# REVIEW ARTICLE

# Mechanisms of lead and chromium hyperaccumulation and tolerance in plant

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## Abstract

*Heavy metal contamination of agricultural lands poses serious threat to food security. Lead (Pb) and Chromium (Cr) are among the most toxic heavy metals reported but least studied. Their presence in soils has detrimental effects on crop productivity. Among different ways of remediating contaminated sites, phytoremediation technology like phytoextraction is now receiving greater attention. It involves the use of plants for cleaning heavy metal polluted media. It is environment-friendly, aesthetically appealing, cost-effective and can be applied in-situ. The plant species that are used for this process are metal tolerant and some of them called hyperaccumulators possess the ability of accumulating high concentrations of specific metals in the above-ground tissue. They have developed several mechanisms both at the genetic and molecular levels for their adaptability and efficiency. The molecular mechanisms could either be enzymatic or non-enzymatic. The enzymatic mechanism involves the participation of different antioxidant enzymes while the non-enzymatic strategies are based on the production of different antioxidant compounds for scavenging reactive oxygen species which are produced in heavy-metal stressed plants. For tolerance and metal accumulation in the above-ground parts of an hyperaccumulator, metal homoestasis through over-expression of different genes have also been reported. In this review, heavy metal toxicity, phytoremediation options and mechanisms of hyperaccumulation and tolerance in plants are discussed with focus on Pb and Cr.*

### Introduction

Rapid industrialization and urbanization have contributed greatly to ecosystem destruction and extensive contamination of the environment (Ogundiran and Osibanjo, 2008). Among the known contaminants, heavy metals are the most dangerous (Gupta and Gupta, 1998). Though there are some heavy metals that have biological uses and are required by plants and animals in minute quantity for metabolism, yet some do not have any known biological uses and are hazardous to both plants and animals. Examples of such metals are lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg) etc. They reduce agricultural productivity by causing phytotoxicity and reduction in soil fertility (Adejumo *et al.,* 2011). Heavy metal contaminated

soils also pose an increasing problem to human and animal health (CDC, 1991; Commission of the European Communities, 2001; ATSDR, 2007). Lead (Pb) is one of the most toxic elements.

Lead is considered as a priority hazardous contaminant by European Union (EU, 2008). According to the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Pb also ranks number two out of 275 toxic substances in the environment (USEPA, 2008). Agency for Toxic Substances and Disease Registry (ATSDR) also reported on the frequency of Pb occurrence and that Pb occurs in 1272 of the 1684 National Priority List (NPL) sites (ATSDR, 2007). This is because Pb has different uses for over 1000 years of human history. It is introduced into the environment through mining and smelting of Pb ore, glass manufacturing, lead-acid batteries, paints, fireworks and gasoline additives specifically, tetraethyl and tetramethyl-Pb (Panich-Pat *et al*., 2004; Amaya-Chavez *et al*., 2006; Padmavathiamma and Li, 2007). United States Geological Survey (USGS, 2006) reported that in 2004, about 3,150,000 tons of Pb were extracted from the earth's crust and brought into circulation. In 1983, 400,000 – 1,000,000 tons of mobilized Pb were disposed of with wastes from metal extraction (Nriagu and Pacyna, 1988). Lead has carcinogenic and genotoxic effects in humans. It is most dangerous to young children (ATSDR, 2007; USEPA, 2007; Pokhrel and Dubey, 2012). It has been reported to cause stress in plant by inducing excessive production of reactive oxygen species thereby causing lipid peroxidation and damage to protein molecules (Mukai *et al*., 2001; Verma and Dubey, 2003).

Chromium is also one of the toxic heavy metals and it is categorized as an important environmental contaminant. It is the seventh most abundant metal due to its wide applications at the industrial scale (Panda and Choudhury, 2005). Chromium toxicity however depends on its oxidation state. Chromium (III) is less toxic than chromium (VI). It has biological uses and is required by animal in trace amounts unlike Chromium (VI). Their oxidation state also affects their solubility and bioavailability. Chromium (VI) is highly soluble in water while Cr (III) is less soluble. This makes Cr (VI) to be more mobile than Cr (III) and also more toxic (Han *et al*., 2004). Chromium is highly phytotoxic and is also capable of inducing oxidative stress in plants and animals (Panda, 2003). It induces lipid per-oxidation and alters the activities of antioxidant enzymes (Zeng *et al*., 2011). Remediation of chromium and lead - contaminated sites is therefore pertinent.

The remediation of metal contaminated soil has been carried out using different engineering and chemical-based techniques which are expensive, not environmentally- friendly and not applicable to large expanse of land (Yang *et al*., 2005; Padmavathiamma and Li, 2007). Effective remediation of contaminated sites is either through total removal or immobilization (Bolan *et al*., 2003). Green technology is now being promoted for successful remediation of metal contaminated media. It involves the use of plants to clean or stabilize contaminants in the soil and water and is called phytoremediation. It is cost-effective, aesthetically pleasing, environmentally friendly and non-intrusive unlike other remediation options. More importantly, it can be used on a large expanse of land and can be applied *in-situ* thereby causing limited destructive impact on soil structure. Phytoremediation technology comprises of different methods among which phytoextraction is the widely adopted strategy. Plants that are capable of tolerating and accumulating high metal concentration in their tissues are used for phytoextraction. These plants are called hyperaccumulators (Baker and Brooks, 1989). In this

review, phytoremediation technology, different tolerants/hyperaccumulator plants that have been reported for phytoextraction of Pb and Cr are discussed. Mechanisms involved in heavy metal hypertolerance and hyperaccumulation, heavy metal distribution in an excluder/non-hyperaccumulator and hyperaccumulator plants are also enumerated.

## Phytoremediation

Phytoremediation is the use of plants for cleaning up of metal-contaminated sites. It is a group of technologies that use plants to degrade, immobilize environmental toxins (Cunningham and Berti,. 2000; Li *et al*., 2003; Peer *et al*., 2003). Phytoremediation science can be traced back to 1980 after the discovery of hyperaccumulator plants (Brooks, 1998; Saxena *et al.,* 1999). It is also known as phytorestoration (the use of plants for complete restoration of contaminated sites). It is rapidly gaining adoption as a green solution to polluted environments. It makes use of the natural processes in plants for ion uptake and absorption (*McGrath et al., 2002)*. Six aspects of phytoremediation have been described. These are: Phytoextraction (the use of plants to remove contaminants from soil); Phytodegradation (degradation of organic pollutants by compounds secreted by plant); Phytovolatilization (volatilization of absorbed contaminants by plants); Rhizofiltration (the use of plants to remove contaminants from water); Phytostabilization (the use of plants to immobilize contaminants or accumulate contaminants in the root) and Rhizophere degradation (the use of rhizophere microorganisms to degrade pollutants) (Shah and Nongkynrih, 2007). The choice of phytotechnology depends on the type of contaminants, site conditions, quantity of contaminants to be removed, and the species of plants to be used for the process. For instance, two types of phytoremediation strategies are commonly employed for restoration of heavy metal polluted land; phytostabilization and phytoextraction (Padmavathiamma and Li, 2007). Successful application of these methods however, depends on the ability of the plant species to tolerate, exclude or accumulate metals in their tissues. Phytoextraction strategy demands that plants must be tolerant and be able to accumulate high concentration of metals in their above-ground tissue. Careful investigation and identification of naturally occurring metal-tolerant plants or hyperaccumulators have been described as effective processes for effective phytoextraction of heavy metals in contaminated media (Mudgal *et al.,* 2010).

## Metal hyperaccumulators

These are particular groups of vascular plants which have been identified and are capable of tolerating and accumulating high metal concentrations in their above-ground tissues (Baker and Brooks, 1989; Prasad and Freitas, 2003). The term "hyperaccumulator" describes a number of plants that belong to distantly related families, but share the ability to grow on metalliferous soils. They can accumulate extraordinarily high amounts of heavy metals in the aerial organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects (Baker and Brooks, 1989; Chaney *et al.,* 2005). More than 450 plant species are known as hyperaccumulators (Reeves, 2006). They are found in 45 different families, with the highest occurrence among the Brassicaceae family (Reeves and Baker, 2000). They include trees, grasses and weeds (Pulford and Watson, 2003). Metal accumulating species are different from non-hyperaccumulators in that they have enhanced rate of heavy metal uptake, a faster root-to-shoot translocation and a greater ability to detoxify and sequester heavy metals in their tissues (Brooks, 1998). According to Bakers and Brooks (1989), plant can be classified as an

hyperaccumulator of a specific metal if it accumulates more than 100 μg g$^{-1}$ dry weight of Cd, 1,000 μg g$^{-1}$ dry weight of either Ni, Cu, Co or Pb, and 10,000 μg g$^{-1}$ dry weight of Zn and Mn. It therefore means that, classification of plant as an hyperaccumulator depends on the metal involved (Brooks *et al.,* 1977).

However, hyperaccumulators are able to perform their roles through morphological, genetical and molecular mechanisms (Blaylock *et al.,* 1989; Wang *et al.,* 2011; Hossain *et al.,* 2012). Generally, unlike non-hyperaccumulators, hyperaccumulator roots appear to be actively involved in transporting metals to the vascular system and up into the shoots in order to keep root concentrations relatively low and shoot levels high, against the concentration gradient (Verbruggen *et al*., 2009; Fahr *et al*., 2013). Some of the processes involved in hyperaccumulation of trace metals from the soil to the shoots by hyperaccumulators include: (a) bioactivation of metals in the rhizosphere through root–microbe interaction, (b) enhanced uptake by metal transporters in the plasma membranes, (c) detoxification of metals by distributing to the apoplasts like binding to cell walls, (d) chelation of metals in the cytoplasm with various ligands, such as glutathiones, phytochelatins, metallothioneins and metal-binding proteins, (e) sequestration of metals into the vacuole by tonoplast-located transporters and (f) constitutive overexpression of metal transporter genes which encode transmembrane movement of metals (Verbruggen *et al*., 2009). These transporters, include, ZIP, HMA, MATE, YSL and MTP families (McDonald, 2006; Revathi, 2013).

Similarly, the general features or characteristics of a good hyperaccumulator as given by Mudgal *et al.* (2010) include; high level of tolerance to a specific metal, high capacity for absorption of metals, efficient root to shoot translocation, ability to detoxify metals or concentrate metals in non-sensitive parts of the cell, rapid growth rate and ability to accumulate metals at a concentration which will be 100 times higher than that of non-accumulator. To overcome the stress of metal toxicity, hyperaccumulators/hypertolerant plants also have selected physiological strategies which help them to remove the toxic ions from the most sensitive subcellular organelles thereby concentrating it in the cell wall or sequestering it in the vacuole (Clemens, 2001). Some excrete metals into the apoplast to reduce internal metal bioavailability (Clemens, 2006).

**Mechanisms of Pb Hyperaccumulation and tolerance**

Among the several plant species reported for metal hyperaccumulation, very few are capable of accumulating Pb (Baker, 1981; Baker and Whiting, 2002). About 5 species of plants have been reported to hyperaccumulate Pb compared to 26, 24, 8 and 145 reported for Co, Cu, Mn and Ni respectively (Peer *et al*., 2003) with Ni having the highest number. These five commonly reported Pb hyperaccumulators are *America mantina, Thlaspi rotundifolium* (L.) Gaudin*, Thlaspi arvense* L*, Allysums species and Polycarpa* spp. Generally, the transportation and detoxification of metals in plants are mediated by different types of ligands which are produced for chelation and sequestration of metals (Abdul *et al.,* 2001; Verbruggen *et al.,* 2009). It is believed that majority of the plants do not have specific channels for Pb uptake unlike other metals, hence the element gets into the plants by binding to the carboxylic acid groups of mucilage uronic acids on root surfaces (Morel *et al*., 1986; Sharma and Dubey, 2005) while the unbound Pb move through Ca channel and accumulate near the endodermis (Huang and Cunningham, 1996; Huang *et al.,*

1997; Antosiewicz, 2005). In some plants, it has been reported that Pb can be transported *via* vascular tissues to aerial parts through complexation with organic molecules (Hanc *et al*., 2009). It is transported in *Sesbania drummondii* (Rydb.) Cory, to the leaves after complexation with acetate, nitrate, and sulfide (Sharma *et al*., 2004). In tobacco, a cyclic nucleotide gated channel (NtCBP4) was suggested to be involved in Pb transport (Sunkar *et al*., 2000). In cytoplasm, Pb is chelates with phytochelatins which in turn enhances its upward movement (Estrell *et al.,* 2009; Fahr *et al*., 2013). The complexes formed are then sequestered in the vacuoles. Some plant species like *Allium cepa* L*, Hordeum vulgare* L. and *Zea mays* L are also tolerant to Pb through these processes of complexation and inactivation (Sunkar *et al*., 2000).

A number of protein and non-protein thiols like gluthathione and phytochelatins together with a network of sulphur containing molecules and related compounds also contribute to plant stress tolerance and metal transport from root to shoot (Álvarez *et al.,* 2012; Zargorchev *et al*., 2013). Glutathione (Glu-Cys-Gly; GSH) and phytochelatin (GluCys)$_n$ Gly (where $n = 2$–11) are major cellular antioxidants reported (Verbruggen *et al.,* 2009). They form complexes with several metals. According to Clemens (2006), phytochelatins are synthesized from glutathione under high metal stress. Gluthathione is therefore the precursor of phytochelatins. Their induced production in metal-stressed plants provides protection against oxidative stress. Hossain *et al.* (2012) reported that GSH by itself and its metabolizing enzymes—notably glutathione S-transferase, glutathione peroxidase, dehydroascorbate reductase, glutathione reductase, glyoxalase I and glyoxalase II - act additively and coordinately for efficient protection against reactive oxygen species induced damage in addition to detoxification, complexation, chelation and compartmentalization of heavy metals. Increased production of glutathione in *T. goesingense* and other *Thlaspi* Ni hyperaccumulators has been reported to be responsible for their protection against oxidative damage under high Ni concentrations (Freeman *et al.,* 2004; Papoyan and Kochian, 2004). Root enhanced detoxification mechanisms through the participation of gluthathione reductase, ascorbate peroxidase and gluthathione S-transferase which induced production of gluthathione has also been reported (Brunet *et al.*, 2009). Similarly, enhanced glutathione synthesis in the leaf and root of *T. caerulescens* was induced under Cd exposure (van de Mortel *et al.,* 2008).

The PIB-type Heavy Metal ATPases (HMAs) are also implicated in the transport of different essential metals and potentially toxic metals across the cell membrane (Sanchez-Fernandez *et al.,* 2001; Gravot *et al*., 2004). They are involved in acquisition and compartmentation of macronutrients as well as toxic heavy metal absorption and detoxification. Expression of the glutahione-Cd vacuolar transporter gene, YCF-1 in *Arabidopsis* has been found to increase the tolerance and slightly increases the accumulation of Pb (Song *et al*., 2003). The Zn cluster proteins which transport Zn also transport Cd and Pb and play a role in metal detoxification (Axelsen and Palmgren, 2001; Mills *et al.,* 2003). The induction of AtHMA3 gene expression under Cd and Pb exposure is also assumed to be playing a role in gluthathione synthesis (Kim *et al*., 2006). The GSH1 transgenic India mustard was found to accumulate 2-3 folds more Cr, Cu and Pb compared to the wild type (Bennett *et al*., 2003). Overexpression of GSH1 genes in *Arabidopsis* spp was also reported to increase phytochelatin synthesis (Guo *et al*., 2008). This increase in phytochelatin synthesis in response to Pb and formation of PC-Pb complexes was confirmed by Piechalak *et al*. (2002). Phytochelatin syhnthesis is also induced in the root and stem of an hyperaccumulator *Sedum alfredii.* Hance, when exposed to 700µM lead (Zhang *et al*.,

2008). It was found that Pb and Cd exposure induced the synthesis of phytochelatin in marine diatom (Phaeo-dactylum tricomutum) (Morelli and Scarano, 2001). According to Mishra *et al*. (2006), production of phytochelatin is very important in Pb tolerance and detoxification. Lead tolerance in Coontail (*Ceratophyllum demersum* L ) was also found to be mediated by PCs with concomitant decrease in gluthathione (its precursor) under Pb exposure. Different phytochelatin synthase genes have been cloned from different crops after exposure to heavy metal stress; Rice (OsPCS1), Wheat (TaPSC1), *Arabidopsis thaliana* (AtPCS1) and *Brassica juncea* (BjPSC1) (Heiss *et al*., 2003). These genes were found to be up-regulated under heavy metal stress. Gisbert *et al*. (2003) reported a genetically modified *Nicotiana glauca* R. Graham (Shrub tobacco) was able to accumulate Pb through enhanced gene expression. It was reported that the gene encoding phytochelatin synthase in wheat (TaPCS1, Accession No. AF093752) increased the tolerance of bioengineered *N. glauca* to Pb.

Gupta *et al*. (2004) however, reported that Pb detoxification in *Sedum alfredii* H is related to gluthathione not phytochelatins. Similarly, Zhao *et al*. (2003), were of the opinion that PCs are generally not essential for the hyperaccumulation phenotype. It was reported that arsenic, which is normally a very effective inducer of PC synthesis in other species, only induces smaller amount of PC in the roots of the As hyperaccumulator, *Pteris vittata (Zhao et al., 2003).* An increase in the GSH concentrations in a hyperaccumulating *S. alfredii* population, and not in a non-accumulating one was also reported and the decrease in GSH of non-hyperaccumulator, was said to be due to PC synthesis (Sun *et al*., 2007). These results therefore suggest that there is a role for GSH in hyperaccumulation than phytochelatin. This was further corroborated by several reports that PCs might have a role in basal metal detoxification but they do not seem to be involved in Cu, Cd, Zn, Co and Ni hypertolerance (Ebbs *et al*., 2002; Schat *et al*., 2002; Hernandez-Allica *et al*., 2006). It was found that PCs are mainly induced in the roots, in particular by Cd, but not (or barely) by Zn or Ni in hyperaccumulators, just as in non-hyperaccumulators. Meanwhile, Zhang *et al*. (2008) reported that using high-performance liquid chromatography (HPLC), HPLC-mass spectrometry, and HPLC-tandem mass spectrometry, PC synthesis and formation in the mine population of *S. alfredii* was induced in the leaf, stem and root tissues upon exposure to 400 μM cadmium, and only in the stem and root when exposed to 700 μM lead. However, no PCs were found in any part of *S. alfredii* when it was exposure to 1600 μM zinc. These results suggest that PC synthesis is a function of metal involved and concentration dependent. Furthermore, the nature of the chelators is said to be different depending on the location within the plant and the age of the plant (Salt *et al*., 1999). Phytochelatin alone might therefore not be as important as was earlier reported for some metal accumulation and tolerance. Other mechanisms might therefore be involved in Pb tolerance and detoxification.

Metallothionein (HiMT2a) and PIB-type-ATPase (HiHMA4) encoding gene, for instance, were also over-expressed or induced by Pb exposure in *Hirschfeldia incana* (L.) Lagr.-Foss, a Brassicae (Auguy *et al*., 2013). This reportedly enhanced Pb tolerance and accumulation by this species and the genes were similar to those reported in *Arabidopsis thaliana* (L.) Heynh.; AtHMA4 and AtHMT2a respectively. AtHMA3 also function as Cd/Pb transporter in yeast (Gravot *et al*., 2004) while OsHMA9 gene was reported to confer tolerance to rice under high levels of Cu, Zn and Pb (Lee *et al*., 2005). Over-expression of *Nicotiana tabacum* plasma membrane protein (NtCBP4) that binds calmodulin in transgenic plants confers $Pb^{2+}$ tolerance

and enhanced $Pb^{2+}$ accumulation. NtCBP4 and its homologous gene in *Arabidopsis thaliana* (AtCNGCI) are also components of a transport pathway responsible for $Pb^{2+}$ entry into plant cells (Sunkar *et al*., 2000). Similarly, for tolerance, expression of PbtABC or PbtA in a metal sensitive *E.coli* are said to induce $Pb^{2+}$ $Cd^{2+}$ and $Zn^{2+}$ tolerance by decreasing accumulation (Hložková *et al*., 2013). The characterization of Pbt genes conferred increased $Pb^{2+}$ and $Cd^{2+}$ tolerance on *Achromobacter xylosoxidans* A8 (Hložková *et al*., 2013). Lead accumulation was in turn enhanced in *E. coli* expressing Pb uptake transporter (PbtT) and Pb resistance was achieved in *Cupriavidus metalidurans* through the cooperation of the Zn/Cd/Pb translocating ATPase (PbrA) and undecaprenyl pyrophosphate phosphatase (PbrB) (Hynninen *et al*., 2009; Morel *et al.,* 2009). The later (PbrB) catalyses the production of phosphate salt which presumably binds with $Pb^{2+}$ after it has been translocated by PbrA and Pb is sequestered as Pb phosphate salt. Pb tolerance and detoxification was therefore achieved through metal sequestration/detoxification and active efflux processes by different enzymes and transporters.

Other metal transporters and accumulation ligands in plants which have been reported include: proline, glycine betaine, histidine, nicotianamine, organic acids (citrate, malate) and metallothioneins. Though information about the involvement of these ligands in Pb hyperaccumulation is few, they have all been reported to be involved in metal hyperaccumulation and tolerance. Histidine (His) and proline are considered as the most important free amino acids involved in metal hyperaccumulation and tolerance. It has been reported to form complexes with different heavy metals in the hyperaccumulator's root (Persans *et al*., 1999; Callahan *et al*., 2006). Enhanced expression of ATP-phospho-ribosyl transferase which is the first enzyme of the histidine biosynthetic pathway was reported in Ni hyperaccumulator *A. lesbiacum*, for Ni tolerance compared with *A. montanum; a non-accumulator (Ingle et al., 2005: Kerkeb and Kramer, 2003).* A dose-dependent increase in histidine in the xylem sap and increased Ni concentrations in xylem sap, which was not found in the non-hyperaccumulator, *A. montanum was also reported in the hyperaccumulator.* Accumulation of proline and glycine betaine in plants growing under different stress conditions has been well reported (Trovato *et al.,* 2008; Adejumo *et al.,* 2015). They are described as potent antioxidants, osmoprotectants and ROS scavengers. High proline accumulation has been reported in plants growing on metalliferous soils most especially the Pb hyperaccumulator, *Gomphrena celosoides* (Adejumo *et al.,* 2015). It is therefore concluded that this osmolyte must be playing a major role in Pb hyperaccumulation and tolerance. Similarly, glycine bentaine which is a quartennary amino acid has also been implicated in Pb tolerance and detoxification (Chen and Murata, 2011).

Though, synthesis of nicotianamine (NA) from 3 S-adenosyl-methionine (SAM) by NA synthase (NAS) is present in all plants, it has also been reported to be involved in metal hyperaccumulation, both in *A. halleri* and *T. caerulescens (Becher et al., 2004; Weber et al., 2004; Mari et al., 2006; Talke et al., 2006; Callahan et al., 2007).* In these plants, several *NAS* genes showed higher expression. It is said to form strong complexes with most transition metal ions (Stephan and Scholz, 1998) where it participates in the distribution of micronutrients. Over-expression of several members of metallothioneins family (type 1, 2 and 3) have also been reported for *T. caerulescens (Roosens et al., 2004; Rigola et al., 2006; Hassinen et al., 2007; Guo et al., 2008).*

**Mechanisms of Cr Hyperaccumulation and tolerance**

Plants vary in their ability to accumulate Cr. The most common methods being employed for Cr uptake and translocation are similar to those reported for other metals. Root exudates, such as organic acids increase the solubility and mobility of Cr in the soil. It is then transported through the root xylem and finds its way into the plants by reduction and/or complexation (Bluskov *et al*., 2005). Shanker *et al*. (2005) reported that both Cr (VI) and Cr (III) enter into the root cells by the symplast pathway where Cr (VI) is reduced and accumulated in the cortex. Few Cr hyperaccumulators have been identified. This is because Cr exists in an insoluble form ($Cr^{3+}$) thereby not available for metal uptake and therefore a few plant species have been reported for Cr. As reported for Pb, majority of Cr hyperaccumulators are from Brassicacae family (cauliflower, kale and cabbage). Indian mustard *Brassica juncea* L. (zem) and sunflower (*Helianthus annus* L.) have been reported to accumulate Pb, Cr, Ni, Cu and Zn (Asuncao *et al*., 2006). The leaves of *Diccona niccolifera* and *Sutera fodina* have been found to accumulate 1500µg /g and 2400µg/g Cr (Wild, 1974; Baker and Brooks, 1989). *Lepertospermum scoparium* contains up to 1% Cr (Lyon *et al*., 1971). *Sutera fadina* and *Diccona niccolofera* accumulate 48000 and 30000 µg/g Cr in the ash respectively (Peterson and Girling, 1981). They absorb more Cr than other plant species with on observable symptoms of toxicity to the plant (Zayed and Terry, 2003).

**Choosing target genes for hyperaccumulation and tolerance**

Since majority of known hyperaccumulators are low biomass, small crawling and difficult to cultivate, the new strategy being proposed for effective phytoremediation is the development of hyperaccumulating plants that will be of high biomass and can be easily cultivated (Mudgal *et al*., 2010; Hassan and Aarts, 2011). Choosing or cloning of the genes of desirable traits for the transformation process is however, a multitasking approach. This is because heavy metal tolerance is not a monogenic trait. Many genes are involved in hyperaccumulation and tolerance (Lee *et al*., 2003). Different genes are involved in metal uptake, translocation, sequestration and detoxification (Verret *et al.,* 2004). To achieve effective phytoextraction therefore, several genes will need to be transferred to the proposed plant species for phytoremediation as there might be no correlation between the tolerance and hyperaccumulation. Therefore, the genes responsible for each process must first be identified. A good hyperaccumulator must constitute an exceptionally high biological material and gene reservoir which makes it adaptable to contaminated environment. Overexpression of existing genes is usually a common process for adaptation (Taji *et al*., 2004; LeDuc *et al.,* 2004). However, according to Asuncao *et al*. (2006), only a few genes are responsible for hyperaccumulation using classic genetic studies and QTL analysis. In all, the complexity of transport mechanism within the plant puts a demand on the study of different metal transporter genes in plants (Hanikenne *et al.,* 2008; Kim *et al.,* 2009). Hyperaccumulation is said to start from metal uptake from soil, metal partitioning to different organs and different cell organelles and detoxification or sequestration process occurring in the vacuole. Enhancement of the activities of metal transporters from soil to root, root to shoot and at the cellular level will go a long way in improving the metal accumulation and tolerance in plant.

### Metal uptake and absorption from the soil

Absorption of either essential or non-essential elements by plants is the most critical process that precedes and determine the translocation and accumulation of mineral ions including heavy metals (Argu¨ello, 2003). Metal absorption or uptake is particularly important in hyperaccumulation process. Several genes encoding transporter proteins mediating the influx and efflux of metals have been identified at the plasma membrane of the root epidermal cells (Awaad *et al.,* 2010). These include Cation Diffusion Facilitator (CDF), Natural resistance-associated macrophage protein (Nramp), ATP Binding Cassette (ABC), Zinc Iron-like Protein (ZIP) and others (Clemens, 2006; Kramer *et al*., 2007).

### Zinc and Iron regulated transporters (ZIP)

These are believed to be playing important roles in the uptake of metal ions by the plant's root. In Zn hyperaccumulators (*Thlaspi caerulescens* and *Arabidopsis halleri)*, these genes have been reported to be over expressed (Kramer *et al*., 2007).  **PIB-**type ATPases (HMAs) are other important transporter enzymes known as P-ATPases which are responsible for the movement of metal ions from root cytosols to the plant vascular system. These are called heavy metal transporting ATPases (HMAs) or PIB-type ATPases (Deng *et al.,* 2013). They use the energy from ATP hydrolysis for transporting metals against concentration gradients (Morel *et al*., 2009). HMAs are involved in metal hyperaccumulation and hypertolerance. Among the 46 genes identified in *Arabdopsis*, eight of them belong to this group (Baxter *et al*., 2003), for example, HMA3 is one of the transporters commonly implicated in metal hyperaccumulation and tolerance processes. Ectopic overexpression of AtHMA3 improved plant tolerance to Cd, Co, Pb, and Zn. Cd accumulation increased by about 2- to 3-fold in plants overexpressing AtHMA3 compared with wild-type plants (Baxter *et al*., 2003). Thus, AtHMA3 likely plays a major role in the detoxification of biological (Zn) and non-biological heavy metals (Cd, Co, and Pb) by participating in their vacuolar sequestration (Verret *et al.,* 2004; Morel *et al*., 2009). HMAs can be divided into two subgroups based on their metal-substrate specificity: a copper/silver group and a zinc / cobalt / cadmium / lead group (Morel *et al*., 2009). *Arabidopsis* AtHMA1-4 are known for transporting Cd, Pb and Zn while HMA 5-8 are for Cu and Ag (Mills *et al*., 2003; Takahashi *et al*., 2012). AtHMA3 is described as a pseudogene in the wild-type Columbia (Col-0) ecotype (Hussain *et al*., 2004; Auguy *et al*., 2013).

### Metal ligands complexes transporters (MATE)

These are involved in the long distance transport of metal from root to shoot of an hyperaccumulator. However, availability of metal for translocation to the shoot is a function of the root's ability to release or withhold this metal. The non – hyperaccumulators retain the highest proportion of metals in their root or sequester the metals in the root vacuoles as observed in *Thlaspi arvense*, a non-accumulator (Lasat *et al*., 1998). Only the hyperaccumulators store heavy metals in the cytosol and then make it available for upward transportation to the shoot.

Availability of metals for translocation to the shoot implies limited sequestration in vacuoles of root cells (Baxter *et al*., 2003; Yang *et al*., 2005; Xing *et al*., 2008). FRD3 which is a member of MATE has been reported to be actively involved in citrate efflux in the root vascular system and is said to be responsible for the upward movement of Fe in plant. Gene responsible for citrate production has also been found to be overexpressed in Zn hyperaccumulators compared to non-hyperaccumulators (Talke *et al*., 2006). More importantly, efficient translocation of metal ions from root to the shoot requires radial symplastic passage and continuous loading of the metals into the xylem (Clemens *et al*., 2006: Xing *et al*., 2008). Salt *et al.* (1999) reported that most of the Zn in the xylem sap of *T. caerulescens* was present as the free hydrated $Zn^{2+}$ ion. Yellow-stripe L-like (Ym vSL) subfamily which belongs to a family of oligopeptide transporters (OPT) gene is also over-expressed in some plants. TcYSL3 and TcYSL7 have been found to be displayed in the root vascular tissue of *Thlaspi caerulescens* (Gendre *et al*., 2007). *Arabdopsis thaliana* OPT also transport Cd-gluthathione complexes in the vascular system (Cagnac *et al*., 2004).

### *Cation Diffusion Facilitator (CDF) family:*

They help in preventing cellular damage. Heavy metals are generally chelated by low molecular weight compounds, sequestered into organelles or expelled to the extracellular space by specific transporters known as Cation Diffusion Facilitator (CDF) family. They are also known as the Metal Tolerance Proteins (MTPs) in plants. They specialize in transporting divalent metals such as $Co^{2+}$, $Pb^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Cd^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ (Persans *et al*., 2001; Delhaize *et al*.,2003). Vascular transporters such as ShMTP (*Stylosanthes hamata* Metal Transporter Protein), AtMIPI or Zn transporters of *Arabdopsis thaliana* are said to be involved in the conferment of tolerance and accumulation traits in *Stylosanthes* and *Arabdopsis* for the accumulation of Mn and Zn respectively (Delhaize *et al*., 2003).

### *ABC (ATP-Binding Cassette) transporters and Cation Exchangers Proteins (CEP)*

They are involved in the vacuolar sequestration of various metals and they may be expected to contribute to trace metal hyperaccumulation, in particular to vacuolar sequestration. CAX is the acronym commonly used for **ca**tion e**x**changer. It consists of two subfamilies, MRP and PRD, and they are involved in the transport of chelated heavy metals or the organic acids necessary for the transport of heavy metals. AtPDR12 which is an ABC transporter has been reported to contribute to $Pb^{2+}$ resistance in *Arabidopsis* (Lee *et al*., 2005). This member of the pleiotropic drug resistance (PDR) subfamily of ABC transporters in *Arabidopsis* according to Lee *et al*. (2005) has a role in $Pb^{2+}$ detoxification. The expression of AtPDR12 was found to be strongly induced by $Pb^{2+}$ treatment and that AtPDR12-knockout plants were more sensitive to $Pb^{2+}$ than wild-type plants while AtPDR12-overexpressing plants are more resistant to $Pb^{2+}$ than wild-type plants. In addition, AtPDR12 knockout plants also contain more $Pb^{2+}$ than wild-type plants. AtPDR12 was able to confer $Pb^{2+}$ resistance by pumping $Pb^{2+}$ out of the cell from the cytoplasm. Two ABC genes were also identified in *T. caerulescens*: the *AtMRP10* and *ATH13* homologues.

*AtMRP10* was shown to be differentially expressed in the shoots of two *T. caerulescens* populations displaying contrasting Zn tolerance and accumulation (Hassinen *et al*., 2007) while *ATH13* was more expressed in the shoot compared with *A. thaliana* (van de Mortel *et al*., 2008). Though, superfamily of ABC (ATP-binding cassette) transporters is involved in many physiological processes but they are mostly involved in metal vacuolar sequestration (Hassinen *et al*., 2007). Members of other CaCA subfamilies may also play a role in metal detoxification (Korenkov *et al*., 2007). AtCAX2 and AtCAX4 have been implicated for the transport of $Cd^{2+}$ into the vacuoles. Under the activity of the constitutive 35S CaMV promoter, overexpression of *AtCAX2* and *AtCAX4* resulted in higher accumulation of Cd in the root vacuoles (Korenkov *et al*., 2007).

**Conclusion**

Plant tolerance and hyperaccumulation mechanisms require the coordination of complex physiological and biochemical processes, including changes in gene expression. Heavy metal (HM) transporters play key roles in the uptake, transport, sequestration and efflux of metals and are among the most studied homeostatic genes relating to metal tolerance and accumulation abilities. Several heavy metal transporters which have been cloned and characterized from various metal tolerant and hyperaccumulating plants have been reported. They regulate metal accumulation and transport in plants and so, are important candidate genes to study in metal tolerant and accumulator plants for their potential use in environmental cleanup.

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