

Review Article

# Cadmium toxicity symptoms and uptake mechanism in plants: a review

## Sintomas de toxicidade de cádmio e mecanismo de absorção em plantas: uma revisão

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### Abstract

Cadmium (Cd) is one of non-essential heavy metals which is released into environment naturally or anthropogenically. It is highly persistent toxic metals that are exceptionally distressing industrial and agriculture activities by contaminating soil, water and food. Its long-duration endurance in soil and water results in accumulation and uptake into plants, leading to the food chain. This becomes a serious global problem threatening humans and animals as food chain components. Living organisms, especially humans, are exposed to Cd through plants as one of the main vegetative food sources. This review paper is concentrated on the symptoms of the plants affected by Cd toxicity. The absorption of Cd triggers several seen and unseen symptoms by polluted plants such as stunted growth, chlorosis, necrosis and wilting. Apart from that, factors that affect the uptake and translocation of Cd in plants are elaborated to understand the mechanism that contributes to its accumulation. By insight of Cd accumulation, this review also discussed the phytoremediation techniques-phytoextraction, phytostimulation, phytostabilization, phytovolatilization and rhizofiltration in bioremediating the Cd.

**Keywords:** cadmium, toxicity, symptoms, translocation, bioavailability, phytoremediation.

### Resumo

O cádmio (Cd) é um dos metais pesados não essenciais que é liberado no meio ambiente de forma natural ou antropogênica. São metais tóxicos altamente persistentes que prejudicam excepcionalmente as atividades industriais e agrícolas, contaminando o solo, a água e os alimentos. Sua resistência de longa duração no solo e na água resulta em acúmulo e absorção pelas plantas, levando à cadeia alimentar. Isso se torna um sério problema global que ameaça humanos e animais como componentes da cadeia alimentar. Os organismos vivos, principalmente os humanos, são expostos ao Cd através das plantas como uma das principais fontes de alimento vegetativo. Este artigo de revisão concentra-se nos sintomas das plantas afetadas pela toxicidade do Cd. A absorção de Cd desencadeia vários sintomas visíveis e invisíveis por plantas poluídas, como crescimento atrofiado, clorose, necrose e murcha. Além disso, são elaborados fatores que afetam a absorção e translocação de Cd nas plantas para entender o mecanismo que contribui para o seu acúmulo. A partir do conhecimento do acúmulo de Cd, esta revisão também discutiu as técnicas de fitorremediação - fitoextração, fitoestimulação, fitoestabilização, fitovolatilização e rizofiltração na biorremediação do Cd.

**Palavras-chave:** cádmio, toxicidade, sintomas, translocação, biodisponibilidade, fitorremediação.

## 1. Introduction

Heavy metals are inevitably drawing global attention due to their well-known toxicity effects on human health and the environment. They are very persistent in our environment, including water, air and soil, instigating their bioaccumulation in the living organism. The non-essential and unknown roles of heavy metals, specifically mercury, cadmium, lead, and uranium, are harmful to plants with promising risks for children and women, notably in

public (Chen et al., 2015; Cárdenas-González et al., 2016). Cadmium (Cd) has, however, been described as the most troublesome heavy metal due to its more significant discharge from industries (Chien et al., 2003). It has been articulated as unnecessary elements by living organisms for any physiological functions. Moreover, it has been identified as one of the most dangerous heavy metals besides lead (Pb) (Ghosh, 2010). Cd enormous presence

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harms the environment by building up a serious threat to human health throughout the food chain (Rafiq et al., 2014). This problem cannot be disregarded, as it inevitably could lead to chronic health problems (Ali et al., 2015a). It is worth known as precarious metals due to the diversity of health effects even being exposed at low concentrations.

Cadmium is manufactured and released into the environment as non-essential elements. It is released into the air by mines, metal smelters, and alloys, batteries, pigments, and plastics industries (Harrison, 2001). Notwithstanding this, the advanced agriculture industry has contributed to an anthropogenic means of aggravating Cd levels in agricultural soil (Leduc and Terry, 2005). Cadmium also has been detected in sewage sludge which is generally employed as plant fertilizer. Thus, Cd could be discovered in different vegetables and animals tissues (Kumar et al., 2007). Zhong et al. (2018) emphasized that poor farming practices and untreated industrial utilization and municipal waste for irrigation have additionally contributed to agricultural soil contamination. Export of phosphate fertilizers, wastewater, Cd contaminated sewage sludge, and manure, metal processing, industrial traffic, and cement factories have also become the primary anthropogenic sources (Yang et al., 2004). Only inorganic salts of cadmium are found in foods. Eventually, the intake of Cd occurs through the consumption of food sources from agricultural products that have been contaminated (UNEP, 2010). High levels of Cd are found in nuts and oilseeds. To minimize the uptake of Cd, most developed countries have set the maximum acceptable limit ( $5 \mu\text{g dm}^3$ ) in drinking water (UNEP, 2010). Apart from that, the breeding techniques have been applied to develop fewer Cd-uptake crops (Smolders and Mertens, 2013).

The plants readily absorb cadmium ions as opposed to lead and mercury ions. They are distributed equally over the plant. Cd is taken up to edible leaves, fruits, and seeds through the plant roots. Physiological mechanism was highlighted to be immensely helpful in carrying out tolerance response to stresses faced by most plant species (Siddiqui et al., 2012). The abiotic stress initiated by Cd at phytotoxicity levels may introduce biochemical and molecular disturbances, subsequently causes oxidative stress. Oxidative stress unnecessarily affects the plant cells result in DNA, cell membrane and protein damage, genetic mutation, lipid peroxidation, growth and development reduction (Hossain et al., 2012). Instead of that, phytotoxicity of Cd triggers the alteration of mineral nutrients uptake and inhibition of stomatal opening by disrupting the water content of the plants (Hossain et al., 2010), photosynthesis, carbohydrate metabolism (Shi et al., 2009) and antioxidants metabolism (Khan et al., 2009).

The most recent remediation strategies including physicochemical alteration and bioremediation are widely applied to alleviate Cd in soil. Application of physical methods and chemical approaches may be successful and efficient in their respective situations. However, they are universally perceived as being prohibitively expensive and failing to completely remove the element from the soil. The drawbacks of physicochemical strategies are restricting the amount of work that can be put into lowering Cd in the environment. Thus, bioremediation of Cd, particularly

phytoremediation, has been extensively practised in which plants can absorb the metal and thereby lower its bioavailability (DalCorso et al., 2019). This review seeks to provide an understanding of cadmium remediation alternatives in the context of phytoremediation approaches as well as factors of plant uptake, mode of absorption and bioaccumulation, and toxic effects of cadmium in plants.

## 2. Materials and Methods

This review study makes use of a collection of journals obtained from a journal database as well as a literature review of worldwide scientific journals written in English to conduct its research. This review examines studies and essential discoveries from years spanning from 2000 to 2021, and it offers a comparison of the research and noteworthy findings. A complete description and summary of prior investigations were provided in the form of tables and figures, which highlighted the findings.

## 3. Soil Properties and Cd Bioavailability

The environment is unearned affected by cadmium, and its toxicity possessions are ongoing distressing humans, plants, and animals. Cd shows high mobility of all other trace elements effortlessly entering the ecological food chain through soil. This exposure happens due to the contribution of intensive modern agricultural activities, the rapid rate of industrialization, and an increase in urbanization (Zhao et al., 2015). The availability of Cd in soil influences the absorption of the element, however not all of the Cd presents in soil is available for plant uptake. Bioavailability and high mobility of Cd in soil increase the capability of the plant to accumulate the elements to other plants parts. However, other factors must be considered like soil pH (Yang et al., 2016), temperature (Silber et al., 2012), reduction potential (Meng et al., 2019), organic matter content (Mohamed et al., 2010), capacity of cation exchange (Jiang et al. 2012), and availability of other elements are noticeable substantial in the process (Helios-Rybicka and Wójcik, 2012).

### 3.1 The role of soil pH

The bioavailability of Cd in soil is apparently influenced by pH level of the soil. Acidic pH is frequently related to increase Cd bioavailability. Lower pH values resulted in a substantial increase of Cd bioavailability, therefore permitting the absorption activity of this element to other plant tissue (Yang et al., 2016). As reported by Rafiq et al. (2014), Cd concentrations in the grains of rice were found to be negatively and significantly correlated with soil pH. The acidic environment might offer higher chances of Cd transformation from immobile form into freely bioavailable form ( $\text{Cd}^{2+}$ ) (Li et al., 2014). In particular, acidic solution affords  $\text{Cd}^{2+}$  that will compete with  $\text{H}^+$  for binding sites of soil particles. Though, less competition from  $\text{Cd}^{2+}$  ions causing the  $\text{H}^+$  ions are more readily adsorbed on soil and organic matter. As a result, Cd desorption from soil particles

into soil solution is enhanced, making  $\text{Cd}^{2+}$  ions more available for absorption by plant root cells (Zhai et al., 2018).

Contrary to the basic environment, Cd is hydrolysed into hydroxy species like  $\text{Cd}(\text{OH})^+$ , enhancing the adsorption affinity to solid-phase exchange sites of soil. Thus, the mobilization and accumulation of Cd in plant will be substantially condensed (Shahid et al., 2017). Furthermore, an increasing soil pH generates superfluous negatively charged adsorption sites on the soil colloid, resulting in increasing  $\text{Cd}^{2+}$  adsorption (Chen et al., 2019). According to Rao et al. (2013), the adsorption of Cd was enhanced by a factor of 0.35-0.37 with each pH unit decline in acidic paddy soils. Moreover, higher pH might also trigger the precipitation of  $\text{Cd}^{2+}$  into immobilized  $\text{Cd}(\text{OH})_2$  form (Bolan et al., 2014).

### 3.2. The role of organic matter

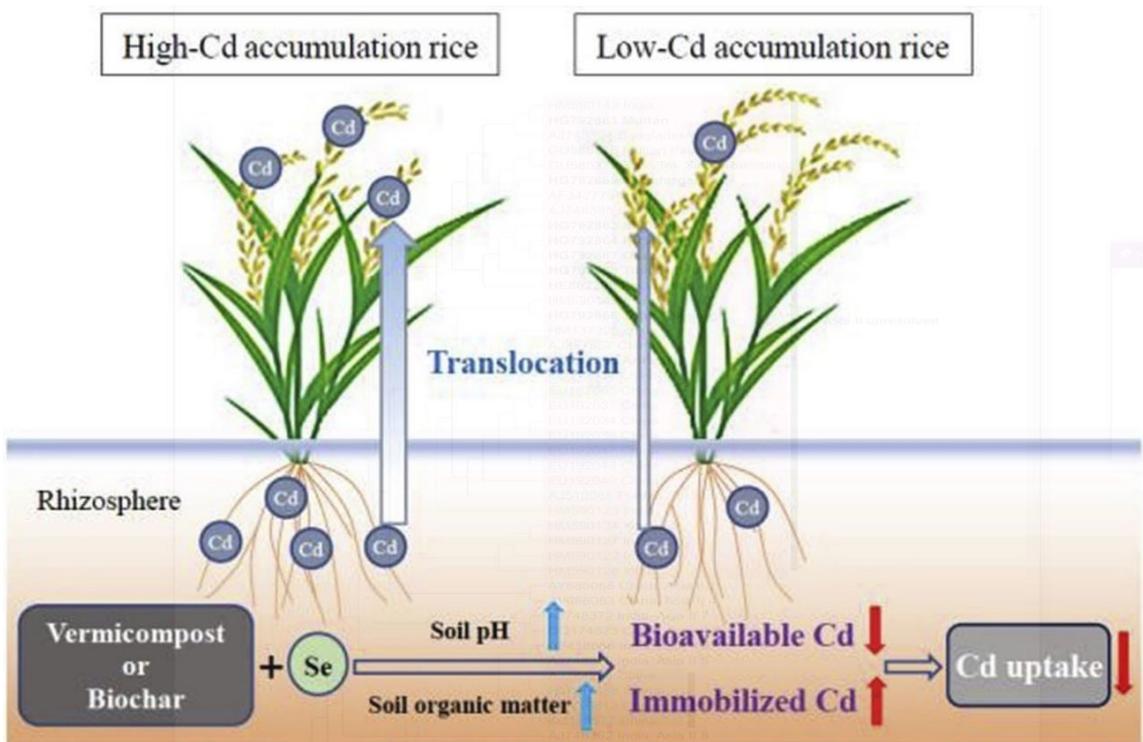
Soil organic matter is the organic component of soil that is composed of three essential components: small plant remnants and small live soil organisms, decomposing organic matter, and stable organic matter or often known as humus. The breakdown of plants and animals as a part of organic matter components contributes significantly to Cd bioavailability through the formation of Cd and soil medium complex. The presence of organic matters in soil promotes the formation of stable organometallic complexes, which can limit the solubility of Cd in soil environment (Mohamed et al., 2010). One of the recent findings reported that the addition of biochar enhanced the organic content of the soil, which improved its potential to stabilize Cd

by transforming the element from labile fraction into less available form. Subsequently, the uptake of Cd by maize plants has been dramatically reduced (Alaboudi et al., 2019). Besides, the collaboration of organic matter and selenium has also proven to attenuate the Cd bioaccumulation in rice plantation system (Figure 1). According to the study, a range of 5.8% to 20.8% of Cd reduction could be attained efficaciously (Liu et al., 2020).

In another research, the introduction of biochar into Cd-contaminated soil also has reduced Cd bioavailability and increased the growth of oak forest seedlings. The amendment of biochar at rates of 1%, 3%, and 5% in  $50 \text{ mg kg}^{-1}$  of Cd-contaminated soil significantly enhanced the plant tolerance index by 40.9%, 56%, and 60.6%, respectively (Amirahmadi et al., 2020). A reduction of total Cd can also be observed in switchgrass (*Panicum virgatum*) with incorporation of compost blend containing either beef cattle manure biochar or poultry litter biochar. Approximately 5% of both compost and beef cattle manure biochar lead to greater reduction in total Cd extracted from shoots and roots (Novak et al., 2019). The combined application of peat and  $\text{Fe}(\text{NO}_3)_3$  showed considerably increased effects on As and Cd immobility, hence restricting Cd bioavailability throughout the rice growth stages (Wang et al., 2019).

### 3.3. The role of reduction potential

The “redox potential” or “reduction potential” is used to describe the combination of reduction and oxidation reactions in the simplest terms. In other words, redox



**Figure 1.** The effects of soil pH and organic matter in soil towards Cd bioavailability. The diagram shows the reduction of Cd uptake and Cd bioavailability by manipulating soil pH and soil organic matter. Adopted from Liu et al. (2020).

potential or reduction potential is the tendency of chemical substances to accept or donate electrons (DeLaune and Reddy, 2005). In fact, redox potential is one of the critical factors manipulating Cd bioavailability by altering the solubility of Cd in soil, organic matter, minerals and rhizosphere microbes regulation (Meng et al., 2019). Under oxidizing conditions (aerobic), Cd is frequently found as soluble salts and cationic ions ( $Cd^{2+}$ ). In contrast, it does exist as precipitates like CdS and  $CdCO_3$  under reducing conditions (anaerobic) (Sebastian and Prasad, 2013). Typically, the change in redox potential condition would change the electron acceptors and the chelating capacity with  $Cd^{2+}$  (Nazar et al., 2012).

A study has been conducted using azolla or known as mosquito fern to reduce the redox potential in soil. The incorporation of azolla resulted in an 80.3% reduction in Cd uptake and a 13.4% increase in plant yield, respectively (Liu et al., 2021). The addition of periphyton has reduced potential reduction of rice cultivation soil by 40-120 mv led to higher Cd concentrations in soils (Lu et al., 2020). In fact, the mobility of Cd in paddy soils was much affected by this reduction that leads to metals reductive dissolution including iron (Fe) and manganese (Mn) oxides (Wang et al., 2019).

#### 4. Cadmium Toxicity Symptoms in Plant

The excess amount of Cd causes the toxicity of this chemical element and usually being measured by monitoring any abnormalities tackling by the plants. Apparent symptoms of Cd stress enable them to diagnose the severity of toxicity which is therefore useful in the sighting of stress effects. This will make it easier to coordinate successful approaches to strengthen the plant endurance to stress (Ahmad et al., 2003). The visual and non-visual symptoms of Cd toxicity have been observed by many researchers. The noticeable identified Cd toxicity symptoms entail growth stunting, chlorosis (leaf discolouration), necrosis, wilting, photosynthesis rate reduction and respiration inhibition (Navarro-León et al., 2019). At the same time, non-visual symptoms include biomass reduction and changes in mineral composition and symptoms at the sub-cellular level (Sanita di Toppi and Gabrielli, 1999). Cd severely reduces biomass production and even leads to integral plant death (Dias et al., 2012). It is believed that Cd might cause growth retardation, photosynthesis inhibition, enzyme induction and inhibition, stomatal action alteration and free radicals generation in plants. However, the symptoms are emerged depending on the level of concentration of absorbed Cd.

##### 4.1. Stunted growth

Stunting of plant can be described as one of the plant diseases because of dwarfing and loss of vigour. This symptom is mainly caused by infectious or non-infectious means. Bacteria, fungi, viruses, and nematodes may infect the plant and cause incurable stunting symptom. In contrast, the non-infectious means are caused by the physical environment, nutrition imbalance, and physical or chemicals injuries. These means can typically readily

be cured. Cd is one of the non-infectious means capable of distressing the process of macro-elements absorption and transport, causing growth inhibition of root and aerial parts of the plant (Souza et al., 2008).

The increasing concentration of Cd caused severely stunted growth of *Helianthus annuus* (Turgut et al., 2004). *Hydrocotyle umbellata* has been discovered to be affected by Cd. Its growth is stunted, and there is no production of new plantlets (Panyakhan et al., 2006). Treatment of Cd onto three *Pisum sativum* L. genotypes has also shown plant growth inhibition symptom (Rahman et al., 2017). Wunder et al. (2009) pointed out that the similar effect can be observed in *Regnellidium diphyllum*, which demonstrated a significant reduction in root and leaf growth with no formation of secondary leaf. Plant growth is inhibited by storing the excess cadmium ion in place of other essential nutrients to the plant growth like Zn and Fe as well as other micro and macronutrients. Furthermore, the Cd stress normally has a negative effect on plant growth because it causes a decrease in chlorophyll concentration, which leads to an obstruction of photosynthesis. This inhibition is believed referring to the Photosystem II (PSII) damage (Chu et al., 2018) which is one of the photosynthetic components. Lipid peroxidation is also one of the plant responses that caused plant growth inhibition. In general, lipid peroxidation will cause cell damage or membrane damage that blocks natural antioxidants in cells and cause water imbalance (Hossain et al., 2012).

##### 4.2. Chlorosis

It is possible to describe chlorosis as the loss of natural green pigmentation of plant leaves. This is a state of plants become impotent to produce enough chlorophyll pigments. Chlorophyll is a pigment accountable for the green colour in leaves. Hence, insufficient chlorophyll in plant results in light greenish, yellowish, or white-yellowish leaves. Since the green colour of the leaves is responsible for chlorophyll, the chlorotic leaves are pale, yellow, or white yellow. Chlorosis may be caused by several factors like insufficient light exposure and deficiency of iron, phosphorus and manganese (Sivasankar et al., 2012) in soil. As a result of chlorosis, sugars or carbohydrates cannot be produced by plant through photosynthesis process. It became worse as the plant may die if being untreated. However, the plant will suffer from other plant disease known as rust though being treated. It was clearly shown by *Arabidopsis thaliana* mutant *ppi2* after being treated with exogenic sucrose to top up the sugars content in the plant (Kubis et al., 2004).

Bioavailability of cadmium in soil contributes to its hyperaccumulation movement in plants, distressing the uptake of important nutrients like calcium, phosphorus, potassium, and water. These agonies readily onset several symptoms, particularly leaf chlorosis and photosynthetic rate reduction. Moreover, the previous study had identified a comparable symptom of chlorosis caused by Cd was determined with a decrease in these essential nutrients (Epstein and Bloom, 2005). This symptom has also been identified in some plant species such as pea (Rahman et al., 2017), oilseed rape (Ali et al., 2015a) and rice grown in soil

contaminated with Cd (Rafiq et al., 2014) and other plants species as shown in Table 1.

#### 4.3. Necrosis

Necrosis is a condition where the tissues and plant cells deplete or generally deteriorate in response to abiotic stress. It is a symptom that appeared due to plant disease or distress that the plant is experiencing. Even though the plant does not require a large intake of nutrients, the absence of nutrients like potassium, nitrogen, iron, and nickel will lead to necrosis. Nutrient deficiency might happen due to soil depletion, imbalance of soil pH or unsuitable fertilizer application. Other factors that lead to necrosis include pathogen invasion, nematode, and fungal problems (Khan, 2008; van Doorn et al., 2011). In the case of Cd, the metal competes with plant nutrients, especially in the root region. Cd ions compete with several mineral nutrients that usually have the same chemical properties to secure a spot in plant absorption. Consequently, the competition between cations causes nutrient depletion of the plant (Barcelo and Poschenrieder, 1990). Necrosis symptoms can be identified with dark watery and dry papery spots on plant parts with possibly dark colour. However, not all spots will appear dark as some may be yellow or wilted, which is one of sign of plant cell death activation. Commonly, necrosis might be caused by a particular disease, but somehow might be due to weather conditions and the quality of water source.

#### 4.4. Biomass reduction

The unintentional absorption of Cd by plant contributes to a necessary unfavourable impact on plant health, including biomass reduction and photosynthetic efficiency (Drava et al., 2012). Reduction of biomass as a result of the Cd toxicity effect can be regarded as phytotoxicity symptoms. The biomass of rice root was drastically reduced when grown in Cd contaminated soil (Yixia et al., 2020).

The biomass of canola and Indian mustard were also identified to be affected with more than 70% of reduction (Turan and Esringu, 2007). The same symptom was observed in the hydroponic system plantation of *Pistia stratiotes*. The plant has tolerated up to 20 mg/L of Cd but still resulted in plant biomass reduction with high Cd treatment (Das et al., 2014). Inhibition of plant growth by Cd is the vital reason for plant biomass lessening.

#### 4.5. Photosynthesis inhibition

Photosynthesis is the most vital activity carried out by plants in order to maintain the existence of humans, animals, and the plant itself. The conversion of water and carbon dioxide into energy-rich compound (which serves as food supply for living organisms) should not be interfered. Nevertheless, the release of Cd into environment has stressed the plants photosynthetic system by destructing the chloroplast structure, at the same time impairing chlorophyll production (Lysenko et al., 2015). Cd also reduced the total chlorophyll and carotenoid content of *Brassica napus* (Ali et al., 2015b). The lower amount of chlorophyll pigments may cause the discoloration of leaves.

In wheat, Cd caused a substantial reduction in net photosynthetic rate, fluorescence efficiency and stomatal conductance but also led an increase of intercellular carbon dioxide (Shafi et al., 2011). The failure of plant to undergo photosynthesis activities will totally inhibit the plant growth. The photosynthesis process of *Schima superba* has likewise been hindered by Cd, which has interfered with electron transport between PSII and PSI, energy distribution of PSI and CO<sub>2</sub> assimilation (Chu et al., 2018). Since carbon fixation or CO<sub>2</sub> assimilation is most probably inhibited by Cd, a build-up of high excitation has been accumulated which has the potential to harm the photosystem (Chu et al., 2018). However, it can be reduced if the excess energy is disbursed through heat dissipation pathway.

**Table 1.** Enlisted plant showing visual symptoms of Cd toxicity.

Plant	Symptoms	References
<i>Lactuca sativa</i>	Necrosis	Azzi et al. (2015)
<i>Helianthus annuus</i>	Stunted growth	Turgut et al. (2004)
<i>Hydrocotyle umbellata</i>	Stunted growth, chlorosis	Panyakhan et al. (2006)
<i>Pisum sativum</i>	Chlorosis, stunted growth, necrosis, root length inhibition	Rahman et al. (2017)
<i>Arabidopsis thaliana</i>	Chlorosis, rust	Kubis et al. (2004)
<i>Brassica napus</i>	Chlorosis, photosynthesis inhibition	Ali et al. (2015b)
<i>Oryza sativa</i>	Chlorosis, necrosis	Rafiq et al. (2014)
<i>Glycine max</i>	Chlorosis, stunted growth	
<i>Nicotiana benthamiana</i>	Chlorosis	Cheng et al. (2018)
<i>Cicer arietinum</i>	Chlorosis, necrosis	Faizan et al. (2011)
<i>Allium schoenoprasum</i>	Wilting, chlorosis, growth inhibition	Golan-Goldhirsh (2006)
<i>Medicago sativa</i>	Chlorosis, necrosis	Chnaya et al. (2015)
<i>Arabidopsis halleri</i>	Chlorosis, photosynthesis inhibition	Verbruggen et al. (2009)

## 5. Cd Translocation and Accumulation in Plants

Cd uptake occurs through transporters of calcium, ferric, magnesium, copper and zinc (Clemens, 2006). Calcium (Ca) and Cd competes for the same Ca channels to transport. Suzuzki (2005) suggested that the low concentration of exogenous Ca in Hoagland media had improved the uptake of Cd, consequently resulting in Cd toxicity. Cd is highly mobile and water-soluble, make it merely reaches the xylem by entering the root's cortical tissues through apoplastic and/or a symplastic pathway, as shown in Figure 2. An event of formation of complexes comprising organic acids and phytochelatin may occur afterwards (Lux et al., 2011). Usually, Cd ions are higher in the roots since the restriction of this metal, thus limiting its translocation to shoots, attributed to a complex system involving adsorption, chelation and compartmentalization (Niaz et al., 2015). The apoplastic region of roots is negatively charged because of carboxylic groups; they act as the first line of defence which is critical for retaining cations such as Cd in roots (Gajdos et al., 2012).

### 5.1. Cell wall binding

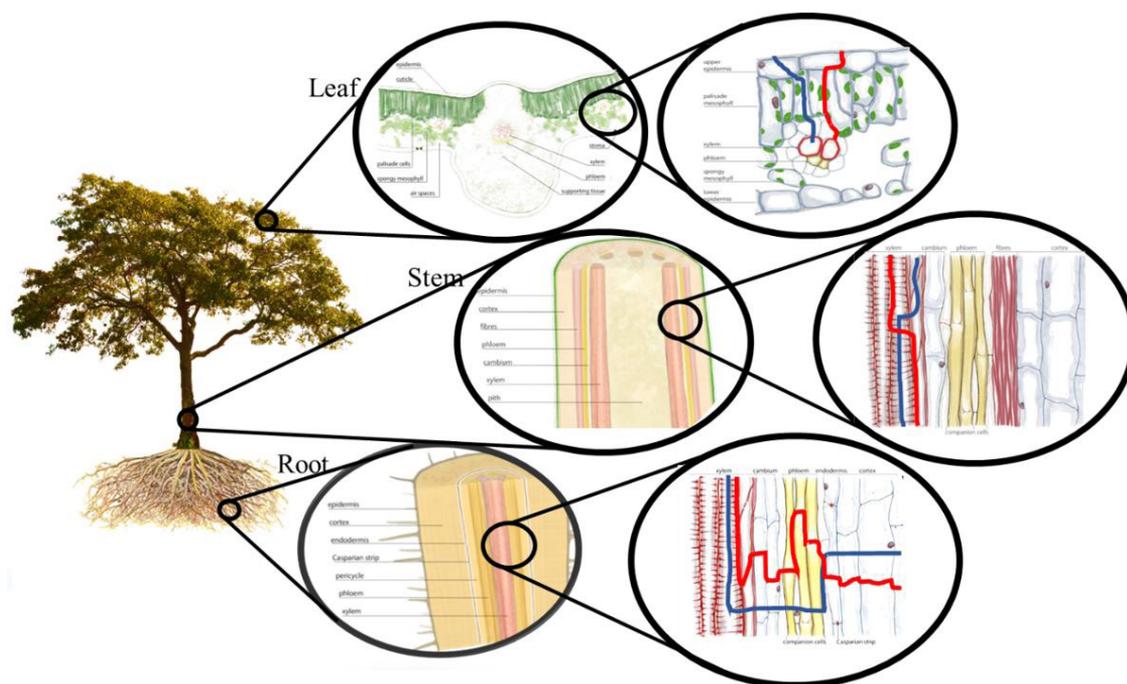
The immobilization of Cd can be prominent protection against Cd translocation from root to upper part of plant that happens mainly at the root stage. The roots cell wall is the first barrier structure that can be successfully infiltrated by metals ion. Being the first living structure as a target of heavy metals toxicity, cell walls may involve in tolerance. An earlier study demonstrated that the Cd tolerance of plants was raised as a result of the cell wall's ability to inhibit Cd absorption (Gutsch et al., 2018).

A remarkably lower metals adsorption onto cell wall is expected to induce tolerance. Nonetheless, a considerable accumulation of metal has occurred between cell wall and cell membrane, as described in previous research (Mehes-Smith et al., 2013). Heavy metals portray various degrees of affinity towards cell wall, which will primarily bind to polygalacturonic acids. Usually, metals cation naturally binds to plant with the presence of functional groups in cell wall. The carboxyls group is a main functional group of pectin components of cell wall that bind to divalent and trivalent metal ions under metal stress (Mehes-Smith et al., 2013; Gutsch et al., 2018).

Boominathan and Doran (2003) identified the hairy roots of *Thlaspi caerulescens* were able to hold most of the Cd in the cell wall. According to a recent study, boron can reduce Cd toxicity in *Brassica napus* by enhancing Cd chelation onto cell walls in the shoots and roots of the plant (Wu et al., 2020). The study found that boron also increased the pectin content of root by decreasing the pectinase activity, hence increased the chelation capacity. Likewise, the amount of cellulose and hemicellulose were significantly reduced resulting in expression of particular genes (expansin, xyloglucan endotransglucosylase, and  $\alpha$ -xylosidase) that improved the cell wall integrity. A study on *Sedum plumbizincicola* also highlighted the importance role of cell walls in Cd hyperaccumulation and detoxification (Peng et al., 2017).

### 5.2. Roots exudates

Almost half of the photosynthesis products are transported to roots and about 12%–40% are released in



**Figure 2.** The diagram is showing the uptake and translocation of cadmium from root-to-shoot through apoplastic (red line) and symplastic (blue line) pathway. Adopted from Science and Plants for Schools (SAPS, 2014) of the University of Cambridge (2014).

rhizosphere as exudates such as polysaccharides, amino acids and proteins (Hinsinger et al., 2006). Additionally, exudates like inorganic ligands are also released into rhizosphere (Dong et al., 2007). These exudates are being secreted as a function of energy sources for microorganisms and may also play a role as ligands responsible for chelating heavy metal in rhizosphere.

Chelation of heavy metals activity influences the changes of pH and Eh (redox measurement) condition in rhizosphere resulting in metals mobilization in soil and metals accumulation in plants. The cadmium uptake is expected to be influenced by the changes in soluble root exudates in rhizosphere activities. Root exudates counter with metal ions causing the changes of metal solubility, mobility and phytoavailability. *Echinochloa crusgalli* secretes root exudates containing oxalic acid and citric acid, enhanced the heavy metals translocation from root to shoots (Kim et al., 2010). Dicarboxylic acids, exudates have been discovered in durum wheat rhizosphere mobilized cadmium in various type of soil (Krishnamurti et al., 1997). Unidentified exudates of *Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L. showed the capability to isolate cadmium from soil, depending on its bioavailability (Mench and Martin, 1991). Römheld (1987) reported the production of phytosiderophores compound by the root's exudates under iron stress. Phytosiderophore from barley roots was observed to mobilize heavy metals from soil and believed might involve cadmium as well (Treeby et al., 1989). The compound might influence the accumulation of cadmium due to Zn deficiency. The presence of cadmium in soil has reduced phytosiderophore production, instigating transition metal enhancement (Fan et al., 2001).

### 5.3. Mycorrhiza

Mycorrhizas are sometimes left out to be considered as extracellular strategy (Jentschke and Godbold, 2000) in coping heavy metals contamination. However, it is qualified as an effective method in ameliorating the phytotoxicity effects of metals on host plant (Hall, 2002). It is believed to tolerate metals at the cellular level with strategies of metals binding to extracellular materials or sequestration in the vacuolar compartment. Mycorrhizae adopt few mechanisms like absorption, adsorption, or chelation, which offer an effective exclusion barrier that limits the entrance of metals into the plant (Hall, 2002). Galli et al. (1994) reported that mycorrhizal fungi infection positively increases the P and N availability. In addition, the toxic level of metals might be reduced or enhanced by the fungus infection depending on fungal isolates, metal elements and condition of experiments. The mycorrhizal association of plant fungi and plant root is the primary factor in the tolerance and accumulation of metal from contaminated soil. It has been acknowledged that ectomycorrhizas and arbuscular mycorrhizae are the most reliable fungal association effectively reduce the harmful effects of heavy metals, thus enhancing plant tolerance in metal-contaminated soil (Jentschke and Godbold, 2000). Arbuscular mycorrhizal fungal application in Cd-contaminated soil has proficiently reduced the Cd uptake even at high concentration. Whereas the Cd uptake

would be enhanced or reduced at lower concentration of exposure or availability (Galli et al., 1994).

Arbuscular mycorrhizae were found to release insoluble glycoprotein or glomalin that can attach with Cu, Cd, and Pb (Göhre and Paszkowski, 2006) where approximately 1g of glomalin could remove up to 4.3 mg copper, 0.08 mg cadmium, and 1.12 mg lead, respectively. Although it would seem achievable tolerance method, there are practical limits to large different response of metal toxicity by plant and fungal species (Hall, 2002). Fungus has been found to increase the host tolerance only if its tolerance is way better than the host plant.

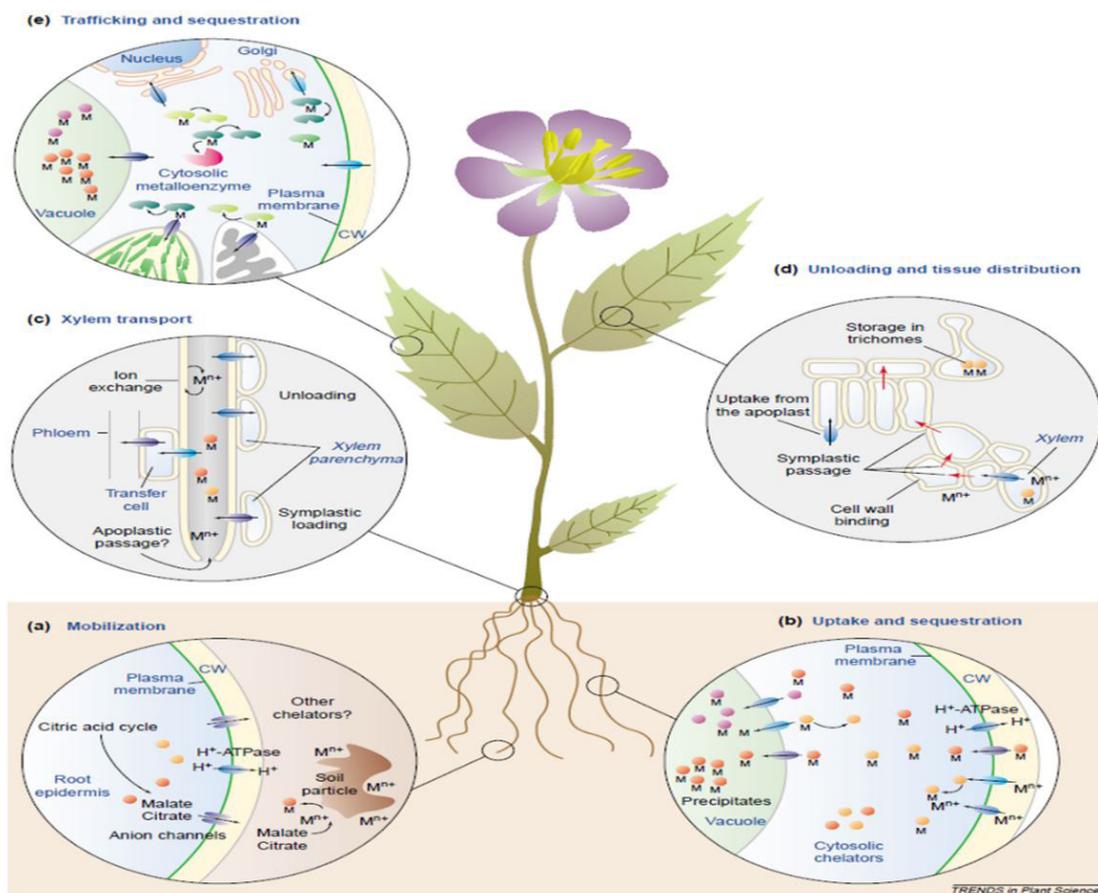
### 5.4. Membrane transport

The toxicity effects of cadmium could be triggered by the activity of oxidation, cross-linking of protein thiols, membrane protein inhibition, and composition and fluidity of membrane lipids changes (Meharg, 1993). Quartacci et al. (2001) reported the toxic effect of cadmium towards lipid composition of membranes. Additionally, cadmium absorption shows the reduction of ATPase activity of wheat plasma membrane and sunflower roots (Fodor et al., 1995). The movements of Cd into cells are aided by membrane transport through passive or active process. The passive process may happen when the concentration of extracellular Cd ions is outwardly high. In contrast, an energy-requiring process or active transport is required at low Cd concentration. Dissociation of  $\text{H}_2\text{CO}_3$  at root plasma membrane occurs during root respiration producing intercellular  $\text{HCO}_3^-$  and  $\text{H}^+$ . Free extracellular  $\text{Cd}^{2+}$  will be then absorbed by root epidermis cells surface after rapid shift with  $\text{H}^+$ . This event may possibly happen through apoplastic pathway (Yamaguchi et al., 2011).

Additionally, the absorption of Cd is readily regulated as roots hairs offer extensive contact of the roots with greater surface area through root tissues via diffusion (Seregin and Ivanov, 2001). The enhancement of ion absorption by root hairs is needed as the ion diffusion in soil is terribly slow. Besides, this extracellular  $\text{Cd}^{2+}$  also carried up by non-selective cation channels, Zn/Fe-regulated transporters (Shahid et al., 2017).

### 5.5. Root-to-shoot translocation

Roots are the first structure involve in cadmium allocation in various plant parts. The accumulation of Cd in shoot is much likely to hinge on the root factors. Shoot may accumulate Cd relying on the level of metals emanated by root. Roots might contain high amount of Cd and released a small portion of it towards shoot or vice versa. There are few intact transport processes that most likely intervene in the accumulation of Cd from root to shoot. The Cd will be (i) taken up by roots, followed by (ii) xylem loading aiding translocation to shoots and might further (iii) being translocated into seeds through phloem as illustrated in Figure 3 (Clemens et al., 2002). To make sure the accurate delivery of heavy metals to the target protein, plant cell uses a combination of compartmentalization, chelation and exclusion of metals (Clemens, 2001) and transmembrane metal-transporting proteins are recognized as essential structure for the operation (Hall and Williams, 2003).



**Figure 3.** The main mechanism of metal ions translocation. (a) Rhizosphere acidification and availability of chelators mobilize the metal elements. (b) Metals are chelated and non-chelated metals will be sequestered into vacuole. (c) Root absorption greatly leads the transportation of metals through xylem loading. (d) Metals are delivered to the shoot exiting the xylem and move from cell-to-cell. (e) Metals uptake is driven by certain transporters that occupying endomembranes. Abbreviations and symbols: CW, cell wall; M, metal; filled circles, chelators; filled ovals, transporters; bean-shaped structures, metallochaperones. Adopted from Clemens et al. (2002).

Plant roots are readily to take up dissolved nutrient mineral from soil solution. However, the chelated  $Cd^{2+}$  is likely not accessible for plant root absorption. During the respiration process, the carbonic acid is dissociated producing  $H^+$  and  $HCO_3^-$  at plasma membrane of root epidermal cells.  $Cd^{2+}$  is rapidly and non-energy driven adsorbed on root epidermal cells surface after swiftly being exchanged with  $H^+$  at plasma membrane. Subsequently,  $Cd^{2+}$  ions are ready to translocate or enter root epidermis layer through apoplast pathway (Yamaguchi et al., 2011). Apart from apoplast pathway,  $Cd^{2+}$  could pass through plant cells through symplast pathway by utilizing ion channels particular for  $Fe^{2+}$ ,  $Zn^{2+}$ , and  $Ca^{2+}$  (Sadana et al., 2003). The combination of  $Cd^{2+}$  and  $Zn^{2+}$  and/or  $Ca^{2+}$  protein transport helps  $Cd^{2+}$  to infringing into root epidermis layer as a substitute of  $Zn^{2+}$  and  $Ca^{2+}$ .

$Cd$  passage through root surface crossing over root cortex and endodermis using apoplast and symplast pathway (Figure 2). Meanwhile, the divalent ions are directly driven by symplast pathway to stele and xylem (Akhter et al., 2014).

Apparently, xylem is a component of apoplastic region which regulate the  $Cd$  translocation into stem and leaves. The occurrence of xylem loading is controlled by sites of root cortex where the cells facilitate the loading of  $Cd$  into root xylem. Moreover, the retention and loading process in root xylem contribute to  $Cd$  translocation which intervened by  $Cd$ -chelating molecules for instance, phytochelatin, vacuolar sequestration and apoplastic barriers (Clemens, 2006; Szopiński et al., 2019). Majorly, the xylem loading is mediated by certain proteins, HMA  $P_{1B}$ -type ATPases that contribute to  $Cd$  translocation (Belcastro et al., 2009). P-type ATPase transporters are one of the transmembrane metal-transporting proteins that can be found in a wide range of organism.

A broad range of evidence implies that rapid and effective heavy metals translocation from root-to-shoot is established with an increase of xylem load as a result of the constitutive genes coding overexpression for transmembrane metal-transporting proteins (Verbruggen et al., 2009). *Arabidopsis thaliana* has eight types of P-type ATPase transporter and

AtHMA4 was determined to mediate the Cd xylem loading (Verret et al., 2004). The homologues of this transporter also can be found in Cd-hyperaccumulator plants *Thlaspi caerulescens* and *Arabidopsis halleri* (Hanikenne et al., 2008). The expression of HMA3 gene into specific transporter protein pump also helps in detoxification of heavy metal by sequestering Cd into the vacuole (Ueno et al., 2005). This expression has been detected in yeasts and plants (Morel et al., 2009). The sequestration process improves in limitation of Cd translocation in *Oriza sativa*. However, a mutation of this transporter gene has prompted high Cd translocation from roots to shoots (Miyadate et al., 2011).

Other transporter like AtPDR8 is an ABC transporter which can be found mainly on the epidermis and root hairs membrane (Kim et al., 2007). Cadmium will be taken up into the xylem and transported into other parts of the plant. Nevertheless, the loading of Cd from xylem parenchyma and vessels of xylem is influenced by the transporter's activity.

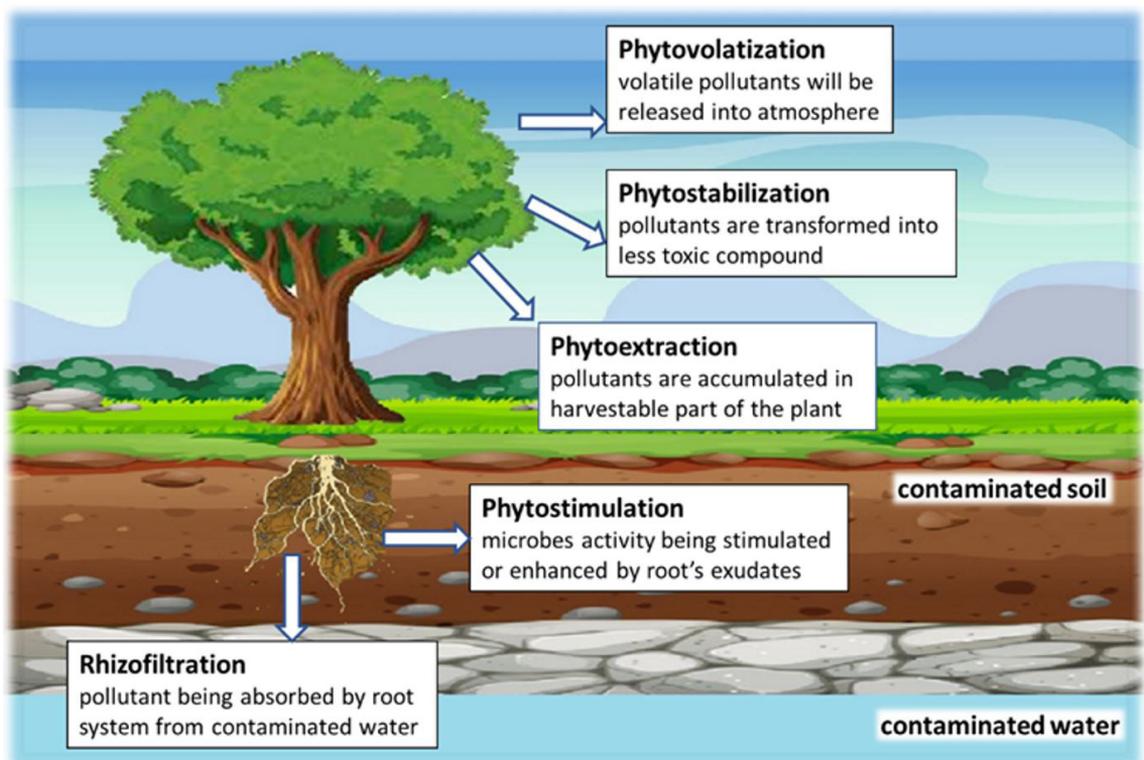
## 6. Phytoremediation Techniques in Cd Remediation

Phytoremediation is a bioremediation technology which utilizes green plants to clean up pollutants from the environment. According to Ali et al. (2013), phytoremediation can be best described as a green technology with cost effective, efficient, environmental eco-friendly technology. Another author defines phytoremediation as environmental remediation of

contaminants using green plants to make them less toxic (Raskin et al., 1997). Cd accessibility in soils, streams and rivers create the chances for Cd absorption and distribution by root to shoot and other plant parts. Different techniques are involved in phytoremediation of Cd, comprising phytoextraction, phytostabilization, phytotransformation, phytostimulation, phytovolatilization, and rhizofiltration as shown in Figure 4.

### 6.1. Phytoextraction

Phytoextraction is a low-cost phytoremediation technique using plants eradicating heavy metals from the soil environment (Evangelou et al., 2007; Shakoor et al., 2017) (Table 2). It is also termed as phytoabsorption or phytoaccumulation, which heavy metals are stored in shoot part after being extracted from plant root by up taking activities from water and soil environment (Rafati et al., 2011). Phytoextraction occurs when the plants absorb the metals and translocate them from roots to other plant parts. It is one of a reliable system to minimize the contamination without obliterating the soil composition and fertility by isolation process. It is extensively used due to the removal efficiency, however depending on few factors like soil properties, contaminant accessibility, plant species and chemical speciation of contaminants (Shabani and Sayadi, 2012). The toxic metals or contaminants are absorbed, concentrated and precipitated from soils into biomass. It is ideally suited for diffusely polluted areas remediation at relatively low concentration and superficially.



**Figure 4.** Phytoremediation of heavy metals and other organic pollutants in ecosystem. Modified from Bandara and Vithanage (2016).

**Table 2.** Implemented phytoremediation techniques in Cd bioremediation.

Phytoremediation	Plants	Reference
Phytoextraction	<i>Brassica juncea</i> L.	Shakoor et al. (2017)
	<i>Helianthus annuus</i> L.	
	<i>Thlaspi caerulescens</i>	
	<i>Medicago sativa</i> L.	Ghnaya et al. (2015)
	<i>Ricinus communis</i>	Yang et al. (2017)
	<i>Sedum alfredii</i>	Yang et al. (2004)
Rhizofiltration	<i>Manihot esculenta</i>	Horsfall et al. (2003)
	<i>Brassica juncea</i>	Dushenkov et al. (1995)
	<i>H. annuus</i>	
	<i>Micranthemum umbrosum</i>	Islam et al. (2013)
	<i>Helianthus annuus</i> L.	Turgut et al. (2004)
	<i>Typha angustifolia</i>	Woraharn et al. (2021)
Pytostabilization	<i>Acorus calamus</i>	
	<i>Pandanus amaryllifolius</i>	
	<i>Vicia faba</i> L.	Piršėlová et al. (2016)
	<i>Brassica juncea</i>	Pérez-Esteban et al. (2014)
	<i>Anthyllis vulneraria, Festuca arvernensis, Koeleria vallesiana, Armeria arenaria</i>	Frérot et al. (2006)
	<i>Iris sibirica</i>	Ma et al. (2017)
	<i>Virola surinamensis</i>	Zgorelec et al. (2020)

There are two types of mechanism involve in exclusion of heavy metals from soil. Hyperaccumulation is the first strategy of phytoextraction utilizing plants to remove the impurities from the soil and water body. The second strategy requires assistance of altered fluids containing chelating agent to enhance metal solubility in soil. This gives the chances for the plants to easily absorb the solubilize metals. Greger and Landberg (1999) have identified the great phytoextraction potential of Willow (*Salix viminalis*) in extracting cadmium, zinc, and copper. In addition, it shows special features of high-energy uptake of heavy metals by root to shoot with a significant amount of biomass production. Phytoextraction is suitable for cadmium removal in agricultural soil as it can be easily implemented due to smooth removal of cadmium in contrast to other heavy metals (Robinson et al., 2000).

### 6.2. Phytostabilization

Phytostabilisation is a technique utilizing plant to immobilize the contaminants in soil reside near the roots through roots adsorption, adsorption or precipitation in rhizosphere. It manipulates the stability of contaminants with long-term containment ensuing contaminants mobility and bioavailability constraint to enter the food chain (Wong, 2003). In fact, it employs different type of plants to stabilize the pollutants from the soil sediment and sludges (Ali et al., 2013). In other view, phytostabilization is selected approaches deactivating the potential of toxic heavy metals from the soil environment

contaminants (Vangronsveld et al., 2009) by turning the metallic toxic state into less toxic state. Therefore, the metals will become immobilize in the resource reducing the migration of metals to other sites (Eapen and D'Souza, 2005). However, it depends on capability of roots in limiting the bioavailability of metals in the soil. The plant roots will create contamination zone and assist in metal immobilization through sorption, precipitation, complexation, or reduction of metal valence (Barcel and Poschenrieder, 2003).

The water erosion can be prevented by plant root system restraining the allocation of contaminants through adsorption or accumulation. Moreover, it provides an area around the roots that induce the precipitation of contaminants. This process being operated by different mechanisms. The leaching ability of contaminants through evapotranspiration and storage of water in the contaminated soil would be likely reduced by the help of vegetative cover. In addition, an increase of aggregation of soil could control the soil erosion when the plants add organic matters in the soil (Robinson et al., 2006). As a result, contaminants will become less biologically thus reducing its exposure towards animals and humans. Subsequently, the pollutants movement and intrusion into food chain and into ground water can be effectively prevented.

It does not require the soil removal and contaminated biomass disposal since it is discovered to be extremely efficient in fine soils and high organic matter content (Berti and Cunningham, 2000). Nevertheless, this technique

perhaps can stop the translocation of heavy metals but not permanently eradicating them from the soil.

### 6.3. Phytovolatilisation

Phytovolatilization is the contaminants uptake by root through absorption process accompanied by their transformation to volatile compounds and subsequent discharge into the atmosphere. It is an approach by which heavy metals are released into air after being removed from the soil or water (Figure 4). This possibly happens due to the phytotransformation process of heavy metals or toxic contaminants into more volatile and less toxic contaminants. These volatile contaminants will be released into the atmosphere along with transpiration process. *Brassicaceae juncea* naturally extracted selenium from the soil where it is frequently found as highly toxic selenocyanate. Part of selenocyanate will eventually be converted into a 500 to 700 time less toxic volatile dimethylselenide (Souza et al., 2002).

While this technique is thought to be more successful in remediating organic contaminants from the soil environment, compared to other techniques, it has more limitations. In fact, it does not permanently remove toxins from the soil, but rather draws them into atmosphere (Padmavathamma and Li, 2007).

### 6.4. Phytostimulation

Phytostimulation is an enhancement of microbial activity in soil through stimulation of root compound exudation into rhizosphere. In other words, microbes or rhizobacteria receive adequate supply of nutrient known as exudates to enhance their growth. In return, this microbe will actively disintegrate pollutants in soil by degradation or biotransformation process. It has become a favourable technology of Cd and other organic pollutants remediation due to cost-effective and eco-friendly (Jia et al., 2016). In addition, it is considerably reliable technique for organic pollutant removal from the soil ecosystem.

### 6.5. Rhizofiltration

Rhizofiltration is one of phytoremediation techniques that involve the removal or filtration of heavy metals from water through the root biomass. Apparently, the contaminants are absorbed or adsorbed through roots surface from soil and water environment (Salt et al., 1995) with the operation of certain chemical techniques such as reverse osmosis, ion exchange, and precipitation (Francis et al., 1999). Unfortunately, these techniques are necessary to be much expensive and hardly operated. Thus, the application of rhizofiltration has been put into account as a promising phytoremediation technique. Various plant roots such as various grasses sunflower and mustard are utilized to get rid of toxic heavy metals like Cd, Ni, Cu, Zn and Pb (Lee and Yang, 2010). The suitability of plants for rhizofiltration depends on root structure and root system. Generally, the plants that have longer and hairy root system providing substantial surface area are most suitable for rhizofiltration technique. This technique has been recognized to retain certain heavy metals within the roots, thus, partially treat industrial waste, and agricultural

runoff (USEPA, 2000). Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*) have been recognized as suitable plants for rhizofiltration in remediating most of toxic heavy metals (Turgut et al., 2004).

## 7. Conclusion

Cadmium is long last pollutant in environment that needed to be diminished as much as possible markedly from food chain to maintain the living's health. Cd is non-essential heavy metals that become a concern because of its bioavailability and mobility in environment is incompetently high. The absorption of Cd by plants depend on the bioavailability of the element in the soil which is regulated by soil conditions or characteristic of plantation medium. Low pH value and poor organic matter content in soils upsurge the Cd bioavailability, hence increases the absorption of the metal. A similar pattern can be observed in the redox potential of the soils. Consequently, the absorption of Cd by plants root followed by translocation to upper plant parts results in Cd bioaccumulation in plants. Due to the fact that this accumulation can not be hindered, the toxicity symptoms visibly appear. This comprehensive literature review has demonstrated the inexorable occurring detrimental toxic symptoms experienced by plants, particularly growth inhibition, leaf discoloration, necrosis, as well as photosynthesis system alteration. Cd interferes the plant biological function by inhibiting the growth and reducing photosynthesis activity abruptly. The plant uptake and translocation of Cd are greatly assisted by multiple mechanisms involving cell wall binding, roots exudates, mycorrhiza, membrane transport and root-to-shoot translocation. The cell wall composition provide a appropriate affinity for ionic Cd. Released exudates also have impact on plant uptake through manipulation of rhizosphere activities, soil pH and soil reduction potential. Though, mycorrhizae have the potential to be quite beneficial when it comes to metals tolerance. The accumulation of Cd from root to shoot occurs when the element is taken up by roots and subsequently translocated into shoot through xylem loading. This involves both apoplastic and symplastic pathways, which begin at the root surface. It is necessary to study on the symptoms of the phytotoxicity or toxicity of cadmium in order to provide basic knowledge that could lead to discovering better approaches to the remedy of Cd, heavy metals and other organic contaminants in plant. Apparently, it is also important to re-establish and remediate the ecosystem using a promising technique. Phytoremediation is one of biological technologies that has been employed to remediate pollutant from soil and water. The most often employed procedures consist of phytoextraction, phytostabilization, phytovolatilization, phytostimulation and rhizofiltration. These various phytoremediation strategies often offer less expensive procedure with extra windfall of most environmental-friendly means. It is also reliable as offering many constructive and desirable results. A positive reintegration can be achieved by utilizing the prominent functional aspects portrayed by hyperaccumulators to extract, transform and stabilize heavy metals, especially

Cd. For the time being, it is necessary to evaluate the efficiency of phytoremediation technologies to incorporate the available resources so that the optimum remediation results can be achieved.

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