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Review

Phytoremediation of cadmium toxicity by *Brassica spp*: A review

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Phytoremediation is a relatively new cost effective and environmental friendly technology that offers clear advantages over traditional methods for site cleanup and intoxication. This green technology can be applied to remediate the polluted soils without creating any destructive effect of soil structure. Members of Brassicaceae family, especially *Brassica spp*., are well-known hyperaccumulators of heavy metals with possible utilization in phytoremediation technologies. The ability of brassicas to bioaccumulate heavy metals can be used to reduce the level of contaminants in the soil (phytoremediation), and thus to clean up and prepare soils for cultivation.

Key words: Phytoremediation, plants, Brassica, heavy metals, substances.

INTRODUCTION

The contamination of the environment with toxic metals has become a worldwide problem, affecting crop yields, soil biomass and fertility, contributing to bioaccumulation in the food chain. In the last few decades, research groups have recognized that certain chemical pollutants such as toxic metals (Cd, Pb, Hg, Zn, etc) may remain in the environment for a long period and can eventually accumulate to levels that could harm humans. The heavy metal toxicity varies greatly with element type, concentration and plant species. Heavy metals, specifically nonessential ones, become toxic because they have no free specific sites and bind to any molecule that cannot chemically effuse them. Thus, they modify the properties of host molecule. Consequently, the general cell metabolism is altered. The roots are damaged first and most severely in many cases. The heavy metal that is translocated slowly accumulates in the roots, affecting the overall plant growth primarily through root damage. In plants that are unable to exclude or inactivate the heavy metals, continuous accumulation of heavy metals led to severe metabolic damage, causing necrotic lesions on leaf margins or blades, leading sometimes to death of plant. Young plants are most susceptible than mature ones and that Cd, Co, Cr and Hg have a particularly negative influence on plant growth even at low concentration.

An ecological *in-situ* remediation technology has been developed involving the use of plants to clean up or remediate soils contaminated with toxic metals and this process is known as “hyperaccumulation” (Jaffre et al., 1976). Certain plants, termed hyperaccumulators, have been shown to be resistant to heavy metals and are capable of accumulating and transporting these soil pollutants to high concentrations. Phytoremediation (“phyto” meaning plant, and the Latin suffix “remedium” meaning to clean or restore) is being developed as an alternative technology for removing or, more accurately, reducing the concentration of toxic pollutants to clean up the environment. Plants affected adversely by heavy metals are more sensitive to drought and can not use soil nutrients efficiently. In fact, heavy metals interfere with the uptake of nutrients and/or induce leakage of nutrients by damaging plant. Plants take heavy metals and degrade them to simpler and useful forms and store them in non sensitive parts of plants like vacuoles or tonoplast or plants directly accumulate the toxic element in vacuole or tonoplast. Over 400 plant species have been identified as natural metal hyperaccumulators representing about 0.2% of all angiosperms. Unfortunately, most of these plants are characterized by slow growth and limited biomass production. Because of these limitations such plants cannot be used to remove certain heavy metals

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from soil. For instance, Cd phytoremediation technology can only be feasible if systems can be developed to employ high biomass plants, which are capable of accumulating more than 1% Cd in shoots and produce more than 20 tonne of biomass ha^{-1} yr^{-1}. The largest number of hyperaccumulators are known for Ni, Zn, and Se, however, the most widespread and hazardous pollutant in the biosphere are Pb, Cd and As.

Based on the literature from 1995 until 2009, it can be stated that the most frequently cited species in phytoremediation studies was *Brassica juncea* (L.) Czern. (148 citations), followed by *Helianthus annuus L*. (57), *Brassica napus L.* and *Zea mays L*. (both 39 citations). The greater interest in Brassicaceae derives from the fact that research on these species started earlier, together with the interesting concentrations they provide, especially for *Brassica juncea* (L.). Among the plants of the *Brassica* species, *B. juneca* deserves special attention because its relevance to the process of phytoextraction of heavy metals from soil was confirmed in many experiments. It has been found that *B. juncea* exhibits a high capacity to accumulate Cd- mainly in the shoots, where Cd level was recorded at level of 1450 µg Cd/g dry wt (Nauari et al., 2006). This is three times more than that reported in *Brassica napus* (555 µg/g dry wt). In addition, this plant exhibits a high removal efficiency of other metals such as Pb (28% reduction) and Se (reduced between 13–48%). In addition, this plant is more effective at removing Zn from soil than *Thlaspi caerulescens*, a known hyperaccumulator of zinc (Salt et al., 1998). This is due to the fact that *B. juncea* produces ten-times more biomass than *T. cearullescens*. However, *B. juncea* needs to be harvested shortly after the plant becomes mature, which causes problems of disposal of obtained biomass. When these plants are dried, they easily crumble and flake off, greatly reducing the yield obtained, and the rest of the plant residues are a source of secondary emissions of toxic substances.

TYPES OF PHYTOREMEDIATION

1. Phytoextraction - The use of plants to remove contaminants, mostly roots, from soils.
2. Phytovolatilization - The use of plants to make volatile chemical species of soil elements.
3. Phytofiltration - The use of plant roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb contaminants (mostly metals) from flowing water.
4. Phytostabilization - The use of plants to transform soil metals to less toxic forms, but not remove the metal from the soil. It reduces the bioavailability of pollutants in the environment.
5. Phytodegradation - The use of plants to degrade organic contaminants.
6. Rhizosphere-bioremediation - The use of plant roots in conjunction with their rhizospheric microorganisms to remediate organics from the contaminated soil.

HYPERACCUMULATING MECHANISM

HM-tolerant plants are often excluders, limiting the entry of root-to-shoot translocation, or retaining the uptaken hyperaccumulating mechanism into the root cells or detoxifying them by their chelation in the cytoplasm or storing them in vacuoles. Translocation in the xylem is probably transpiration driven. They hyperaccumulate HMs even from low external HM concentrations, and most of the HM is translocated to the shoot (Salt and Krame, 2000). At the root membrane level, HM uptake is unusually high in hyperaccumulators. This may be due to constitutive high expression of an HM transporter in the plasma membrane, as was found for the Cd and Zn hyperaccumulator; different chelators may be involved in translocation of HM cations through the xylem (Figure 1) such as organic acid chelators (Salt et al., 1995) or nicotianamine (Vonwiren et al., 1999). Uptake of HM ions from the xylem apoplast into the shoot symplast is mediated by HM transporters in the shoot cell membrane.

MECHANISM OF HEAVY METAL TOLERANCE AND DETOXIFICATION

Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants and is manifested by an interaction between a genotype and its environment (Macnair et al., 2000). Plants have developed potential mechanisms at the cellular level that might be involved in the detoxification and thus imparting tolerance to heavy metal stress. Some adaptive mechanisms evolved by tolerant plants include immobilization, plasma membrane exclusion, restriction of uptake and transport of metals, synthesis of specific heavy metal transporters, chelation and sequestration of heavy metals by ligands such as phytochelatins and metallothioneins, induction of mechanisms contrasting the effects of ROS and MG (such as up-regulation of antioxidant and glyoxalase systems), induction of stress proteins, biosynthesis of proline, polyamines, and signalling molecules such as salicylic acid and nitric oxide (Sharma and Dietz, 2009) (Figure 2).

MOLECULAR MECHANISM OF CD ACCUMULATION IN *BRASSICA SPP.*

Family Brassicaceae contains some members, in which metabolism of heavy metals is intensely investigated. *Arabidopsis thaliana* represents the most important member of family and model plant in molecular biology. Cadmium is rapidly absorbed, translocated and accumulated in the aerial parts of many plants of *Brassica spp*. Tolerance of heavy metals is closely connected to regulation of sulphur uptake. Sulphate transporters are proteins involved in sulphate transport across a membrane (Kumar et al., 2011).

Two classes of sulphate transporters were established:

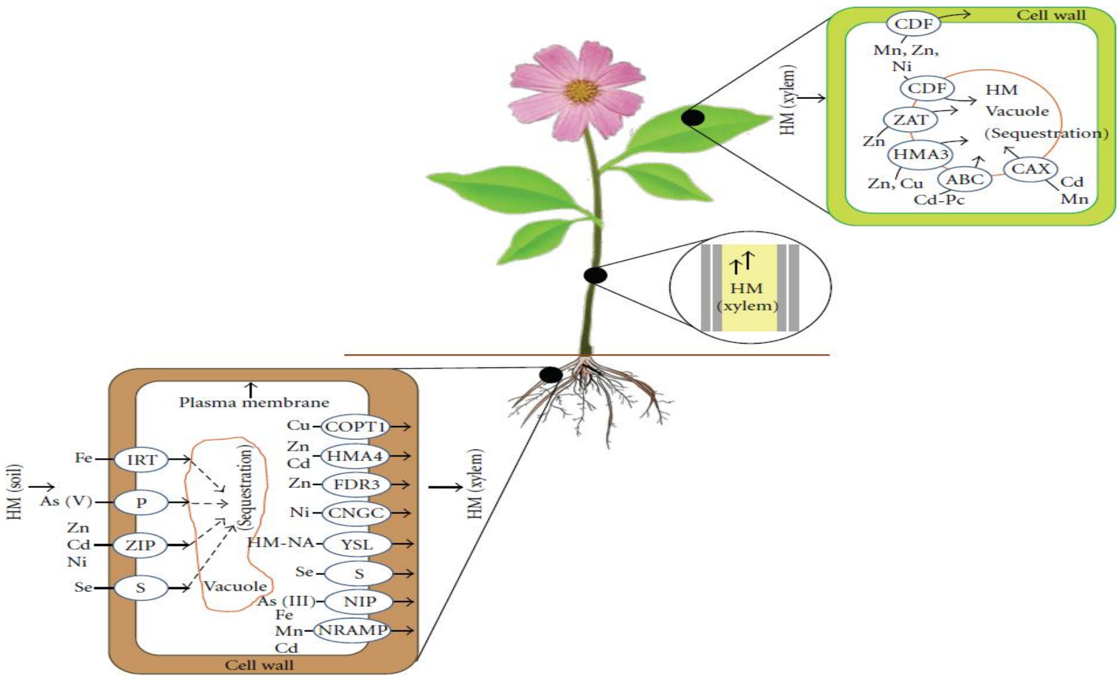

Figure 1. Diagrammatic representation of uptake and transport of heavy metals in plants through metal transporters (Rascio and Navari-Izzo, 2011).

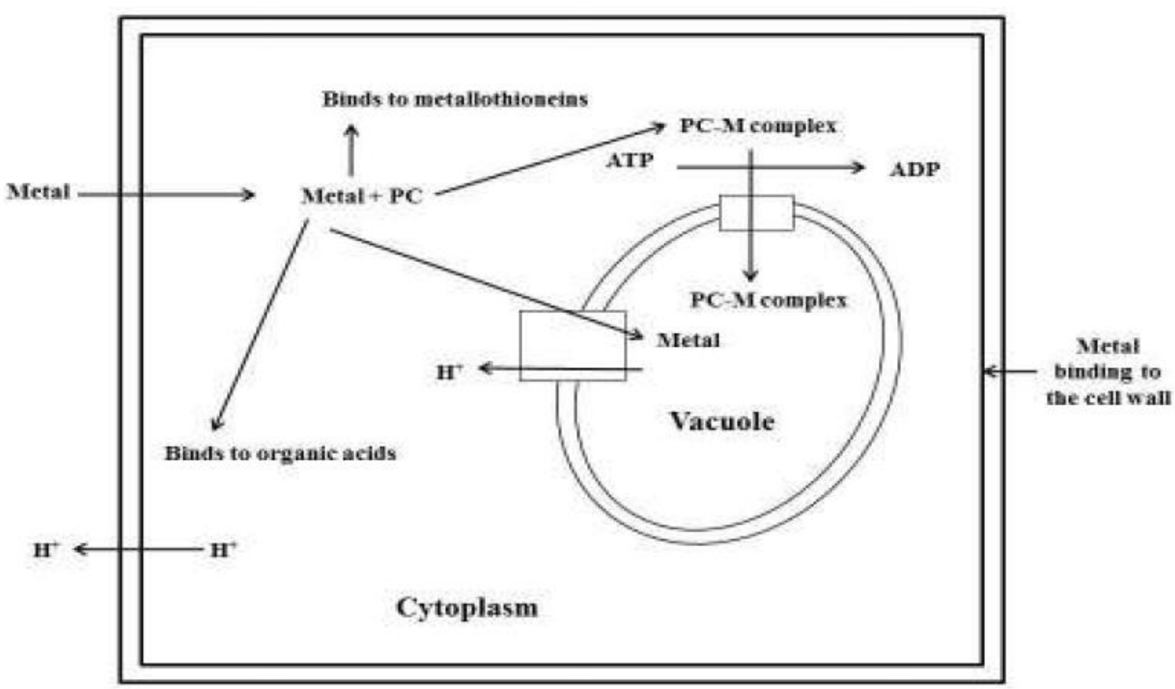

Figure 2. Cellular mechanism metal detoxification in plant (Jentschke and Godbol, 2000).

low-affinity and high-affinity. They differ not only in condition, but under their work (sulphur-replete/sulphur/deficient conditions) and in selectivity. Some of them are responsible for uptake of not only sulphate, but also chemically similar, but toxic analogues, such as selenate. High-affinity sulphate transporters are

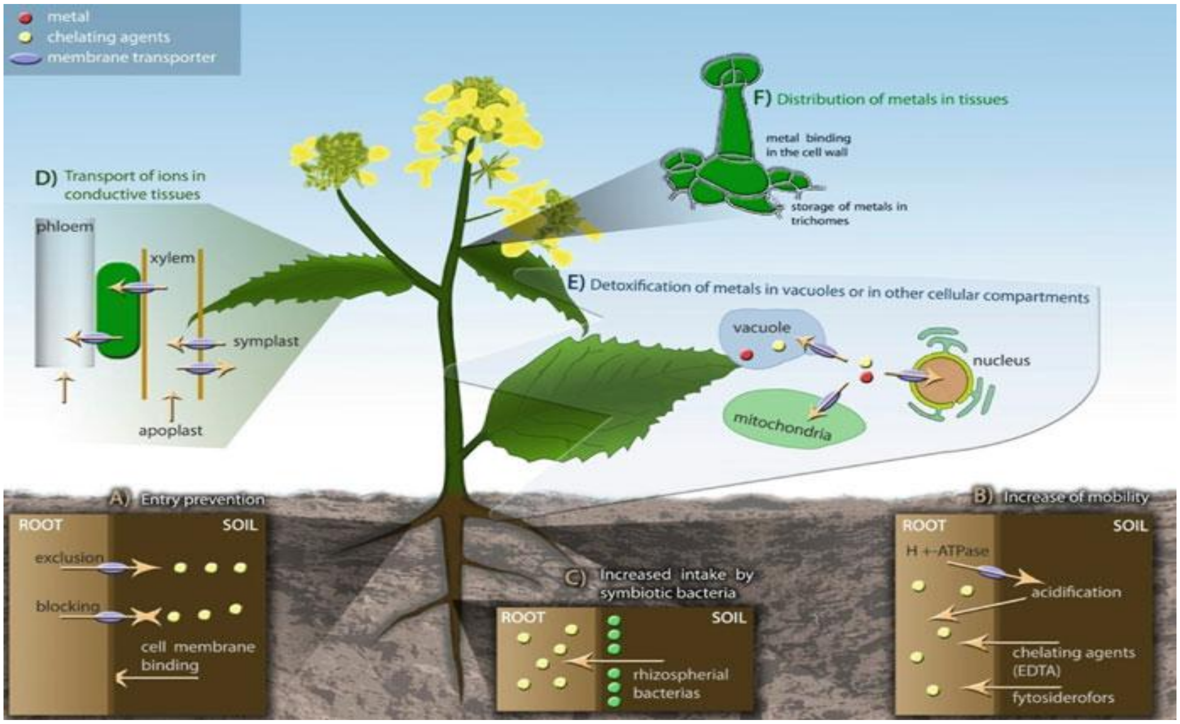

Figure 3. Molecular mechanisms proposed to be involved in transition metal accumulation by plants. (a) Metal ions are mobilized by secretion of chelators and by acidification of the rhizosphere. (b) Uptake of hydrated metal ions or metal-chelate complexes is mediated by various uptake systems residing in the plasma membrane. Inside the cell, metals are chelated and excess metal is sequestered by transport into the vacuole. (c) From the roots, transition metals are transported to the shoot via the xylem. Presumably, the larger portion reaches the xylem via the root symplast. Apoplastic passage might occur at the root tip. Inside the xylem, metals are present as hydrated ions or as metal-chelate complexes. (d) After reaching the apoplast of the leaf, metals are differentially captured by different leaf cell types and moved cell-to-cell through plasmodesmata. Storage appears to occur preferentially in trichomes. (e) Uptake into the leaf cells again is catalysed by various transporters. (f) Intracellular distribution of essential transition metals (¼ trafficking) is mediated by specific metallochaperones and transporters localized in endomembranes (Clemens et al., 2002).

the best known and characterized sulphate transporters. They have been identified especially in model plant *Arabidopsis thaliana* with tissue specificity. Recent works describe newly discovered sulphate transporters in different plants. For example, low-affinity sulphate transporter BnSultr2:2 together with high-affinity sulphate transporter BnSultr1:1 has been identified in *Brassica napus*. Increased tolerance to cadmium ions based on the efficient sulphate uptake and assimilation has been identified (Sun et al., 2007). Some genes involved in cadmium uptake have been identified. BjCdR15 expression was detected mainly in the epidermis and vascular system of cadmium treated *B. juncea* plants (Farinati et al., 2010). Further transport of cadmium (and next metal ions) into aerial parts is significantly affected by: (i) interactions with cell walls, (ii) chelation by thiols, and (iii) compartmentation into vacuoles (Nocito: Cadmium retention). Transport of cadmium ions is via cells (across cell membranes) closely connected with transporters (Figure 3). In addition, plenty of transporters associated with transport of next heavy metals (zinc, lead, arsenic, etc.) were described (Song et al., 2010). It was observed that cadmium is usually compartmented in vacuoles. As such, cadmium accumulators: *Arabidopsis halleri*, *Arabidopsis thaliana, Thlaspi caerulescens, Thlaspi praecox and Brassica juncea*, are intensely studied due to their abilities to accumulate cadmium ions.

STRATEGY OF GENETIC ENGINEERING FOR PLANT ENHANCING PHYTOREMEDIATION

Plants have the innate capabilities of remedying hazardous contaminants from the environment (bioremediation), but the rate of bioremediation is directly proportional to plant growth rate and the total amount of bioremediation is correlated with a plant total biomass, making the process very slow. This necessitates the identification of a fast growing (largest potential biomass and greatest nutrient responses) and more strongly metal accumulating genotypes (Shah and Nongkynrih, 2007).

Genetic engineering approach has successfully facilitate to alter the biological functions of plants through modification of primary and secondary metabolism and by adding new phenotypic and genotypic characters to plants with the aim of understanding and improving their phytoremediation properties (Davison, 2005). Many reports have supported the increase of valuable natural products through the over expression of biosynthetic genes with a strong promoter and a suitable signal sequence to control the preferred subcellular localization (Ohara et al., 2004). Use of tissue culture to select genes having enhanced biodegradative properties (for organics) or enhanced ability to assimilate metals, and regenerate new plant varieties based on these selected cells is also helping to select plants with desired characters. Molecular techniques such as the analysis of molecular variance of the random amplified polymorphic DNA markers are also useful to investigate the genetic diversity and heavy metal tolerance in plant populations, providing the opportunity to investigate the first steps in the differentiation of plant populations under severe selection pressure and to select plants for phytoremediation (Mengoni et al., 2000). Metal-hyperaccumulating plants and microbes with unique abilities to tolerate, accumulate and detoxify metals and metalloids, represent an important reservoir of unique genes (Danika and Norman, 2005). These genes could be transferred to fast-growing plant species for enhanced phytoremediation (De Souza et al., 1998). It has been established after a number of thorough genetic studies that the adaptive metal tolerance has been shown to be governed by a small number of major genes and perhaps contribute to some minor modifier genes (Schat et al., 2002). Probably, it is this adaptive metal tolerance that gears a plant species for hyperaccumulation. For example, a genetic analysis of copper tolerance with Cu-tolerant and susceptible lines of *Mimulus guttatus* showed that a modifier gene that is active only in presence of the tolerance gene is responsible for the difference in Cu-tolerance in this species (Smith and McNair, 1998). Similar studies with Zn-hyperaccumulator *Arabidopsis halleri* and the nonaccumulator *Arabidopsis petrea* suggested that Zn tolerance is also controlled by a single major gene (McNair et al., 2000). Therefore, the desired characters for phytoremediation can be improved by identifying candidate protein, metal chelators, and transporter genes for transfer and/or over expression of particular gene. Through genetic engineering modification of physiological and molecular mechanisms of plants, heavy metal uptake and resistance is successfully achieved by implanting bacterial gene or mutant cells on the basis of desired phenotype in plant genome which enhances the very process of uptake of metals. One promising approach for manipulation of plants character is through recombinant DNA technology. Recombinant DNA technologies combine the potentially more powerful ability to more selectively and proactively choose the traits to be introduced into the plant cell, via the introduction of DNA encoding enzymes or other proteins from other living organisms, or even completely synthetic genes designed to encode enhanced enzymes. DNA or gene of interest is spliced into a small, circular carrier DNA molecule known as a vector. The vector is introduced into plant cells either by physical means (electroporation or via high-velocity microprojectiles shot inside the cell), or biological means (utilizing natural biological systems where bacteria such as *Agrobacterium* can insert DNA into plant cells, and cause the DNA to be incorporated into plant chromosomes). Upon entry into the cell and integration into the plant chromosome, the desired gene is “expressed” in a subset of the cells (that is, its genetic code is read by the plant cell to cause the synthesis of a protein encoded by the gene); these cells are selected in tissue culture and used to regenerate whole plants for subsequent breeding.

GENETIC ENGINEERING IMPLEMENTATIONS IN FEW PLANTS

The genetic and biochemical basis is becoming an interesting target for genetic engineering. A fundamental understanding of both uptake and translocation processes in normal plants and metal hyperaccumulators, regulatory control of these activities, and the use of tissue specific promoters offers great promise that the use of molecular biology tools can give scientists the ability to develop effective and economic phytoremediation plants for soil metals (Chaney et al., 1997).

B. juncea was genetically engineered to investigate rate-limiting factors for glutathione and phytochelatin production. To achieve this, *Escherichia coli* gshl gene was introduced. The ECS transgenic seedlings showed increased tolerance to cadmium and had higher concentrations of phytochelatins, -GluCys, glutathione, and total non-protein thiols compared to wild type seedlings (Ow, 1996). The study showed that c-glutamylcysteine synthetase inhibitor, L-buthionine-[S,R]-sulphoximine (BSO), dramatically increases As sensitivity, both in non-adapted and As-hypertolerant plants, showing that phytochelatin-based sequestration is essential for both normal constitutive tolerance and adaptative hypertolerance to this metalloid (Schat et al., 2002). Isolation of the quantitative trait loci (QTL) associated with this trait holds great promise for the identification of the main genes responsible for this adaptation (Schat et al., 2002).

CONCLUSION

In recent years, public concerns relating to ecological threats caused by heavy metal (Cd, Pb, Zn, Cu, Hg and As), herbicides, pesticides, tars and PCBs have led to intensive research of new economical plants based remediation technologies. Conventional methods used for reclamation of contaminated soils, namely chemical, physical and microbiological methods, are costly to install and operate. Phytoremediation is an environmental friendly, cost-effective and plant-based solution for the remediation of heavy metal-contaminated soils. Low biomass production and slow growth of the plants and the low availability of heavy metals in soil limited effective remediation. Plants that are intended to be used for phytoextraction need to be both tolerant and able to accumulate the metals in their above ground parts. Brassica species are tolerant to heavy metals due to their fast growth, high biomass production, and ability to uptake greater amount of heavy metals. This study provides a promising start for biomass-based phytoextraction; it includes high biomass production species, and growing these species is practically easier than the production of hyperaccumulators.

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