ACCUMULATION CHARACTERISTICS OF *Centella asiatica L.* AS A POTENTIAL Cd-HYPERACCUMULATOR IN SOIL

by

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To my amazing husband,

without whom none of my success would be possible;

thank you for your eternal love, Abang.

Another achievement unlocked!

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In the name of Allah, The Most Gracious, The Most Beneficial.

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LIST OF ABBREVIATIONS AND SYMBOLS

Cd	Cadmium
EDTA	Ethylene diamine triacetic acid
et al.	et alia; and others
AAS	Atomic absorption spectrophotometer
HNO_3	Nitric acid
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
n.a	not available
Fe	iron
Pb	lead
Ni	Nickel
GLU	L-glutamic acid
GSH	Glutathione
CYS	L-cysteine
OM	Organic matter
Ti	Tolerance index
PC	Phytochelatin
OAS	O-acetyl-L-serine

CIRI-CIRI PENGUMPULAN *Centella asiatica L.* SEBAGAI PENGUMPUL BERPOTENSI TINGGI BAGI KADMIUM DALAM TANAH

ABSTRAK

Dewasa ini, para penyelidik berminat dengan penggunaan penyerap hiper bagi proses pembersihan tanah, terutamanya, yang terkesan dengan pencemaran logam berat. Kajian ini memberi maklumat mengenai tindak balas pengaplikasian kadmium serta kebolehserapan *Centella asiatica L.* ke atas kadmium di dalam sebuah ujikaji terkawal. Tiga kumpulan ujikaji telah dijalankan iaitu bekas yang berisi tanah yang telah dicampur dengan kadmium sebagai kadmium klorida hemi pentahydrate $CdCl_2.2.5H_2O$ di dalam 15 bekas (diameter = 20 cm, tinggi = 13 cm, 2 kg tanah kering setiap bekas) yg mana dibahagikan kepada tiga kumpulan dan dicampur 10 mg/kg, 25 mg/kg dan 40 mg/kg kadmium di dalam setiap kumpulan. Agen pemangkin yang merupakan garam asid ethylene diamine triacetic (EDTA) juga ditambah dan telah menunjukkan kesan tumbesaran yang menggalakkan. Kepekatan konsentrasi kadmium telah dianalisa menggunakan atomic absorption spectrophotometer (AAS) pada gelombang 228.8 nm. Kemudian, pertumbuhan relative, produktiviti biomass dan factor biokonsentrasi telah diambilkira. Kepekatan kadmium yang dikesan pada sampel akar *Centella asiatica* dalam kajian ini didapati jauh lebih rendah daripada had yang sepatutnya. Sepanjang tempoh 60 hari, terdapat peningkatan sisa kadmium yang dikesan di dalam tumbuhan berbanding kumpulan kawalan. Pengaruh kadmium adalah tinggi di bahagian akar berbanding pucuk namun faktor pengayaan adalah lebih daripada 1. Kesimpulannya, *Centella asiatica* tidak menepati ciri-ciri tanaman penyerap hiper dan sememangnya bukan tanaman penyerap hiper yang berpotensi.

ACCUMULATION CHARACTERISTICS OF *Centella asiatica L.* AS A POTENTIAL Cd-HYPERACCUMULATOR IN SOIL

ABSTRACT

Researchers are becoming fascinated in using hyperaccumulators for sanitization of heavy metal polluted soils. In this study, *Centella asiatica L.* has been proposed as a potential cadmium (Cd) hyperaccumulator. A series of pot experiments were conducted in this study. Three groups of treatments were emerged which are soil spiked with 10 $mgkg^{-1}$ of cadmium as cadmium chloride hemi pentahydrate ($CdCl_2.2.5H_2O$), 25 $mgkg^{-1}$ Cd and 40 $mgkg^{-1}$ Cd into total 15 plastic pots (diameter = 20 cm, height = 13 cm, 2 kg air-dried soil per pot), added with chelating agent which was ethylene diamine triacetic acid (EDTA) and showed the favorable growth of the plants. The concentrations of heavy metals in digested solutions were determined using an atomic absorption spectrophotometer (AAS) using the wavelength of 228.8 nm. Later, the relative growth, biomass productivity and bioconcentration factor were taken into consideration. However, the Cd concentration obtained from the root of *Centella asiatica* was below detection limit. Addition to that, the accumulation of Cd trace was at large in the root of the plant compared to the stem and leaves. After a period of 60 days accumulation, there were increasing traces of cadmium detected. Compared to the control treatment, there were no values of cadmium detected. However, the enrichment factor (EF) of Cd in *C.asiatica* shoots for each treatment was found to be greater than 1. To conclude,

Centella asiatica did not has the basic characteristics of a Cd-hyperaccumulator and definitely not a potential Cd-hyperaccumulating plant.

CHAPTER 1

INTRODUCTION

1.1 Cadmium and Environmental Issues

On average, 25,000 to 30,000 tons of cadmium is released into the environment each year (ATSDR, 1999). Approximately half of this release is due to weathering of rocks. Human activities release between 4,000 to 13,000 tons cadmium per year with some causes include mining and fossil fuel processing (Kanakaraju *et. al.*, 2007). Cadmium is extremely toxic in water. Even trace levels of cadmium can result in adverse effects in the kidneys (Madrid *et al.*, 2003). The estimated half-life of cadmium in the environment is 18 years and 10 years within the human body (Yang *et al.*, 2005). There is no known function of cadmium for vascular plants (Roosens *et al.*, 2003). Generally, cadmium is very toxic to most plants when present between 3 and 8 mg/kg soil (Murillo *et al.*, 1999).

Since ethylenediamine tetraacetic acid (EDTA) is among the most common chelator used (Nacimiento *et al.*, 2006), it was chosen for use in these experiments. EDTA has been used as an additive for micronutrient fertilizers since the 1950s (Madrid *et al.*, 2003; Meers *et al.*, 2005a) and can also be used as a supplement to soil washing techniques (Lim *et al.*, 2005). EDTA is poorly biodegraded in the soils (Meers *et al.*, 2005a). The increased metal mobility also increases potential leaching of metals furthering contamination into the soil and groundwater (LeDuc *et al.*, 2005; Meers *et al.*, 2005b). There is also a possibility of the toxicity of the EDTA itself decreasing plant biomass enough to minimize its metal mobilizing and translocation benefits (Nacimento *et al.*, 2006).

Heavy metals that persist in the ambient environment are non-biodegradable and have the tendency to accumulate in different organs through food crops consumption. Excessive accumulation of dietary heavy metals such as zinc, cadmium, copper, chromium and lead over time may lead to serious health problems (Kanakaraju *et al.*, 2007). Heavy metals are ubiquitous in environment and exist in various forms. However, only cadmium is stressed in this study. According to (Jiao *et al.*, 2012), long-term use of phosphate fertilizers and micronutrients could cause the arsenic (As), cadmium (Cd) and lead (Pb) content of the cropland soils to rise if the products used contains high level of these elements (for Cd, greater than 10 mg kg^{-1}).

On the other hand, (UNEP, 2006), in its report stated that input of cadmium to farmland by atmospheric deposition and application of phosphate fertilizers and sewage sludge has been an important environmental and health concerns. The significance of cadmium accumulation in agricultural topsoil has been demonstrated in several European countries. According to (ATSDR, 2011), chronic exposure to cadmium may lead into serious health problems such as renal nephropathy, skeletal lesions, *Itai-itai* disease and even cancer. In Malaysia, research on vegetable consumption in Cameron Highlands prove that acute symptoms of cadmium poisoning include gastrointestinal problems, skin problems and mild anemia (Munisamy *et. al.*,2013).

In Malaysia, heavy metal contamination in soil is widespread and contributed by human activities (Najib, *et. al.*, 2012). Some of the most prevalent metals are cadmium, chromium, copper, mercury, lead and zinc (Tangahu, *et. al*., 2011). Chelators are used to mobilize metals in soil for enhanced phytoremediation (Madrid *et al*., 2003). Although the effectiveness of chelators is well documented, the effect of chelators on the plants themselves has not been examined in depth.

1.2 *Centella asiatica L.* towards Phytoremediation

Centella asiatica L is a small herbaceous annual plant and is native to Sri Lanka, northern Australia, Indonesia, Iran and also Malaysia. It is also known as Gotu Kola, Asiatic Pennywort, Takip-kohol, and our very own local name 'pegaga'. The stems are slander, creeping stolons, green to reddish green in colour, interconnecting one plant to another. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pericladial petioles, around 20 cm. The rootstock consists of rhizomes, growing vertically down. They are creamy in colour and covered with root hairs. The crop can matures as fast as 2 weeks to 3 months and usually its study only restrain on its properties as antioxidant, wound healing treatment, oxidative stress and other physiological characteristics of human (Gupta and Flora, 2006).

Centella asiatica L. urban, synonym *Hydrocotyle asiatica*, belongs to the family Apiaceae (Umbelliferae). This herb is found almost all over the world, particularly during rainy season and in damp and marshy areas. It is a popular medicinal plant in several traditional systems of medicine.

Centella asiatica L. is a slender trailing herb, rooting at the nodes. It has long, reddish, prostate stem emerging from the leaf axils of a vertical root stock. Leaves are orbicular, reniform, entire, crenate, glaborous, 1.3-7 cm in diameter. Flowers are sessile, white or reddish, covered by bracts and 3-6 flowers are arranged in an umbel. Fruits are small, compressed, 8 mm long, mericarps are curved, rounded at the top, broad and 7-9 ridged. Seeds are compressed laterally. This has a characteristic odour, greyish green colour and bittersweet taste (Plate 2.1).

Plate 2.1: *Centella asiatica L.*

The plant is indigenous to the warmer regions of both the hemispheres, including Asia, Africa, Australia, southern United States of America, Central America and South America. It is especially profuse in the swampy areas of India, up to an altitude of approximately 700 meters. It is abundantly found during rainy season.

Among the various plant species, aquatic macrophytes including *Centella asiatica* has generated great interest in phytoremediation of heavy metals. These aquatic plants can accumulate heavy metals up to 100,000 times greater than the amount in the associated medium (Irshad *et. al.,* 2016). Therefore, these macrophytes have been reported for heavy metal remediation from a variety of sources (Mokhtar *et al.* 2011). But to the best of our knowledge there is no study pertaining to iron remediation from red soil by using abundantly available *C. asiatica* in the region. Since, Malaysia is a tropical country with high rainfall and high soil water holding capacity, thus, it generates interest to further explore potential of aquatic *C. asiatica* as phytoremediator in the soil medium.

1.3 Phytoremediation as an Environmental Friendly Heavy Metal Cleaning Channel

Phytoremediation is the use of vegetation for *in situ* treatment of contaminated soils, sediments, and water (Lim *et. al.,* 2005). It is best applied at sites with shallow contamination of organic, nutrient, or metal pollutants that are amenable to one of five applications: phytodegredation, phytovolatization, phytostabilization, phytoextraction, or phytostimulation (Lone *et. al.,* 2008). It is an emerging technology that should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long-term applicability. Phytoremediation is well-suited for use at very large field sites where other methods of remediation are not cost-effective or practicable; at sites with low concentrations of contaminants where only "polishing treatment" is required over long periods of time; and in conjunction with other technologies where vegetation is used as a final cap and closure of the site. (Schnoor, 1997). Phytoremediation is a viable, relatively low-cost approach to removing heavy metals from soil and groundwater (Salido *et al.*, 2003).

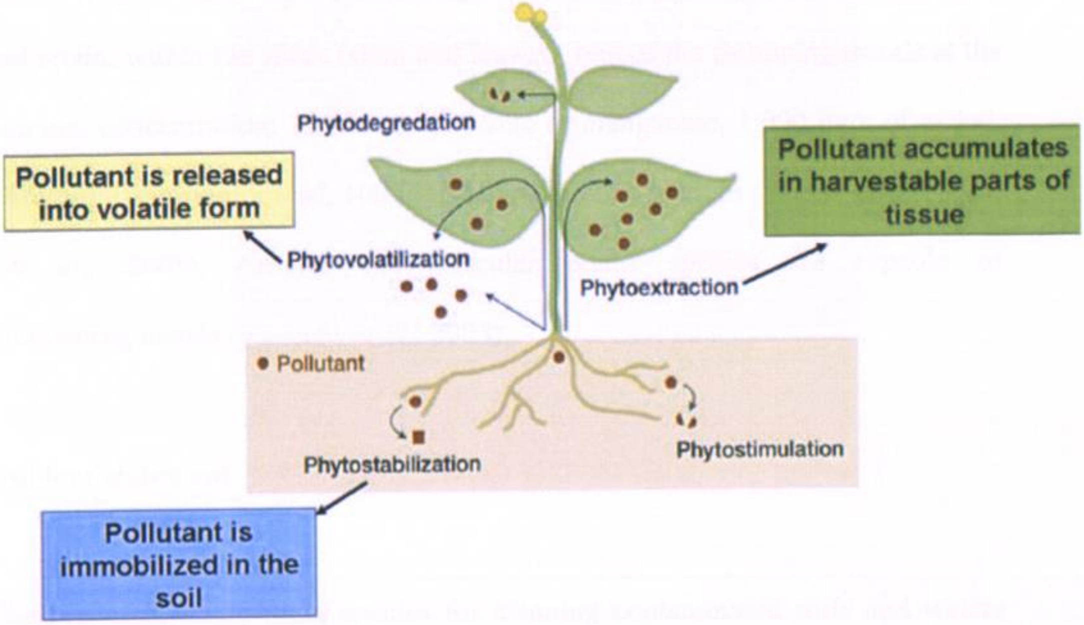

Figure 1.1: Five main applications in phytoremediation (Pilon-Smits, 2005)

Phytoextraction refers that plants absorb metals from soil and translocation them to the harvestable shoots where they accumulate. The roots and shoots are subsequently harvested to remove the contaminants from the soil. It can be applied in mineral industry to commercially produce metals by cropping (Sheoran et al., 2009). Nascimento and Xing (2006) expressed that phytoextraction may be considered as a commercial technology in the future. Jiang *et al.* (2004) determined the growth performance and ability for copper phytoextraction of *Elsholtzia splendens.* According to report, in the presence of vegetation, the exchangeable form of cadmium was partly removed by plant uptake that accompanied with the intake of nutrition (Zhang *et al.*, 2009). Zhang *et al.* (2009) expressed that as cadmium phytoextraction is observed by maize, the percentage of exchangeable form of cadmium decreased in the planted soil. Similar finding of decrease in cadmium level in soil planted with maize have also been reported by Mojiri (2011).

Hyperaccumulators are plants that are able to take up large quantities of metals (Roosens *et al.*, 2003). The classification of a hyperaccumulator is based on the ability to uptake and retain, within the shoot (stem and leaves), one of the following metals at the listed minimum concentration: 10,000 μg/g of zinc or manganese, 1,000 μg/g of nickel, copper, chromium, cuprum or lead, 100 μg/g of arsenic or cadmium (Prasad *et al.*, 2003; Turgut *et al.*, 2005). Around 400 vascular plants species are capable of hyperaccumulating metals (Roosens *et al.,* 2003).

1.4 Problem statement

The use of different plant species for cleaning contaminated soils and waters which namely as phytoremediation has gained increasing awareness since last decade, as an emerging more environmental friendly technology (Chen & Cutright, 2002; Fayiaga *et al.,* 2004). For this study, *Centella asiatica* or local name known as 'pegaga' was selected to evaluate its potential to uptake cadmium from contaminated soil in different concentrations of cadmium assisted with different concentrations of chelating agent that is ethylenediamine tetraacetic acid (EDTA).

Among the various plant species, aquatic macrophytes including *Centella asiatica* has generated great interest in phytoremediation of heavy metals (Irshad *et. al.,* 2016). These aquatic plants can accumulate heavy metals up to 100,000 times greater than the amount in the associated medium (Mokhtar *et al.* 2011). But to the best of our knowledge, this plant has the history of hyperaccumulative study on iron, zinc, lead, copper and nickel (Irshad *et. al.,* 2016; Ong *et. al.,* 2011) but there is no study specifically pertaining to cadmium remediation from sandy clay loam soil by using abundantly available *C. asiatica* in the region. Since Malaysia is a tropical country and high soil water holding capacity, thus, it generated interest to further explore potential of *C. asiatica* as phytoremediator in the soil medium. Thus, the current research deals with the study of phytoextraction capability of *C. asiatica* at various cadmium concentration of soil treatments by analyzing cadmium content in roots, shoots and leaves of the plant with the addition of EDTA usage as the chelating agent.

1.5 Objectives of the study

This study embarks the evaluation of the capability of *Centella asiatica L.* as hyperaccumulator for cadmium in contaminated soil by:

1) Identification of the cadmium uptake capability and accumulation.
2) Determination of the cadmium accumulation in plant parts of *Centella asiatica* at different cadmium and EDTA concentrations at different time exposure.
3) Determination of the bioconcentration translocation factor and biomass productivity of cadmium in *Centella asiatica* at different cadmium concentrations.

1.6 Scope of the study

The herbaceous plant *Centella asiatica L.* had been considered in this study as a potential cadmium hyperaccumulation plant. It is due to this plant vast availability as food source and its presence in large amount at swampy, wet in the industrial area. The study was carried out in the laboratory using the pot experiment. Generic cadmium contaminated soils with varying concentrations was constructed for this purpose. The study was focused on cadmium as the heavy metal but in different concentrations since cadmium is among the most commonly occurring metals at contaminated sites and pose a significant impacts to human health. The collected samples were washed and dried before digested with solvent dosage. The sample solution then will undergo dilution before being analyze using Atomic Absorption Spectroscopy (AAS) to determine the concentrations of heavy metals. By using the data obtained from AAS, their ability to uptake the heavy metals from contaminated soil will be analyzed. This research study also will covers on the effect of chelating agent (EDTA) to uptake heavy metals. Different concentrations of cadmium, EDTA and different time exposure for the plant was taken into consideration in this research.

1.7 Significant Contribution of the Study

The findings in this study will redound to the benefit of the environmental research field about *Centella asiatica* as a cadmium hyperaccumulator. It is proven that this plant is not a great hyperaccumulator for cadmium as the accumulation concentration is not far above 100 mg cadmium per kilo soil. Anyhow, this plant is a great phytoextractor as it can accumulate cadmium from the roots through its stem and leaves. Thus, as *Centalla asiatica* is not great hyperaccumulator for cadmium, consumer can still devour this plant to be used as food source or for cosmetic reasons.

CHAPTER 2

LITERATURE REVIEW

2.1 Metal and Heavy metal

Metal is a material with high reflectivity and conductivity that can usually be deformed plastically (Grey, 2012). A metal reflects light like a mirror unless the surface has been corroded. The high conductivity of metals, which depends on mobile electrons, is a critical property for their use in electrical and electronic devices. The variety of shapes produced by different production technologies, such as extrusion and rolling, attests to their plasticity. Meanwhile, heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water (Tchounwou *et. al.*, 2012). Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals or even plants. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance (Bradl *et. al.*, 2002)

Heavy metals are natural constituents of the earth's crust, but indiscriminate human activities have drastically altered their geochemical cycles and biochemical balance. This results in accumulation of metals in plant parts having secondary metabolites, which is responsible for a particular pharmacological activity. Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel, and zinc can cause deleterious health effects in humans too. Molecular understanding of plant metal accumulation has numerous biotechnological implications and also, the long term effects of which might not be yet known (Singh *et. al.*, 2011).

These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (WHO, 1996). They are also classified as human carcinogens according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer. Heavy metals are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10ppm) in various environmental matrices.

Heavy metals are common inorganic pollutants in the environment and soil. At high concentrations, heavy metals in the soil particles cause serious hazardous effect, restrict microorganism activities, unsuitable for plant growth and destroy biodiversity (Ali *et al.* 2013). Heavy metal contamination of soil can be remediated by various physical, chemical and biological techniques (Yao *et al.,* 2012). Most of the conventional remediation technologies are costly to implement and cause further disturbance to the already damaged environment (Ghosh and Singh 2005). Heavy metals are a group of environmental chemicals that are ubiquitous and non-biodegradable. Though adverse effects emanating from their exposure (like lead, mercury, cadmium, and arsenic) are widely known, their usage and concentrations in the environment is increasing (Alloway, 2013).

2.1.1 Heavy Metal Contamination in Soil

Heavy metal contamination in soil and groundwater is widespread and hazardous to human and animal life (Lombi *et al.*, 2000; Zavoda *et al.*, 2001). For instance, large input of chicken manure, chemical fertilizer and other agro biocides are added to sustain vegetable cultivation as part of intensive cropping cycle. Much of these inputs contain heavy metals and these elements tend to accumulate over time in soil by which the overall process of plant growth depending on the nutrients cycle; absorbing trace elements from soil to plant. Vegetable consumption is one of the pathways by which these said heavy metals gain access into our body and subsequently increase health risks (Roosens *et. al.,* 2003)

Heavy metal contamination occurs from a variety of sources. Mine tailings, industrial practices, pesticides and sewage sludge treatment used as a fertilizer are major contributors to soil contamination (Nacimiento *et al.,* 2006; Jacob and Otte, 2004; Liphadzi *et al.,* 2003; Madrid *et al.,* 2003; Yang *et al.,* 2005). It is also possible for metals to enter the environment from natural processes such as weathering of rocks, volcanic activity and continental dusts (Schützendübel and Poole, 2001). However, the primary source is still from anthropogenic activities. Illegal dumping, accidental spills, poor day to day practices and improper storage of metal based materials can lead to soil and water contamination (Nacimiento *et al.*, 2006).

Once the heavy metals are present in the soil they may leach into groundwater or run off into surface water. From the soil and water, the metals may be taken into plants which may then be consumed by living organisms, including human. The most common route of human exposure to heavy metals is through ingestion from both food and water sources, although inhalation is also possible (Bordajandi *et al.*, 2004).

2.2 Cadmium as Heavy Metal

Cadmium (Cd) is a heavy metal naturally present in soil at concentration of slightly more than 1 mg/kg (Irwin *et al.,* 1991). Atmospheric levels of cadmium range up to 5 nanograms per cubic meter (ng/m3) in rural areas, from 0.005 to 0.015 micrograms per cubic meter (μ/m3) in urban areas, and up to 0.06 μg/m3 in industrial areas (WHO 1992). Concentrations may reach 0.3 μg/m3 weekly mean values near metal smelters (WHO 1987).

Cadmium is a heavy metal of considerable environmental and occupational concern. It is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. The highest level of cadmium compounds in the environment is accumulated in sedimentary rocks, and marine phosphates contain about 15 mg cadmium/kg (Tchounwou *et. al.*, 2012). Cadmium is frequently used in various industrial activities. The major industrial applications of cadmium include the production of alloys, pigments, and batteries (Wilson, 1988). Although the use of cadmium in batteries has shown considerable growth in recent years, its commercial use has declined in developed countries in response to environmental concerns. In the United States for example, the daily cadmium intake is about 0.4μg/kg/day, less than half of the U.S. EPA's oral reference dose (USEPA, 2006). This decline has been linked to the introduction of stringent effluent limits from plating works and, more recently, to the introduction of general restrictions on cadmium consumption in certain countries.

Cadmium also is one of the most hazardous and ubiquitous contaminants in soil and water generated from industrial and agricultural activities such as mining and smelting of metalliferous ores, electroplating, wastewater irrigation, and abuse of chemical fertilizers and pesticides (Zhou & Huang, 2000 and Wu *et al.*, 2006) Therefore, cleanup of Cd-contaminated soils is emergent and imperativebelimov. Over the past five decades, the worldwide release of cadmium has reached 22,000 metric ton (Singh *et al.*, 2003). Cadmium contamination in soils has become a global concern as Cd is not only absorbed by plants or other life forms, but it is easily transferred to human food chain. Therefore, it is important and urgent to develop methods to cleanup Cd-contaminated soils.

Cadmium is toxic at very low exposure levels and has acute and chronic effects on health and environment. Cadmium is not degradable in nature and will thus, once released to the environment, stay in circulation. New releases add to the already existing deposits of cadmium in the environment. Cadmium and cadmium compounds are, compared to other heavy metals, relatively water soluble. They are therefore also more mobile for instance in soil, generally more bioavailable and tend to bioaccumulate.

2.2.1 Physical and Chemical Properties of Cadmium

Cadmium has a relatively high vapor pressure. Its vapor is oxidized rapidly in air to produce cadmium oxide. When reactive gases or vapor, such as carbon dioxide, water vapor, sulfur dioxide, sulfur trioxide or hydrogen chloride are present, cadmium vapor reacts to produce cadmium carbonate, sulfate or chloride, respectively. These compounds maybe formed in stacks and emitted to the environment. Basic information of cadmium is shown in Table 2.1.

Table 2.1: Basic information on cadmium (Yap *et. al.,* 2002)

Name:	Cadmium
Symbol:	Cd
Atomic number:	48
Atomic weight:	112.411 (8) g
Melting point:	320.9 °C
Boiling point:	765.0 °
Number of proton/electrons:	48
Number of neutrons:	64
Classification:	Transition metal
Crystal structure:	Hexagonal
Colour:	Silver grey metallic
Solubility:	Insoluble 5 mg/L in water

Cadmium can form a number of salts those are cadmium chloride, oxide, sulfide, carbonate, selenide and sulphate (Zelicoff and Thomas, 1998). Its mobility in the environment and effects on the ecosystem depend to a great extent on the nature of these salts. Since there is no evidence that organocadmium compounds, where the metal is covalently bound to carbon, occur in nature, only inorganic cadmium salts will be discussed. Cadmium may bound to proteins and other organic molecules and form of salts with organic acids, but in this form, it is regarded as inorganic.

Some of the cadmium salts, such as sulfide, carbonate or oxide, are practically insoluble in water. However, these can be converted to water-soluble salts in nature under the influence of oxygen and acids; the sulfate, nitrate, and halogenates are soluble to water.

2.2.2 Sources and Uses of Cadmium

Unlike mercury and lead, cadmium (Cd) is not an 'ancient' metal, at least in terms of its use by man. It is only in recent years that it has found widespread industrial application, mainly in the metal plating and chemical industries. However, it is quite likely that it was unsuspected used, and that humans experienced its highly toxic effects, for many centuries. Its presence in zinc, for instance, is well known and in many instances in which 'zinc poisoning' was believed to have occurred, it was probably cadmium that was the toxic agent. Even minute amounts of cadmium are sufficient to cause poisoning.

Moreover, since the metal is soluble in organic acids, it easily enters acid foods with which it comes in contact. Cadmium is a highly toxic element. It has been described as 'one of the most dangerous trace elements in the food and environment of man. Cadmium is a divalent metal, homologous with zinc and mercury in the periodic table. Cadmium is generated in waste streams from pigment works, textiles, electroplating and chemical plants. Natural cadmium is a mixture of 8 isotopes with mass numbers between 106 and 116, the most abundant being 112 Cd (24.07 %) and 114 Cd (28.86%).

Cadmium is one of the most hazardous and ubiquitous contaminants in soil and water generated from industrial and agricultural activities such as mining and smelting of metalliferous ores, electroplating, wastewater irrigation, and abuse of chemical fertilizers and pesticides (Zhou & Huang, 2000 and Wu, et al., 2006). It can reduce the yield of crops and may pose a potential hazard to human health by way of food chain, in particular, induce some fatal diseases such as the "*itai-itai* disease". Therefore, cleanup of Cd contaminated soils is emergent and imperative (Zhou & Song, 2004 and Belimov, *et al.*, 2005).

The boiling point of cadmium is 765 °C. Only one, very rare, ore of cadmium is known, namely genocide (sulphide), the metal is normally extracted from zinc ores. Concentration of cadmium in coal is 0.01 to 65 mg kg^{-1} and more than 1 mg kg^{-1} in crude oil. In marine sediments it was around 0.1 to 1 mg/kg. Soil normally contains less than 0.5 mg/kg (Herber, 1994).

2.3 Plant Uptake Mechanisms of Heavy Metal

There are several plant mechanisms employed in reaction to exposure to contaminations. These include phytoextraction, rhizofiltration, phytovolatlization, phytostabilization, phytotransformation, and phytodegredation (Yang *et al.*, 2005). Phytoextraction is the uptake of contaminants into the shoot (aerial portion) of the plant. It is the process used by the plants to accumulate contaminants from soil into root and to above ground shoots and leaves (0.01 to 1% dry weight, depending on the metal). This technique yields a mass of plants and pollutants that must be transported for recycling. Usually, the shoot biomasses are harvested for proper disposal in special site or are burnt to recover the metal. *Elsholtzia splendens, Alyssum bertolonii, Thlaspi caerulescens* and *Pteris vittata* are known examples of hyperaccumulator plants for copper, nickle, zinc, cadmium and arsenic, respectively (Prasad, 2004).

Meanwhile, rhizofiltration (i.e., phytofiltration) is the absorption or adsorption into the roots of the plant (LeDuc *et al.*, 2005; Yang *et al.*, 2005). Rhizofiltration involves the decontamination of polluted waters and sewage by adsorbing or up taking roots of plants. Rhizofiltration in other word, is similar to phytoextraction but the plants are used primarily to address contaminated ground water rather than soil. The plants to be used for cleanup are raised in greenhouses with their roots in water rather than in soil. Many plants such as sunflower, Indian mustard, tobacco, rye, spinach, and corn are able to remove lead from water. Rhizofiltration also serves to precipitate and concentrate metals within the rhizosphere, reducing contaminant migration (Chen and Cutright, 2001).

Apart from that, phytovolatilization entails the evaporation of metal ions or volatile organics. In the process of phytovolatilization, plants are used to absorb the contaminants from the soil and transferred it to volatile forms and finally into the atmosphere through transpiration process. In laboratory experiments, tobacco (*N. tabacum*) and a small model plant (*Arabidopsis thaliana*) that had been genetically modified to include a gene for mercuric reductase converted ionic mercury to the less toxic metallic mercury and volatilized it. This technique can also be used for organic compounds.

In phytostabilization, roots exude or release materials to cause metals to precipitate, reducing metal mobility. It is the technique in which plants reduce the mobility and migration of contaminants and contaminated soil through absorption and precipitation by plants, thus reducing their bio availability. It is very effective when rapid immobilization is needed to preserve ground and surface waters. This process reduces the mobility of the contaminant and prevents migration to the ground water or air, and it reduces bioavailability for entry into the food chain. Species of genera *Haumaniastrum, Eragrostis, Ascolepis, Gladiolus* and *Alyssum* are examples of plants cultivated for this purpose.

Phytotransformation is the uptake of organic contaminants for consumption within the plant. It refers to the uptake of organic contaminants from soil, sediments, or water and, subsequently their transformation to more stable, less toxic or less mobile forms via the action of various enzymes produced by the plant tissues. *Populus* species and *Myriophyllium spicatum* are examples of plants that have these enzymatic systems (Rylott and Bruce, 2008).

Phytodegradation is a microbial assisted process where a consortium of microorganisms degrades contaminants within the enhanced environment provided in the rhizosphere (Itanna and Coulman, 2003). It is the plant assist bioremediation wherein stimulation of microbial degradation takes place. The application of phytostimulation is limited to organic contaminants. The microbial community in the rhizosphere is heterogeneous due to variable spatial distribution of nutrients, however species of the genus *Pseudomonas* are the predominant organisms associated with roots (Ali *et. al.*, 2013).

Plants are also able to uptake aerial contaminants within their leaves (Yang *et al.*, 2005). There are two main functions involved in facilitating the uptake of metals. The first is the production of metal chelating compounds to form complexes that are both more mobile and less toxic to the plants. The second is the solubilization of metals from exudates that acidify the rhizoshpere (Chen and Cutright, 2001). When plants are exposed to heavy metal contamination, they produce phytochelatins which assist in both functions for facilitating metal uptake (Baker, *et. al.*, 1994)

Phytochelatins are thiol-reactive peptides (Li, 2004) composed of glutathione (GLU), cysteine and glycine (amino acids) (Gupta *et al.*, 2004; Yang *et al.*, 2005). Glutathione is a natural antioxidant and is consumed in enzymatic reactions during the formation of phytochelatins (PCs) (Gallego *et al.*, 2002; Gupta *et al.*, 2004; LeDuc *et al.*, 2005). Environmental contaminants, including cadmium and arsenic, have thiol-reactive species (Li *et al.*, 2004). Arsenate (AsO_4 III-) is a phosphate analog and arsenite (AsO_3 III-) is thiolreactive.

Cadmium, in divalent cation form, is highly thiol-reactive. As such, both arsenic and cadmium uptake are directly dependent on the binding to the thiol component of phytochelatins. The PCs then sequester heavy metals within cell vacuoles, storage sites within plant cells (Schützendübel and Polle, 2001; Nouiari *et al.*, 2006). EDTA has been shown to increase or recover glutathione reductase activity (Schützendübel and Polle, 2001). This is important since GLU depletetion may serve as a mechanism for metal tolerance (Alkorta *et al.,* 2004).

For instance, cadmium has no known function within plants but is mobile in soils and therefore easily transported into root cells. The depletion of GLU and glutathione reductase in the presence of Cd limits uptake of the metal into the roots and reduces toxicity reactions within the plant (Alkorta *et al.*, 2004). Since PC is necessary for cadmium uptake and PC is produced from glutothiones, cadmium uptake should be limited by GLU depletion. The addition of EDTA and subsequent increase in GLU should increase the plant's ability to uptake cadmium. However, the GLU depletion serves to limit the cadmium toxicity to the plant, so the EDTA should increase the plant's toxicity reactions to cadmium as well.

2.3.1 Phytoremediation of Heavy Metals

Phytoremediation is the technique of using plants to remove contaminants from soil or water (Rajakaruna, *et. al.*, 2006). It is a relatively low-cost about USD5.00 per/sqft of land (about RM20 per/sqft land) option compared to other remediation techniques such as stabilization, electro-osmosis, and excavation and reburial (Davies, *et. al.*, 2002; Li, *et. al.*, 2004). Phytoremediation is also viewed more favorably by the public due to its low environmental impact and improved the overall contaminated site (Chen *et al.*, 2002; Fayiaga *et al.,* 2004; Lyubun *et al.,* 2002).

The principal application of phytoremediation is for lightly contaminated soils and waters where the material to be treated is at a shallow or medium depth and the area to be treated is large. This will make agronomic techniques economical and applicable for both planting and harvesting. In addition, the site owner must be prepared to accept a longer remediation period. Plants that are able to decontaminate soils does one or more of the following: 1) plant uptake of contaminant from soil particles or soil liquid into their roots; 2) bind the contaminant into their root tissue, physically or chemically; and 3) transport the contaminant from their roots into growing shoots and prevent or inhibit the contaminant from leaching out of the soil (Nouiari *et al.*, 2006)

Moreover, the plants should not only accumulate, degrade or volatilize the contaminants, but should also grow quickly in a range of different conditions and lend themselves to easy harvesting. If the plants are left to die *in situ*, the contaminants will return to the soil. So, for complete removal of contaminants from an area, the plants must be cut and disposed of elsewhere in a nonpolluting way. Some examples of plants used in phyoremediation practices are the following: water hyacinths (*Eichornia crassipes*); poplar trees (*Populus spp*.); forage kochia (*Kochia spp*); alfalfa (*Medicago sativa*); Kentucky bluegrass (*Poa pratensis*); Scirpus spp, coontail (*Ceratophyllum demersum L.*); American pondweed (*Potamogeton nodosus*); and the emergent common arrowhead (*Sagittaria latifolia*) amongst others (Lasat, 2000).

Heavy metals are easily taken up by the plants through their roots and transported to other aerial parts. Uptake of these metals depends on several factors such as soil pH, temperature, organic contents and the presence of chelating agents (Singh and Singh, 2016). Among various soil factors, soil pH being the most important factor to affect the availability of heavy metals. The success of metal remediation process depends on those plants which can accumulates desired levels of metal concentration in their aerial parts (100-1000 folds) without any visible symptoms and these plants are termed as hyperaccumulators and the phenomenon is termed as hyperaccumulation. About 500 plant species have metal hyperaccumulation characteristic, among these approximately 0.2 % belong to angiosperm (Sarma, 2011).

The ideal plants for phytoremediation should have the ability to accumulate high metal content, tolerate high salt concentration, having fast growth rate, higher biomass production, easily harvestable and must translocate metals to their above ground parts efficiently (Chandra, *et. al.*, 2015). For selecting model plant species for phytoremediation, the ratio of metals between soil and plant parts (metal transfer factor) is measured and this ratio should be more than one. It means higher accumulation of metals in plant parts than soil (Barman, *et. al.,* 2000). Gupta *et al.* (2008) have been studied the multiple metal accumulation characteristics of three wild macrophyte species for instance *Ipomea* sp., *Eclipta* sp. and *Marsilea* sp. It was recorded that *Ipomea* sp. Suitable for cadmium (Cd), copper (Cu), manganese (Mn) and zinc (Zn), while *Eclipta* sp. and *Marsilea* sp. shows transfer factor more than one for iron (Fe), copper (Cu) and cadmium (Cd) (Singh and Singh, 2016).