. The main factor determining the Cd content of soil is the chemical composition of the parent rock. The major causes of Cd contamination in soils are mining and smelting of Cd and Zn.

## 3) Cadmium in plant

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 such as pH, CEC, redox potential, OM, other metals, fertilization, and others. Plant factors such as 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). Leafy 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 of 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, ambient temperature, and light intensity, can affect the distribution of the metal between the shoots and roots of the rice plant (Chino and Baba, 1981).

## 2.1.2 Zinc (Zn)

## 1) General properties of zinc

Zinc is a bluish white, relatively soft metal with a density of 7.13 g cm<sup>-3</sup>. It belongs to Group II-B of the periodic table and has an atomic weight of 65.38 and melting point of 420 °C. Zinc is divalent in all its compounds. The  $Zn^{2+}$  ion is colorless and exists in a hydrated form in acidic and neutral aqueous solutions; however, the hydroxide is precipitated in alkaline solution. With excess base, the hydroxide redissolves to form zincate ion,  $Zn(OH)_4^{2-}$ . Due to its amphoteric nature, Zn forms a variety of salts; the chlorates, chlorides, sulfates, and nitrates are readily soluble in water whereas the oxides, carbonates, phosphates, silicates, the sulfides are relatively insoluble in water. The oxidation state of Zn in nature is II (Adriano, 2001).

Zinc is mainly used as a protective coating for iron and steel. The automobile industry accounts for almost one-third of United States slab Zn consumption. Main pollutant sources for Zn in soils are mealliferous mining activities, agricultural use of sewage sludge and composted materials, and the use of agrochemicals such as fertilizers and pesticides (Alloway, 1995).

#### 2) Zinc in soil

Zinc is the 24<sup>th</sup> most abundant element in the earth's crust, with the average value at ~70 ppm (Krauskop, 1979). Zinc is abundant in magma and premetamorphic rock (Wedepohl, 1978). The most common impurities in Zn are minerals, Fe, Cd, and Pb Usually Cd is about 0.05% as abundant as Zn. Thus, processing 1 tonne of Zn ore produces about 3 kg of Cd (Adriano, 2001).

The Zn content in soils depends on the nature of the parent rock, Organic Matter (OM), texture, and pH. Total Zn in normal soils is 10 to 300 ppm (Swine, 1955). The most common of Zn mobilization in soil is believed to be in forms of free and complexed ions in soil solutions, but several other ionic species that occur as nonspecifically and specifically adsorbed cations which can also be easily mobilized in soils. From the Lindsay studied (1979), it has been shown that clay and soil organic matter are capable of holding Zn quite strongly; thus the solubility of Zn in soils is less than that of Zn(OH)<sub>2</sub>, ZnCO<sub>3</sub>, and Zn(PO<sub>4</sub>) in pure experimental systems. The clay minerals, hydrous oxides, and pH are likely to be the most important factor controlling Zn solubility in soils, while organic complexing and precipitation of Zn as hydroxide, carbonate, and sulfide compounds appear to be of much lesser importance.

## 3) Zinc in plant

Zinc is an essential element for plant nutrition. Unlike the major nutrients ordinarily supplied in mined fertilizer, it is required in only minute amounts. Zinc is very important in plant nutrition because it is involved in a number of metalloenzymes, is essential in the stability of cytoplasmic ribosomes and roots cell plasma membrane, catalyzes the process of oxidation, and the transformation of carbohydrates (Kochian, 1993; Romheld and Marschner, 1991).

In the studies of plants, Chinese tea leaves contain Zn from 26 to 40 ppm, of which up to 50% is extracted by hot water. Zn in grass and clover throughout the world is also relatively stable, and mean levels in grasses ranged from 12 to 47 ppm (DW), and in clovers ranged from 24 to 45 ppm (DW) (Adriano, 2001).

#### 2.1.3 Lead (Pb)

## 1) General properties of lead

Lead (atom. no. 82) is a bluish-gray metal that is found in small quantities in the earth's crust. It is of bright luster, soft, highly malleable, ductile, and a poor conductor of electricity. It is very resistant to corrosion and belongs to Group IV-A in the periodic table, has an atomic weight of 207.2, melting point of 328 °C, and specific gravity of 11.4. Lead has II or IV of oxidation state but in most inorganic compounds, Pb is in the II oxidation state. Pure Pb is insoluble in water; however, its chloride and bromide salts are slightly soluble (~1%) in cold water, whereas carbonate and hydroxide salts are almost insoluble. Because of their use as antiknock agents in gasoline, tetramethyl and tetraethyl, Pb are the most important organolead compounds (Adriano, 2001). The Pb metal is very soft and tends to creep or flow under sustained pressure: it is therefore readily cut and shaped, and since ancient times it has been used on roofs or as pipes. Metallic Pb is relatively opaque to ionizing radiation, and makes a valued shield material in X-ray and radioisotope work. Lead readily alloys with other metals: the Pb/Sb alloy is chiefly used to make battery plates and are often used as solder. Lead metal, in combination with PbO<sub>2</sub>, is used to fabricate the lead acid accumulator battery. Other inorganic compounds are widely used, e.g., the yellow chromate is used in road markings, and many paints contain Pb oxides or Pb sops to promote polymerisation (Kabata-Pendias, 2001).

### 2) Lead in soil

Lead concentration is presented in all soils at levels ranging from < 1.0 ppm in normal soils to well over 10% in ore materials. The average abundance of Pb in the Earth's crust is estimated at about 15 ppm. In the terrestrial environment, two kinds of Pb ore known: primary and secondary. Primary Pb is of geogenic origin and was incorporated into minerals at the time of their formation, and secondary Pb is of a radiogenic origin from the decay of U and Th. Pb occurs mainly as Pb<sup>2+</sup>, although its oxidation state, +4, is also known, and it forms several other minerals which are quite insoluble in natural waters (Kabata-Pendias, 2001). Soil is a sink for anthropogenic Pb and there are several well-recognized major sources, namely, mining and smelting activities, sewage sludge usage in agriculture and combination from vehicle exhausts. Lead arsenate (PbHAsO<sub>4</sub>) has been applied to orchard trees to control insect pests, and orchard soils may therefore contain elevated concentrations of Pb (Alloway, 1995).

#### 3) Lead in plant

Plants can accumulate Pb from soil or foliar application. However, the results vary according to plant species, source term, and experimental conditions (Adriano, 2001). Pb uptake by plants from soil is a passive process that proceeds rapidly until exchange sites in the root-free space are equilibrated with solution concentration (Zimdahl and Arvik, 1973). Solute transport from the external root environment to the central root xylem and to the shoot takes place through two major pathways: apoplastic (cell wall space between cell membranes) and symplastic (crossing many cell membranes along the path) (Adriano, 2001).

# 2.1.4 Copper (Cu)

### 1) General properties of copper

Copper is reddish, takes on a bright metallic luster, is malleable, ductile, and a good conductor of heat and electricity (second only to silver in electrical conductivity). It belongs to Group I-B in the periodic table, has an atomic weight of 63.55, a melting point of 1083 °C, and a specific gravity of 8.96. In nature, Cu occurs in the I and II oxidation states. In the II, it is ismorphous with  $Zn^{2+}$ ,  $Mg^{2+}$ , and  $Fe^{2+}$  ions. It is found in minerals such as cuprite, malachite, azurite, chalcopyrite, and bornite. The most important Cu ores are the sulfides oxides, and carbonates (Adriano, 2001). In nature, Cu forms sulfides, sulfated, sulfosalts, carbonates, and other compounds and also occurs as the native metal. Chalcopyrite (CuFeS<sub>2</sub>, 34% Cu) is by far the most abundant Cu mineral, being found widely dispersed in rocks and concentrated in the largest Cu ore deposits. Estimates for the average crystal abundance of Cu range from 24 to 55 ppm (Cox, 1979).

Copper is principally used in the production of wire and copper alloys: brass (with zinc) and bronze (with tin). Copper is also alloyed with Au, Pb, Cd, Cr, Be, Ni, Al, and Mn. The electrical industry is one of the major users of Cu in the production of electrical wires and other electrical apparatus because of its high thermal conductance and relative inertness. Furthermore, Cu is extensively used in containers such as boilers, steam pipes, automobile radiators, and kitchenware. It is also extensively used in agriculture in the form of fertilizers, bactericides and fungicides, and as algicides in water purification. It is used as a feed additive, such as in antibiotics, drugs, and selected chemical compounds, as a growth promoter, and as an agent for disease control in livestock and poultry production (Adriano, 2001). Sources of Cu pollution are smelters, fertilizers, sewage sludge and other wastes, fungicides or bactericides, and manures from swine and poultry (Alloway, 1995).

## 2) Copper in soil

In soil profiles, the common characteristic of Cu distribution is its accumulation in the top horizons. This phenomenon is an effect of various factors, but above all, Cu concentration in surface soils reflects the bioaccumulation of the metal and also recent anthropogenic sources of the element (Kabata-Pendias, 2001).

The average value of 30 ppm (range of 2 to 250 ppm) for total Cu content of world soils has been reported (Adriano, 2001). For normal arable soils, total Cu is expected to range from 1 to about 50 ppm (Gilbert, 1952). A mean of 33 ppm total Cu was reported for tropical Asian paddy soils, ranging from 11 ppm for Malaysian soils to 50 ppm for Sri Lanka soils (Domingo and Kyuma, 1983). In China, a total Cu content of soils having a geometric mean of 23 ppm was reported (Chen et al., 1991). For Japanese soils, Shiha (1951) reported an average of 93 ppm total Cu, the range being 26 to 151 ppm.

## 3) Copper in plant

Copper is one of seven traditional micronutrients (Zn, Cu, Mn, Fe, B, Mo, and Cl) essential for normal plant nutrition. Copper is required only in very small amounts; 5 to 20 ppm in crop tissue is adequate for normal growth, while <4 ppm is considered deficient, and >20 ppm is considered toxic (Jones, 1972). Copper has been established as a constituent of a number of plant enzymes. It occurs as part of the prosthetic groups of enzymes, as an activator of the enzyme systems, and as a facultative activator in enzyme systems (Gupta, 1979). Insufficient Cu affects a multitude of physiological processes in plants: carbohydrate metabolism (photosynthesis, respiration, and carbohydrate distribution), N metabolism (especially

lignin synthesis), water relations, seed production (especially pollen viability), and disease resistance (Bussler, 1981; Romheld and Marschner, 1991). It may also affect ion uptake and plant differentiation in early developmental stages. Copper deficiency in legumes depresses nodulation and  $N_2$  fixation, leading to N deficiency symptoms that can be corrected by application of Zn fertilizers. In general, Cu deficiency depresses reproductive growth (i.e., formation of seeds and fruits) more than vegetative growth.

## 2.2 Phytoavailability

Phytoavailability 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 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 Cunningham, 1998).

Since soils 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 plants 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 2.1). Excluders include members of the grass family (e.g., Sudan grass, brome grass, 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, oats, etc.), and accumulators include the mustard and *Compositae* families (e.g., lettuce, spinach, chard, etc.) and tobacco. There are extreme accumulators (know as hyper accumulators) 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). Hyper accumulators are plants and/or genotypes that accumulate metals above certain concentrations in leaves. Greger (1991) suggested

hyper accumulators 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.

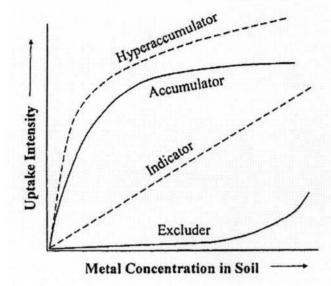
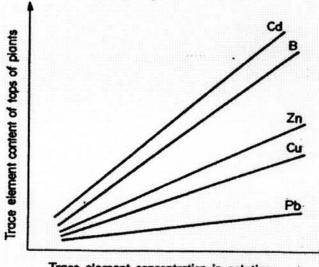


Figure 2.1 Relative uptake and bioaccumulation potential among plant species Source: Adriano, 2001

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 are shown in Figure 2.2. Following root absorption, the extent to which elements decrease follows in the order Cd>B>Zn>Cu>Pb (Alloway, 1995).



Trace element concentration in solutions

Figure 2.2 Trace element uptakes by plants as a function of their concentrations in nutrient solution

Source: Kabata-Pendias, 2000; Alloway, 1995

#### 2.2.1 Factor affecting mobility and bioavailability

## 1) pH

In general, the retention capacity of soils for trace metals increases when pH increases. The heavy metals, 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 2.3), 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.

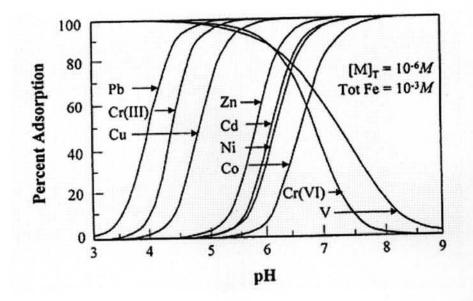


Figure 2.3 Modeled adsorptions of certain trace elements onto hydrous ferric oxide Source: Adriano, 2001

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, blueberries, etc.) species. Above pH 7, the risk of micronutrient deficiency including for Fe, Zn, Mn, and B increased.

The effect of pH on chemical speciation of trace elements in soils and sediments is illustrated in Table 2.1.

Element	Acid soils/sediments	Alkaline soils/sediments	
Cd (II)	Cd <sup>2+</sup> , CdSO <sup>0</sup> <sub>4</sub> , CdCl <sup>-</sup>	Cd <sup>2+</sup> , CdCl <sup>-</sup> , CdSO <sup>0</sup> <sub>4</sub> , CdHCO <sub>3</sub> <sup>+</sup>	
Cu(II)	Cu <sup>2+</sup> , CuCl <sup>-</sup>	CuCO <sup>0</sup> <sub>3</sub> , CuHCO <sub>3</sub> <sup>+</sup>	
Pb(II)	Pb <sup>2+</sup> , PbSO <sup>0</sup> <sub>4</sub> , PbHCO <sub>3</sub> <sup>+</sup>	PbCO <sup>0</sup> <sub>3</sub> , PbHCO <sub>3</sub> <sup>+</sup> , Pb(CO <sub>3</sub> ) <sup>2-</sup> , PbOH <sup>+</sup>	
Zn(II)	$Zn^{2+}$ , $ZnSO_4^0$	ZnHCO <sub>3</sub> <sup>+</sup> , ZnCO <sup>+</sup> <sub>3</sub> , Zn <sup>2+</sup> , ZnSO <sup>0</sup> <sub>4</sub>	

Table 2.1 Expected dominant oxidation states and chemical species of trace elements in aqueous solution.

Source: Adriano, 2001

#### 2) Cation exchange capacity

The cation exchange capacity (CEC) of soils is largely dependent of 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 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., clays, OM, and oxides), which may limit the solubility and mobility of metals instead of being a specific factor in the bioavailability of these metals.

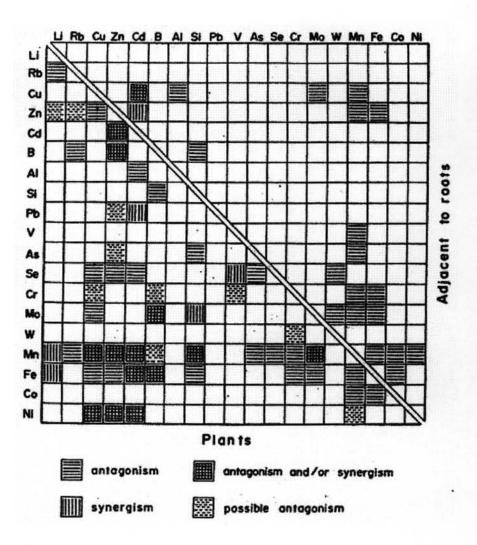
The mix of clay, silt, and sand influences the CEC of soils. In general, high clay content causes high CEC. The CEC of soil is largely proportionate to the surface area of individual components. The degree of CEC by type of soil is clay > silt > sand.

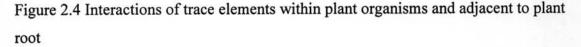
### 3) Redox potential

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 sulfides form. The metalbearing sulfides are quite insoluble so metal mobility and bioavailabilities 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).

#### **2.2.2 Interactions between metals and other elements**

Figure 2.4 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.





Source: Kabata-Pendias, 2001; 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 heavy metal may competitively inhibit root absorption of the other.

Cu-Cd interaction is reported as both antagonistic and synergistic in the element uptake by roots. Synergism may be a secondary effect of the damage to membranes 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 uptaketransport 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.

## 2.3 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):

- Changes in the permeability of the cell membrane: Ag, Au, Br, Cd, Cu, Hg, I, Pb, UO<sub>2</sub>.
- 2) Reactions of sulphydryl (SH) groups with cations: Ag, Hg, Pb.
- 3) Competition for sites with essential metabolites: As, Sb, Se, Te, W, F.
- Affinity for reacting with phosphate groups and active groups of ADP or ATP: Al, Be, Y, Zr, lanthanides and, possibly, all heavy metal.
- 5) Replacement of essential ions (mainly major cations): Cs, Li, Rb, Se, Sr.
- 6) Occupation of sites for essential groups 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.

The types of metal tolerance mechanisms in plants include:

- 1) Selective uptake of ions.
- Decreased permeability of membranes of other differences in the structure and function of membranes.
- 3) Immobilization of ions in roots, foliage and seeds.

- Removal of ions from metabolism by deposition (storage) in field and/or insoluble forms.
- Alteration in metabolic patterns-increased enzyme system that is inhibited or increased antagonistic metabolite, or reduced metabolic pathway bypassing an inhibited site.
- Adaptation to toxic metal replacement of a physiological metal in an enzyme.
- 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 2.2.

Table 2.2 General effects of trac	e element toxicity on common cultivars
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Element	Symptoms	Sensitive Crop
Cd	Brown margin of leaves, chlorosis, reddish veins and	Legumes (bean, soybean)
	petioles, curled leaves, and brown stunted roots	spinach, radish, carrots,
	Severe reduction in growth of roots, tops, and number of	and oats
	tillers (in rice).	
	Reduced conductivity of stem, caused by deterioration of	
	Xylem tissues. Reduction of chlorophyll and carotenoids	
Cu	Dark green leaves followed by induced Fe chlorosis, thick,	Cereals and legumes,
	short, or barbed-wire roots, depressed tillering. Changes	spinach, citrus
	in lipid content and losses of polypeptides involved in	seedlings, and gladiolus
	photochemical activities	
	disease of rice)	
Pb	Dark green leaves, wilting of older leaves, stunted foliage,	-
	And brown short roots	
Zn	Chlorotic and necrotic leaf tips, interveinal chlorosis in new	Cereals and spinach
	leaves, retarded growth of entire plant, and injured roots	
	resemble barbed wire	

Source: Kabata-Pendias, 2000.

### 2.4 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). 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 soils as they cannot be degraded (Audet and Charest, 2007).

#### 2.4.1 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 plants (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 does excavation of the soil or other media. This technology is most often applied to metal-contaminated soil as shown in Figure 2.5 (USEPA, 2000).

#### 2) Phytodegradation

Phytodegradation (also known as phytotransformation) is the breakdown of contaminants taken up by plants through metabolic processes 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 2.5, 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 plant root is considered biodegradation.

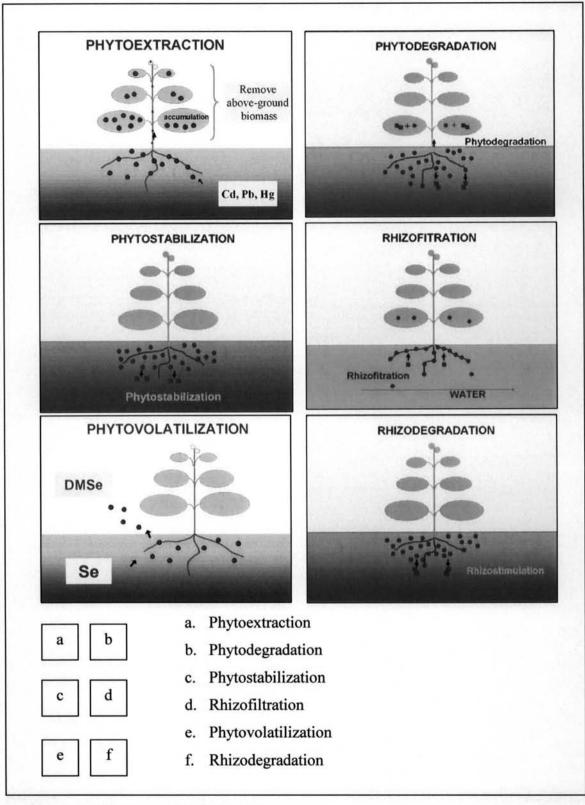


Figure 2.5 Phytoremediation technology Source: Sampanpanish, 2006

## 3) Phytovolatillization

Phytovolatilization (Figure 2.5) is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant through contaminant uptake, plant metabolism, and plant transpiration. Phytodegradation is a related phytoremediation process that can occur along with phytovolatilization.

## 4) Rhizodegradation

Rhizodegradation is the breakdown of an organic contaminant in soil through microbial activity that is enhanced by the presence of the root zone (Figure 2.5). Rhizodegradation is also known as plant-assisted degradation, plant-assisted bioremediation, plant-aided in situ biodegradation, and enhanced rhizosphere biodegradation.

## 5) Rhizofiltration

Rhizofiltration is the adsorption or precipitation onto plant roots, or absorption into the roots of contaminants that are in solution surrounding the root zone, due to biotic or antibiotic processes (Figure 2.5). Plant uptake, concentration, and translocation might occur, depending on the contaminant. Exudates from the plant roots 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 2.5) is defined as (1) immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants, and (2) the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching, and soil dispersion.

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 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 humic material in soils in a process likely related to phytostabilization in its use of plant material.

### 2.4.2 The process of metal accumulation in plant

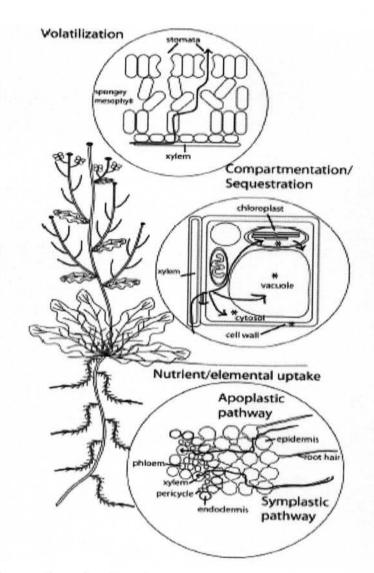
## 1) Solubilization of the metal from the soil matrix

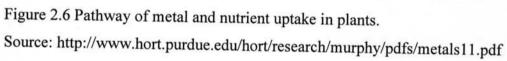
Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential 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 solublize Pb from the soil matrix, where most of soil Pb exists in an insoluble form (Blaylock and Huang 2000). Experiments demonstrating Pb hyperaccumulation have used Pb(NO<sub>3</sub>)<sub>2</sub>, a soluble form of Pb, though it must be questioned whether this is the

most appropriate form 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 metals 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 metals 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 2.6). 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 2.6). Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. Excluder plants survive by enhancing specificity for the essential element or pumping the toxic metal back out of the plant (Hall 2002; Mecharg and Macnair 1992a, 1992b).





#### 3) Transport to the leaves

Once loaded into the 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 2.6). The cell types where the metals are deposited vary between hyperaccumulator 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 thiol groups 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 hyperaccumulators allow tolerance of higher concentrations of metals (Freeman et al. 2004).

# 5) Sequestration/Volatilization

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes 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 sites on the cell walls may interact with polyvalent cations (Wang and Evangelou 1994). Selenium may also be volatized through the stomata.

#### 2.5 Advantages and Disadvantages of Phytoremediation

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

#### 2.5.1 Advantages

- 1) Works on a variety of organic and inorganic compounds
- 2) Can be either In Situ/Ex Situ
- 3) Easy to implement and maintain
- 4) Low-cost compared to other treatment methods
- 5) Environmentally friendly and aesthetically pleasing to the public
- 6) Reduces the amount wastes to be land filled

#### 2.5.2 Disadvantages

- 1) May take several years to remediate
- 2) May depend on climatic conditions
- 3) Restricted to sites with shallow contamination within rooting zone
- Harvested biomass from phytoextraction may be classified as a RCRA hazardous waste
- 5) Consumption of contaminated plant tissue is also a concern
- 6) Possible effect on the food chain

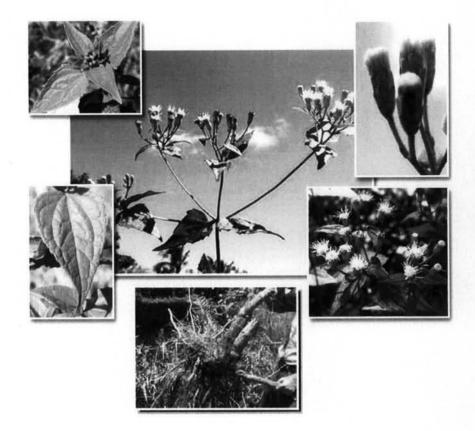
## 2.6 Type of plants

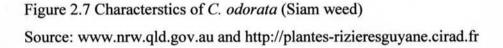
King Monkut's University of Technology Thonburi researchers surveyed a nearby site to the site for our experimental soil which involved zinc exploration. Two types of weed were generally found growing there in both normal and highly contaminated soils. These were siam weed (*Chromolaena odorata*) and vetiver grass (*Vetiveria zizainoides*), (Sasithorn, 2005). Therefore, these plants were selected to use for heavy metals removal in this experiments.

#### 2.6.1 Siam weed (Chromolaena odorata)

Siam weed is a perennial shrub that forms dense tangled bushes 1.5-2.0 m in height, occasionally reaching 6 m as a scrambler up trees. Due to its fast growth rate, and prolific, wind-dispersed seed production, the plants can spread very easily (Tanhan et al., 2007). Its roots are fibrous with a few well formed anchor roots and many laterals, the stems are round, yellowish, hairy or almost smooth and profusely branched. Its leaves are opposite, with toothed margins and are conspicuously three veined. The flowers are at the tips of all stems, in clusters of 20 to 60, white or pale lilac. The achenes consist of 5 mm-long seeds with hooks on their angles, together with a pappus of 5 mm-long white bristles. The leaves have a pungent odor when damaged. Seed production is prolific (as many as 2 million per plant) and seeds provide the main mode of reproduction. The achenes float long distances in the air and the seed hooks cling to hair and clothing. Germination occurs as soon as there is adequate moisture, although some 66% of seeds are not viable. Buried seeds lose up to 50% of their viability after 2 years (Waterhouse, 1994; McFadyen, 1996).

Siam weed is widespread throughout Southeast Asia, India, Africa, Australia, Iran, Java, Papua New Guinea, New Britain, Mariana and Caroline Is, southern China, Taiwan, Sri Lanka, Bangladesh, India, West, Central and South Africa (Figure 2.7) (Waterhouse, 1994; McFadyen, R.E., 1996; Tanhan et al., 2007). It is grown best in the tropics and subtropics, and though not tolerant of frost it can be found at altitudes up to 1000 m. It grows on most soil types but prefers well drained soils in full sun. It has a competitive advantage over other plants in climates with distinct wet and dry seasons because it survives fires and grows back vigorously following rain. As an invasive, fast growing plant, siam weed thrives in disturbed areas such as pastures, plantations, clearings, roadsides and riverbanks, particularly those in well-lit sites (CRC weed management, 2003). In Thailand, siam weed is widely distributed throughout the country especially in those areas with a pronounced dry season (Tanhan et al, 2007).





## 2.6.2 Vetiver grass (Vetiveria zizanioides)

Vetiver grass is a tropical plant which grows naturally in Thailand. Vetiver grass can be generally found growing in a wide range from highlands to lowlands and in various soil conditions (Figure 2.8). It is most commonly known in scientific terms as *Vetiveria zizanioides* for its characteristic appearance in a dense clump. It grows fast through tillering. The clump diameter is about 30 cm. and the height is 50-150 cm. The leaves are erect and rather stiff with 75 cm. of length and 8 mm. of width.



Figure 2.8 Characterstics of *V. zizanioides* (Vetiver grass) Source: http://www.chaipat.or.th/chaipat\_old/vetiver/body\_vetiver\_e.html

## 1) Culm

Vetiver grass is a prolific tiller growing in clumps with thin, long, and erect leaves. Clumps may grow densely tufted in a big cluster or scattered over the nearby space. The base makes it distinctive from other types of grass. The leaves lie on top of one another and the culm base looks flat. The genuine culm is a tiny shoot hidden in the leaf sheath at the culm base near the soil.

The growth of vetiver is controlled by tillering which occurs regularly by producing new shoots on the sides, making the clump bigger and bigger. However, it has a normal short culm with unclear joints and pedicels. Aerial branching and culm rising slightly above the ground is not commonly found in fertile conditions, but does occur with vetiver tillers grown in bags, in plots with old tillers or in critical conditions.

## 2) Leaf

Vetiver leaves will sprout from the bottom of the clump. Each blade is narrow, long and coarse. The edge of the blade is parallel and the apex is acute. Particularly on old leaves, the edge and midrib are spinulose. The base and the middle of the blade have few spines whereas the apex has numerous spines. All spines are pointing diagonally towards the apex.

The ligule at the base of the leaf is observable in a strikingly bending form with short silky hair which sometimes cannot be noticed.

#### 3) Roots

Vetiver roots are important and the most useful part. In general, grass has fibrous roots which spread out from the underground part of the culm and hold the soil in a horizontal pattern, but not penetrate vertically deep into the soil. In contrast, the Vetiver root system does not expand horizontally but penetrates vertically deep into the soil, whether it be the main roots, secondary roots or fibrous roots.

### 4) Inflorescence / Spikelets

Vetiver inflorescence is erect and it appears in the form of a panicle. The panicle and the stalk which is round and long are about 100-150 cm. above the ground. However, for a mature culm, the stalk can be as high as 200 cm. The inflorescence or the panicle alone is about 20-40 cm. high and can spread out at a maximum width of 10-15 cm. The inflorescences of *Vetiveria zizanioides* are mostly purple, the colour which is an ordinary attribute of this species.

The spikelet appears in pairs with similar features and size, except for the base of the stalk which has 3 spikelets. Each pair consists of both sessile and pedicelled spikelets. The sessile spikelet is at the middle, whereas, the pedicelled one is at the tip. Each spikelet is similar in appearance to a spindle. The edge is parallel and oval. The cuneate apex is 1.5-2.5 mm. wide and 2.5-3.5 mm. long. The surface of the back of the spikelet is rough and consists of minute spines, especially at the edge where it can be clearly seen. The lower part of the spikelet is smooth.

## 5) Seed and Seedling

After breeding, the sessile spikelet which is a hermaphrodite flower produces seeds. Each seed is light brown and spindle shaped. The surface is smooth and the apex and base is round. The inner texture is like sticky flour which turns stiff when exposed to strong wind, concentrated sunlight or other critical climatic conditions. Stiffness enables the seed to enlarge, thus improve its chance to germinate. Since the seed can germinate only within a limited period of time and some ecotypes which are imported do not seed at all, the vetiver grass cannot spread like a serious weed.

### **2.7 Literature Reviews**

Yang et al. (2003) compared the growth and metal accumulation of vetiver (*Vetiveria zizanioides*) and two legume species (*Sesbania rostrata* and *Sesbania sesban*) grown on tailing amended with domestic refuse and/or fertilizer. He found that the lead (Pb)/zinc (Zn) tailings contained high concentrations of heavy metals (total Pb, Zn, Cu, and Cd concentration 4164, 4377, 35 and 32 mg/kg, respectively), and low content of major nutrient elements (N, P, and K) and organic matter. Roots of these species accumulated similar levels of heavy metals, but the shoot of the two Sesbania species accumulated higher concentrations of Pb, Zn, Cu and Cd than shoots of *V. zizanioides*. Most of the heavy metals in *V. zizanioides* were accumulated in roots, and the translocation of metals from roots to shoots was restricted.

Somsanguan (2003) studied the phytoextraction of lead (Pb) in two species of vetiver grass (*Vetiveria zizanioides* and *V. nemoralis*) irrigated with increasing levels of Pb(NO<sub>3</sub>)<sub>2</sub> (5, 7, 9 and 11 g/L) for 12 weeks in a soil-culture study. In the laboratory, the removal of lead from soil was correlated with lead accumulation by roots and shoots of both species of vetiver. High concentration of lead (9-11 g/L)

resulted in decrease in growth, total chlorophyll content and biomass of *V. zizanioides* while *V. nemoralis* died after one week of application. Toxicity symptoms (e.g., burning leaf margins, shoots dying back) occurred in vetiver in high concentrations of lead. Based on the data, *V. zizanioides* best tolerated and accumulated the greatest amount of lead. A simulated field experiment was conducted to examine the usefulness of vetiver in removing lead from contaminated soil. The vetiver, *V. zizanioides* and *V. nemoralis* were grown in soil contaminated with Pb(NO<sub>3</sub>)<sub>2</sub> (5, 7, 9 and 11 g/L) for 3 months. The removal of lead from soil was correlated with lead accumulation by roots and shoots of both species of grasses. The grass roots took up more lead than the shoot. *V. zizanioides* could uptake lead from soil at higher levels than *V. nemoralis*. The effects of lead on relative growth of *V. zizanioides* and *V. nemoralis* showed that both species attained similar height and did not differ significantly from the controls.

Chen et al. (2004) studied the use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. In the pot experiment, the uptake and transport of Pb by vetiver from Pb contaminated soils under EDTA application was investigated. The results showed that vetiver has the capacity to tolerate high Pb concentrations in soils. With the application of EDTA, the translocation ratio of Pb from vetiver roots to shoots was significantly increased. The shoot Pb concentration reached 42, 160, 243 mg/kg DW and the root Pb concentrations were 266, 951, and 2280 mg/kg DW in the 500, 2500 and 500 mg Pb/kg soil, respectively. In the short soil leaching column 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 of soil application. In the long soil leaching experiment, soil columns were packed with uncontaminated soils and planted with vetiver. Heavy metal leachate from the short column experiment was applied to the surface of the long soil column, the artificial rainwater was percolated, and the final leachate was collected at the bottom of the soil columns. The results showed that soil matrix with planted vetiver, could re-adsorb 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), zinc (500 mg/kg), and lead (1,000 mg/kg) in pot experiments. Three concentrations of Na<sub>2</sub>-EDTA solution (0, 5, and 10 mmol/kg 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.

Xia, H. P. (2004) studied the ecological rehabilitation and phytoremediation with Vetiver grass (Vetiveria zizanioides), Bahia grass (Paspalum nottum), St. Augustine grass (Stenotaphrum secundatum), and Bana grass (Pennisetum glaucum x P. purpureum) in oil shale mined land. And found that Vetiver had the highest survival rate, up to 99%, following by Bahia, St. Augustine and Bana, 96%, 91% and 62%, respectively. Fertilizer application significantly increased biomass and tiller number of the four grases, of which St. Augustine was promoted most, up to 70% for biomass, while Vetiver was promoted leas, only 27% for biomass. Lead (Pb) and cadmium (Cd) tested in this trial had different concentrations in the oil shale residue, and also had different contents and distributions in the four grass species. Concentrations of Pb and Cd in the four grasses presented a disparity of only 1.6-3.8 times, but their uptake amounts of the two metals were u 27.5-35.5 times different. This was chiefly due to the significantly different biomasses among them. Fertilizer application could reduce the ability of the four species to accumulate heavy metals because concentration of heavy metals in plants decreased as fertilizer was applied. The total amount of metals accumulated by each plant under the condition of fertilization did not increase due to an increase of biomass. In summary, vetiver may be the best species to use for vegetation rehabilitation in oil shale disposal sites.

Yanqun et al. (2004) studied the accumulation of Pb, Cd, Cu and Zn in plants seeking a hyperaccumulator in the Lanping lead-zinc mine area, China. Field surveys of higher plants growing in the area were conducted to identify species accumulating large concentrations of heavy metals. 20 samples of 17 plant species were studied. Concentrations of Pb and Zn in soil and in plant were higher than that of Cu and Cd. For the enrichment coefficient of the four heavy metals in plant, the order of average was Pb<Cu<Cd<Zn. Between four heavy metals, the only significant difference was observed between the enrichment coefficient average of Cd and Cu (p<0.05). The enrichment coefficients were higher than 1 in Llex plyneura and Rhododendron annae in Paomaping for Pb, Salix cathayana, L. plyneura and R. annae in Paomaping for Cd, and R. annae in Paomaping for Zn, respectively. Concentrations and enrichment coefficient of Pb, Cd and Zn of Rhododendron were higher than that of Gramimeae. Enrichment coefficient of Pb, Cd and Zn were bush>tree>herbaceous. Herbaceous grew in soil with the highest concentrations of the four heavy metals. In different areas, the concentrations of Pb, Cd, Cu and Zn in plants and soils and the enrichment coefficient were different. Plants in Paomaping had more accumulating ability to Pb, Cd and Zn, and plants in Jinfeng River had more accumulating ability to Cu. Six plant species, i.e. S. cathayana, Lithocarpus dealbatus, L. plyneura, Fargesia dura, Arundinella yunnanensis and R. annae in Paomaping, had high accumulation capacity. R. annae in Paomaping had hyperaccumulating capacity to Pb, Cd and Zn, L. plyneura to Pb and Cd, and S. cathayana to Cd, respectively.

Sampanpanish (2005) studied the possibility of using phytoremediation and biosorption with weed plant species in Thailand to remove chromium (Cr) from soil and water. Six plant species, *Cynodon dactylon, Pluchea indica, Phyllanthus reticulates, Echinochloa colonum, vetiveria nemoralis* and *Amaranthus viridis*, were chosen for their abilities to accumulate total chromium (TCr). TCr accumulation capacities of these plants were 152.1, 151.8, 101, 77, 69 and 0 mg/kg, respectively, at a hexavalent Cr[Cr(VI)] concentration of 100 mg Cr(VI)/kg soil. Within 30 days of dosing, Cr(VI) accumulation by *Pluchea indica* occurred mainly in roots, stems and leaves at 29, 35, and 73 mg/kg biomass on a dry weight basis, respectively, whereas 38, 18, and 0 mg/kg accumulated in the roots, stems and leaves of *Cynodon dactylon*, respectively. Biosorption experiments were conducted in both batch and column reactors. A synthetic solution containing 50 ppm of Cr(VI) was used to represent Cr(VI) contaminated water. *Phyllanthus reticulates, Pluchea indica* and *Echinochloa* column showed the maximum Cr(VI) adsorption capacities of 53, 45, and 37 mg/g biomass, respectively, at a pH of 2 and an equilibrium time of 24 hours. Leaves were

found to have the maximum adsorption capacity. In the column experiments, leaves of *Pluchea indica* had the maximum Cr(VI) adsorption capacity of 51.3 mg/g biomass at a pH of 2, a breakthrough time of 102 hours, and a flow rate of 1.3 mL/min. The relationship of Cr removal capacities of phytoremediation with living plants and biosorption using non living biomass are discussed. Leaves of *Pluchea indica* had greater Cr(VI) accumulation and adsorption than the other plants and are therefore the most effective for Cr phytoremediation and biosorption.

Yanai et al. (2006) studied the effect of soil characteristics on Cd uptake by the hyperaccumulator *Thlaspi caerulescens*. And found that on soils with total Cd concentration of 0.6-3.7 mg/kg, *T. caerulescens* (the Ganges ecotype) produced greater biomass in the pH range 5.1-7.6 than at pH 4.4. The highest plant Cd concentration (236 mg/kg) and Cd uptake (228  $\mu$ g/pot) were observed at pH 5.1. On soils with total Cd concentrations of 2.6-314.8 mg/kg, shoot Cd concentrations were texture were associated with higher Cd concentration and Cd uptake by *T. caerulescens*.

Tanhan et al. (2007) studied the uptake and accumulation of cadmium, lead, and zinc by Siam weed in the lead contaminated site (Bo Ngam lead mine, Thongphapum district, Kanchanaburi province) and also conducted hydroponic studies. Hydroponic experiments looked for relative growth rate and accumulation of heavy metal by plants from the lead mine area and from the uncontaminated area (Sai Yok district, Kanchanaburi province). Plants were grown in a hydroponic system which contained one of the following heavy metals: Cd (0.25 and 0.5 mg/L), Zn (10 and 20 mg/L), and Pb (5 and 10 mg/L). They found that Siam weed from field collection accumulated 1,377 and 4,236 mg Pb/kg in their stems and roots, respectively, but very low concentrations of Cd and Zn were found in these plants collected from the field. The hydroponic experiments found that the percentage uptake of Pb, Cd, and Zn by Siam weed increased with increasing metal concentrations. Pb concentrations in shoots and roots reached the highest value 1,772.3 and 60,655.7 mg/kg, respectively. These results confirm that Siam weed is a hyperaccumulator which grows rapidly, has substantial biomass, wide distribution and has a potential for the phytoremediation of Pb contaminated soil.