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REVIEW

# Tracing the role of plant proteins in the response to metal toxicity: a comprehensive review

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**ABSTRACT**

Plants are sessile in nature, but are capable to evade from high level concentration of heavy metals like Cd, Hg, Cu, through various metabolic pathways. Some of the pathways regulate normal metabolism in plants, whereas others are required for for their survival under metal toxicity. Different plant proteins act as transporters to transfer metal from one organelle to the other and further eliminate it out from the plants. Initially, exposure of heavy metals/metalloids to plants lead to over expression of proteins which in turn stimulate other stress-related genes. Further, they activate signalling mechanism like MAPK cascade, Cd-Calmodulin signalling pathway, and oxidation signalling pathway that lead to generation of ROS (reactive oxygen species). Once these ROS (highly unstable) are formed, they generate free radicals which react with macromolecules like proteins and DNA. This has negative impact on plant growth and leads to ageing and, eventually, cell death. The uncontrolled, destructive processes damage plants physiologically and ultimately lead to oxidative stress. Activation of antioxidant enzymes like SOD (superoxide dismutase) and CAT (catalase) allows plants to cope under oxidative stress conditions. Among plant proteins, some of the antioxidant enzymes like glutathione, and APX (ascorbate peroxidase) play defensive roles against abiotic stress in plants. Chaperones help in protein folding to maintain protein stability under stress conditions. With this background, the present review gives a brief account of the functions, localization and expression pattern of plant proteins against metal/metalloid toxicity. Moreover, the aim of this review is also to summarize the cutting edge research of plant protein and metal interfaces and their future prospects.

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## Introduction

Metals occur naturally in the earth's crust at different depths. These metals are classified on the basis of their density. Heavy metals are generally defined as metals with relatively high density; for example, zinc, copper, lead, iron, etc. Light metals have low density and include magnesium, aluminium and titanium. Another division is metalloids which include germanium, arsenic, selenium etc. Heavy metals occur in the d-block of the periodic table.[1] During the process of evolution of angiosperms, only 19 elements such as Mg, C, P, H, S, K, N, O and Ca (macronutrients) and Cu, Mn, Fe, B, Zn, Mo, Co, Ni, and Cl (micronutrients) were selected for basic metabolism.[2] In addition, Si is also a beneficial element, and it was reported to be involved in the maintenance of the cell wall structure in some plants.[3] Macro- and micronutrients play an important role in both biochemical and physiological processes of plants.

However, the problem comes during their excess discharge into the environment due to various human activities and natural phenomena. According to statistics, it was estimated that around 147000 tonnes of copper and 38900 tonnes of cadmium are discharged annually and liberated out of industries to the environment.[4] Due to the non-degradable nature of the metals, they are being transferred from one trophic level to the other, through the food chains, further leading to bio-magnification. Due to the presence of metals in the soil, these plants cannot grow optimally and their photosynthetic functions are dramatically affected. Most of the plants cannot grow efficiently in soil and water contaminated with metals. Eventually, some plants have evolved specific molecular and physiological processes to withstand metal stress.[5] Different studies at the gene and protein levels have identified mechanisms regulating metal tolerance.[6] Some of the essential entities to identify correct protein functions are protein folding and protein-protein interactions.[7] Hence, studies using proteomics offer an unprecedented analytical depth for the identification and characterization of the array of proteins which control metal detoxification, and for the study of pathways involving networks of proteins.[8] Further, functional studies carried out on these plant proteins are equally important to decipher the

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molecular mechanisms underlying plant metal-tolerance. Additionally, such studies merging proteomics and functional studies can inspire biotechnological strategies which will help in bioremediation.

## Plant proteins regulating the plant response to metal stress

As plants are static, they cannot escape from unfavourable environmental conditions. The exposure of plants to these toxic metals triggers various biochemical and physiological processes, which eventually lead to adaptation and ability to survive under adverse conditions.[9] Different mechanisms are involved in the plant response to abiotic stresses like heavy metal toxicity (Figure 1).

These include:

(1) Signal transduction in response to metals
(2) Entry of metal ions from the soil into the root
(3) Movement of metals from the root to the shoot
(4) Metals chelated in the cytoplasm
(5) Distribution of metal ions to other cell organelles
(6) Increased abundance of other types of proteins under metal toxicity

### (1) Signal transduction in response to metals

It is difficult to detect all the changes, even subtle, in signal transduction after metal exposure in the different plant tissues and organs. Eventually, the early response like gene expression and eventual changes in protein abundances, as well as oxidative stress and production of metabolites can be useful in thorough understanding of signalling pathway.[10,11] Plants have innate capability to respond to the metal stress by complex networks. In order to grow healthy in metal toxic environments, the plant initially synthesize some proteins and molecules/metabolites which then induce the expression of particular genes.[9] It has been reported that abundant proteins involved in energy, disease and defense-related functions are differentially regulated in response to cadmium stress in sunflower.[12] Further, in some cases, metal stress affects the proteins linked with nutrient metabolism with an increase in proteins associated with transcription and translational regulation, antioxidant pathways, molecular chaperones and biosynthetic metabolism.[13,14]

The different networks involved are: Ca-Calmodulin system, hormonal synthesis, ROS signalling and the mitogen-activated protein kinase (MAPK) which further stimulates the genes associated with metal toxicity. These networks are activated according to the specific type of metal toxicity.[15] Hereafter is a comprehensive summary of the pathways stimulated under abiotic stress.

a. The Ca-calmodulin system

Calcium ions are secreted under abiotic stress conditions like metal toxicity, high oxidative conditions, high or low temperature conditions and low oxygen.[16] Metal accumulation in plants leads to inward movement of $Ca^{2+}$ ions through calcium channels. Subsequently, the calcium ions which have been transported inside the plants bind with the calmodulin protein. This process generate signals which further control the activation of genes associated with detoxification of these metals.[17] Large amounts of calcium ions are detected in plants under cadmium toxicity which help in metabolism and tolerance under these stress conditions.[18] This pathway is also activated under other types of metal stress conditions like lead and nickel toxicities. For

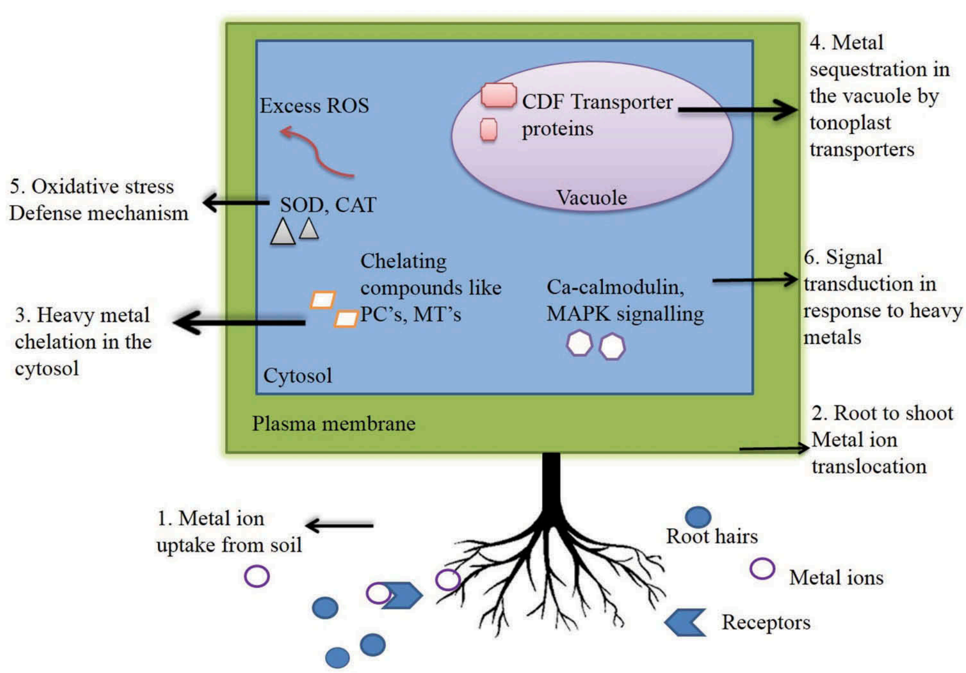


Figure 1. Different mechanisms involved in plants in response to heavy metal toxicity (modified from Maksymiec [9]).

instance, over expression of the *NtCBP4* gene in tobacco plant allows growth under high concentrations of nickel ions, whereas it prevents growth in lead-contaminated soil.[19]

b. Hormones in the Metal Response

These are chemical messengers released by plants for many growth and metabolic activities. They allow plants to efficiently withstand metal toxicity.[20] Various types of phytohormones are synthesized in response to different types of metals. Ethylene is released in plants grown in soil with high levels of zinc, copper, and iron.[9] Furthermore, copper and cadmium toxic levels lead to the production of ethylene by activation of the ACC synthase gene.[21] Some other hormones, like jasmonic acid, are synthesised, for example in *Phaseolus coccineus*,[22] under toxic levels of cadmium and copper ions.

c. Mitogen-Activated Protein Kinase (MAPK) Cascade

The proteins responsible for cascade reactions transducing the external signal to the cell's interior are stimulated under abiotic stress conditions. During metal stress conditions, these messengers like some of the protein kinases[23] enable production of chemical messengers. The kinase are MAPK kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK, which are activated by phosphorylation. *Arabidopsis* contains over 60 MAPKKK, 10 MAPKK, and 20 MAPK.[24] The rapid activation of multiple MAPKs, including MAPK3, 4, and 6, has long been observed in plants in response to biotic as well as abiotic stimuli and in response to growth and developmental signals.[24] As these kinases are phosphorylated, they further phosphorylate other proteins which in their turn get activated to perform different processes. MAPK cascades also exert positive feedback regulation on ROS production. A cascade OXI1-MPK6 activated by ROS also positively regulates ROS production.[25,26] Some proteins of MAPK are stimulated under Cu or Cd toxicity.[27] This signalling process stimulates other genes which are responsible for translation of metal transporters and removal of toxic metals by degrading them. However, studies in response to other metals like lead, zinc, iron are very few.[26]

d. ROS Signalling

The major drawback of high deposition of toxic metals is the generation of free radicals or reactive oxygen species (ROS), leading to a cascade of reactions favouring the ageing process in plants. Some signalling molecules are generated during this cascade process.[28,29] The deposition of free radicals in plants depends on the ratio between their rate of generation and removal. ROS generation also depends on other plant developmental conditions like pH, temperature, and properties of soil. Due to the presence of free radicals in plants, less carbon dioxide fixation takes place in chloroplasts and a dysfunction and over-reduction in the electron transport chain (ETC) in mitochondria[30] are triggered. Consequently, electron leakage takes place, which enhances ROS production by reducing molecular oxygen to superoxide and hydrogen peroxide. Hydrogen peroxide is also produced as a signalling molecule during oxidation in peroxisomes.[30]

ROS are synthesised by heavy metals directly by the Fenton and Haber–Weiss reactions, or indirectly by inhibiting the activity of antioxidants.[31] An excessive amount of peroxide is produced under heavy metal toxicity by copper and cadmium in *Arabidopsis*,[32] exposure to mercury in *Lycopersicum escluentum*[33] and high levels of manganese in *Hordeum vulgare*.[33] ROS are unstable species which undergo continuous reactions to form more free radicals.[34] Free radicals interact with other biomolecules like proteins and DNA, leading to negative changes collectively known as "oxidative stress".[35-37]

These reactive oxygen species also have positive effects in plants, as they regulate cell growth and development, closing and opening of stomata,[38] when efficiently controlled by antioxidants. As reported, free radicals produced by NADPH oxidases under stress enable production of signals for adaptation and protection against oxidation.[39-42] Hence, the outcome of free radical production depends on the end products of various reactions which include stimulation of the antioxidant system.[32] Plants respond to oxidative stress conditions by enhancing antioxidant enzymes like SOD, CAT, glutathione peroxidase (GPX) and glutathione *S*-transferase (GST), as well as molecules like proline and glutathione.[35-37,43] Glutathione acts as cofactor for antioxidant enzymes which help in the removal of free radicals and allow plants to grow under adverse conditions.[36,37] These antioxidants maintain the ratio of ROS in plants and help to escape oxidative stress. Hence, they contribute to the regulation of genes associated with the response to heavy metal stress.[44] In *Arabidopsis*, Mittler et al[30] have discussed that ROS like $H_2O_2$ and $O_{2-}$ act as signalling molecule requiring a wide gene network consisting of around 152 genes. Further, there are certain essential components involved in ROS signalling in *Arabidopsis* such as receptor proteins, redox-sensitive transcription factors, as well as ROS-induced inhibition of protein phosphatases. A chloroplast nucleus retrograde signalling is activated under ROS generation. A well studied pathway is for example Mg-Protoporphyrin IX (Mg-PPIX).[45] This Mg PPIX pathway in *Arabidopsis* has revealed that around 35% of the identified proteins including glutathione *S*-transferases (AtGST10, AtGSTT1 and AtGSTF3) and peroxidases (ATP15, APX1, PER22 and ATP3) are involved in a variety of stress responses.[46] Moreover, a number of studies proposed a close relationship between ROS and G proteins in plant signalling under abiotic stress conditions.[47] However, in depth studies are still required to unravel the role of G proteins and their signalling in stressed plants.

**(2) Entry of metal ions from the soil into the root**

Root exudation and the presence of some microbes in the vicinity of the rhizosphere allow movement of metal ions from soil.[48] Many mechanisms are involved in the transfer of these toxic metals across the cell membrane in roots. Different transporters which are substrate specific are used to transport toxic metals.

a. Movement of ions from soil to root secretions and roots' outer coating

Root secretions are released in the soil and act as protective agents against toxic metals.[49] These secretions have the property to chelate metal ions and stop their entry inside the root hairs. For example, the entry of nickel ions into plants from soil is prevented, roots release some nickel-chelating histidine and citrate which do not allow its movement.[50] Some heavy metals, like zinc and copper, are bound to the cell wall, which actively partakes in the regulation of ion movement inside the plants.[51] The cell wall of plants (and more precisely, cell wall polysaccharides, like pectins) is not only a site for the adsorption of heavy metals (like Cd)[52] but also a structure responding to heavy metal stress. Cell wall receptors (like the wall-associated kinases, WAKs) sense the status of the cell (as for example perturbations due to exogenous stresses) and can transduce the signal to the cell's interior via a cytoplasmic kinase domain.[53] These receptors create a continuum between cell wall and membrane and respond to changes in turgor pressure, a parameter that is affected upon metal stress. The presence of sites of attachment of ions on the root surface allows interchange between ions which helps in efflux and movement into the apoplast.[54] The presence of pectic and histidyl groups of the cell wall and sugar molecules, like mucilage, as well as amorphous linear polysaccharides, like callose, regulate the movement of toxic ions and restrict entry of toxic ions into the plant cell. Various genotypes of tobacco having difference in the chemical composition of root cell walls can restrict entry of toxic manganese ions into the plants.[55] Hence, the intrinsic properties of the cell wall control the entry of metal ions into plants. But cell walls have limited number of heavy metal binding sites.[56] For example, *Silene vulgaris* has the property of absorbing a large amount of metals attributed to the presence of proteins or bound as silicates in the epidermis.[57]

b. Movement of ions across cell membrane

Plants have different types of membrane transporter proteins which help in metal absorption of toxic ions and metabolic processes. These proteins are responsible for transport of metal ions and stabilize the concentration of toxic metals under abiotic stress. These transporter proteins include the heavy metal ATPase transporter protein, the NRAMP, the CDF (cation diffusion facilitator),[58] and the ZIP families.[59] Some of the specific functions and properties of these transporter proteins are still under investigation. These transporters have been studied in *Saccharomyces cerevisiae* mutants[58,59] for metal ion transport (Figure 2).

(i) The ZIP Family

These transporter proteins perform a chief role in metal ion movement in plants. They are present in most plants, microbes and animals and are responsible for the transport of ions across the plasma membrane. Some of the ZIP transporter proteins are involved in the transport of iron and zinc in roots and shoots of *A. thaliana* under metal toxicity. The function of *A. thaliana* IRT (iron regulated transporter) was validated by *S. cerevisiae* fet3fet4 double mutant showing poor $Fe^{2+}$ ions transport.[60] In *Arabidopsis*, the protein shows significant expression under iron deficiency conditions and allows the entry of $Fe^{2+}$ from soil.[61] In *A. thaliana, IRT1* is expressed in root cells and accumulates in response to iron deficiency, suggesting a role in $Fe^{2+}$ uptake from the soil.[61] Although expressed during iron deficiency, it was reported in yeast that AtIRT1 also helps in zinc, cadmium and manganese ion translocation.[62] It additionally plays a role in the response to heavy metals, like cadmium and zinc[63] and is responsible for nickel uptake and transfer from the soil.[64] *S. cerevisiae* zinc transporters ZRT1 and ZRT2 show sequence similarity to IRT1 and could efficiently transport zinc.[65,66] Double mutant of *zrt1* and *zrt2* in yeast helped in the functional analysis of *AtZIP1, AtZIP2* and *AtZIP3* transporters.[67] Furthermore, these proteins are produced under zinc ion deficiency conditions and help in its absorption.

(ii) The NRAMP Family

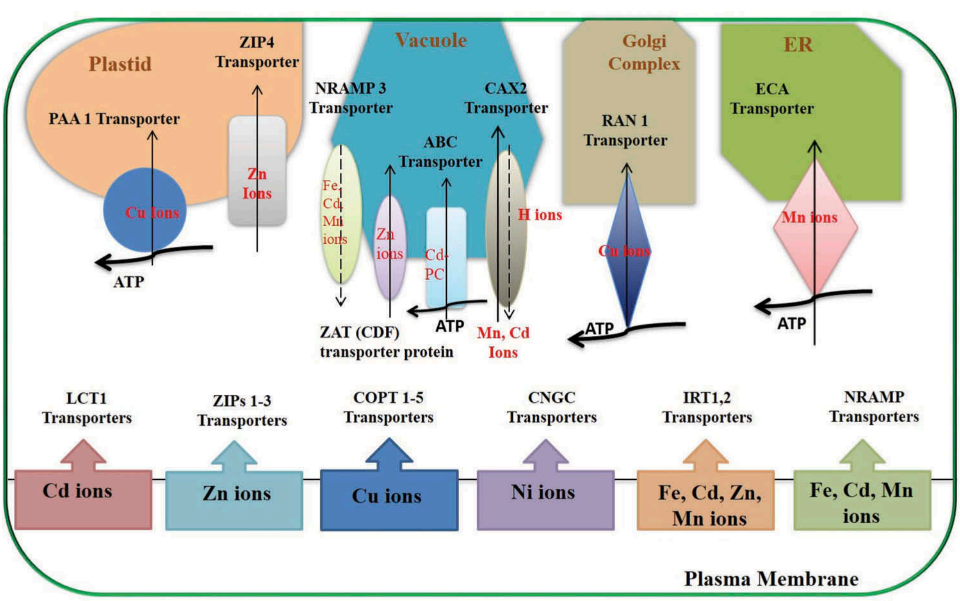


Figure 2. Different transporter proteins involved in heavy metal ion translocation in plants (modified from Williams et al. [58]; Guerinot [59]).

These transporters are used to transfer different ions, like iron, nickel, manganese, zinc, copper in different kingdoms.[68] The plant proteins help in the transfer of toxic metal ions from the roots and shoots across cell membrane and vacuolar membrane.[69] In the case of *Arabidopsis*, it helps in the transfer of iron and cadmium, where NRAMP1 protein maintains its metabolic level.[70] These transporter proteins are highly unregulated during iron deficiency in plants and hence help in Fe uptake. Molecular studies revealed that IRT1 helps in the initial movement of iron from the soil to the roots (in non-graminaceous monocots and in dicots)[61] and that the other YS1 transporter allows initial movement of siderophore–Fe complexes in graminaceous monocots.[71] The similarity in NRAMP genes in the graminaceous and dicots species points to the same function of NRAMP proteins in maintaining iron metabolism in plants.[72-74]

(iii) The copper transporters family (CTR)

These transporter proteins were first identified in mammalian and yeast cells and then in plants.[75] They are associated with the transport of $Cu^{2+}$ from the soil. The CTR proteins are made up of metal binding sites and trans membrane domains. As reported in *Arabidopsi*s, COTP1 is responsible for $Cu^{2+}$ uptake and is required for development. *A. thaliana* copper transporter COPT1 was identified by functional complementation of the *S. cerevisiae* mutant *ctr1-3*, which is defective in copper uptake.[75] In *A. thaliana*, COPT1 has been shown to transport copper, and it also has a role in growth and pollen development.[76]

c. Slow movement and removal of metal ions from the cell

The cell membrane in plants controls the rate of metal intake and with the help of specific proteins, it enhances the removal of toxic ions by eliminating them from the cell.[73,77,78] The As-resistant plant *Holcus lanatus* does not absorb much As with respect to non-resistant types and this is due to the presence of phytochelatins which form complexes with As.[72] The enhanced removal of toxic ions prevents the entry and translocation into the different plant organs and tissues, as already reported in bacteria [79] and animal cells.[80]

**(3) Movement of metals from the root to the shoot**

Metals are transported from the roots to the aerial part via conductive tissues in the form of metal conjugates. For the removal of iron and nickel, major organic acids form the complexes.[81] Some of the derived substances from methionine are used for transportation of copper ions.[82] Some chelating agents like histidine are involved in the movement of nickel ions. Transporter proteins help in the translocation of toxic metals from the root to the stem.

a. The HMA Family of Transporters

These are ATPases grouped according to their metal-substrate selectivity for copper/silver and zinc/cadmium/lead/cobalt groups.[83] *Arabidopsis* HMA4 (belonging to the Zn/Cd//Pb/Co group) is involved in zinc loading in the xylem, as well as cadmium translocation, together with AtHMA2.[83] AtHMA5 is required in copper removal and transportation from the roots to the shoot.[84]

b. The MATE Family of Efflux Proteins

MATE Family of efflux proteins are required in the elimination of various drugs and harmful substances from plants. For example, FRD3 is a protein involved in the removal of iron via the formation of Fe – citrate complexes in xylem in roots. The iron complexes formed help in the translocation of iron to the leaves through the vessels.[85]

c. The Oligopeptide Transporters Family (OPT)

These are transporters which include YSL protein for iron transport in plants in the form of conjugates.[71] *Zea mays* Yellow stripe 1 protein (*ZmYS1*) is involved in the transport of chelated forms of iron, copper, nickel by methionine derivatives.[86] The OPT proteins include a particular type of YSL subfamily which is present in plants. The protein AtYSL2 present in cell membrane is required for translocation of toxic ions along stems via the xylem and phloem.[87,88] The synthesis of this protein is altered by the presence of copper and iron.[87]

**(4) Metals chelated in the cytoplasm**

Some of the chemicals are not dissolved immediately; therefore, their concentration increases in the plant cell. Plants produce phytochelatins and metallothioneins which bind these metals and sequester them to stable forms.

a. Phytochelatins

Phytochelatins (PCs) are widely studied toxic metal chelating proteins which bind and compartmentalize heavy metals.[89] The metal binds to form a structure as (c-Glu-Cys)nGly (n = 2–11) (Figure 3).[89] Phytochelatins are synthesized in the cytoplasm and translocated as conjugates to the particular cell compartment. Their production is enhanced under heavy metal toxicity of silver, gold, zinc, lead and copper ions.[89,90] PCs are activated in brown mustard during cadmium contamination and enable photosynthesis to take place; however, the leaf size and transpiration rate are decreased.[91] It is seen that cadmium ion tolerance in different mutants of *Arabidopsis* is associated with the capability to activate phytochelatins.[92] Heavy metal contamination with copper and cadmium can be correlated with the production of glutamate synthase which further stimulates phytochelatins.[93] *Arabidopsis* cadmium-sensitive mutants (where *CAD1* is responsible for phytochelatin synthesis) produced glutathione but are incapable of synthesizing phytochelatins and consequently were sensitive to cadmium ions.[92]

b. Metallothioneins and Ferritins

Metallothioneins (MTs) are low molecular weight proteins containing large amounts of cysteine and bind to metals.

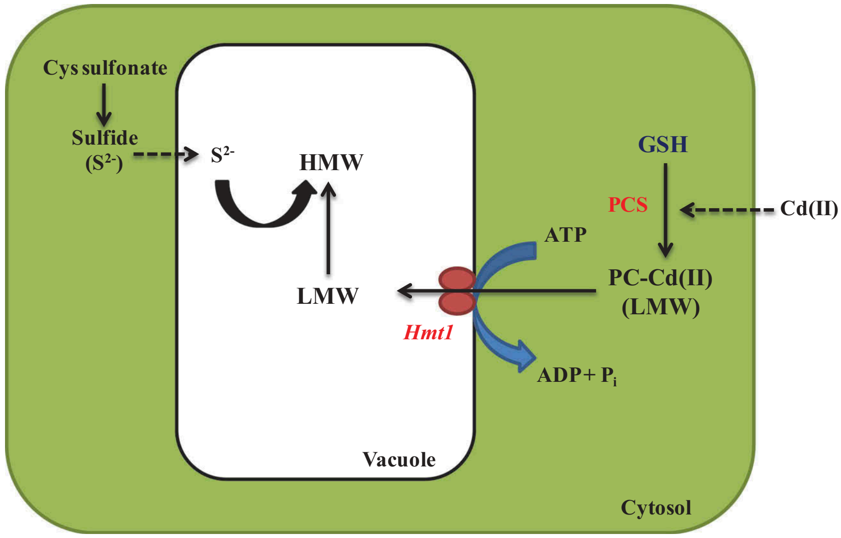


Figure 3. Phytochelatins activate an important mechanism responsible for $Cd^{2+}$ tolerance (modified from Clemens [134]). HMW-High molecular weight, LMW- Low molecular weight, PCS- Phytochelatin synthatase, PC-Phytochelatins.

Different classes of MTs are present: class 1 MTs shows similarity with horse liver MT, class 2 has no clear similarity to horse liver MT[94] and class III comprises phytochelatins and Cys-rich peptides of enzymatic origin. In plants, metallothioneins are activated under different abiotic stress conditions and also during growth.[95] Ferritins are widespread proteins having the ability to store huge amounts of $Fe^{2+}$ and $Fe^{3+}$and releasing them in a controlled manner.[96] Plant ferritins have the property to store only iron but, in animals, these proteins can store other metals like cadmium, zinc, aluminium and copper atoms.[97] These plant proteins are produced under various abiotic stresses like metal toxicity by Fe.[98,99] This gene in plants is controlled by abscisic acid and antioxidants.[100] Hence, the protein acts as a protective element for plants by preventing continuous reactions of free radicals stimulated by Fe ions.[101]

### (5) Distribution of metal ions to other cell organelles

After high accumulation of metal ions in the cytoplasm, they are further transported to other cell organelles in order to remove them from plant cells. During the process, these plants use protein as transporters to transfer the metal ions (Figure 2). The plant proteins are:-

a. ATPase transporters:

The CPx-type ATPases help in the movement of toxic metals like copper, lead, cadmium across the plasma membrane by using energy in the form of ATPP1B-type. ATPases also perform the same function of transporting toxic metals from the roots to the organelles. They control plant metabolism to eliminate toxic metal ions.[102] They have been classified into two subtypes: multidrug resistance proteins (MDRs) and multidrug resistance associated proteins (MRPs). Furthermore, the first one to be cloned was the MDR-like gene in *Arabidopsis*, but there were few numbers of MRPs which were functionally characterized.[103,104]

b. NRAMP (Natural Resistance-Associated Macrophage Proteins)

NRAMP are transporters involved in the transport of toxic metals. Data on three rice NRAMPs, known as OsNramp1, OsNramp2, and OsNramp3 reported their presence in various parts and a major role in transporting metal ions.[73,105] The role of NRAMP is mainly related with transport of iron and to a lesser extent, manganese.[106] In thale cress, AtNRAMP1, 3, 4, and 6 encode functional plant heavy metal transporters.[107] Further, over expression of AtNRAMP1 in *Arabidopsis* increases plant resistance to toxic Fe concentrations.[74]

c. Metal tolerance proteins (MTPs)

Cation diffusion facilitator (CDF) is responsible for the transfer of zinc, nickel, cadmium and cobalt and sequester these ions away from the cytoplasm into the vacuole. Hence, they are also known as "cation efflux transporter"[72] and metal tolerance proteins (MTPs) which promote metal ions movement from the cytoplasm to the extracellular space or into organelles.[108-110] Around ten MTP genes have been found in *O. sativa*[111] and 12 in *A. thaliana*. The first CDF gene identified in *A. thaliana* was originally named Zinc Transporter 1 gene (ZAT1) and later called METAL TOLERANCE PROTEIN 1 (AtMTP1).[112,113] The corresponding gene is expressed in roots as well as shoots and, when over expressed in *Arabidopsis*, it helps in zinc tolerance.[113]

d. ZIP transporters

ZIP transporters are referred to as ZRT, IRT-like proteins used in translocation of different ions like cadmium, zinc, iron, manganese depending on the type of substrate and their particular identity.[59,72] They are involved in the transport of cadmium from the soil to roots and its further translocation from the root to the shoot.[69] Some of the ZIP family transporters described in *Arabidopsis* are, IRT1, IRT2, and

IRT3 ZIP 1–12.[114] Further, detailed description of these transporters AtIRT1, AtIRT2, and AtIRT3 have been reported. Among these three transporters, AtIRT1 is mostly involved in the movement of zinc and iron in plants.[60,115,116] Other transporters of the ZIP family are required for metal transportation in and out of specific organelles.[114]

e. MATE Transporters

Another class of protein family includes MATE transporters which also help in the movement of toxic metals. Studies revealed that FDR3 (Ferric reductase defective 3) was abundant in the roots of the heavy metal hyper accumulators *Thlaspi caerulescens* and *Arabidopsis hallerit*.[107,115,116] Hence, metal transporters help in the transport of the metals in plants and in the removal of these toxic substances from the plants.

**(6) Other types of proteins stimulated under metal toxicity**

a. Glutathione

Reduced glutathione (GSH) is a tripeptide and has a sulfhydryl group (SH) which is required for scavenging metal from plants.[117] The protein known as glutathione reductase (GR) maintains the redox potential of cells by changing the oxidised glutathione (GSSG) form to the reduced one (GSH) (Figure 4). A substantial increase in GR content was observed in *Luffa* seedlings due to arsenic toxicity.[118] Conversion of oxidised to reduced form is dependent on NADPH.[119] Glutathione helps in various plant cellular mechanisms like heavy metal detoxification,[120] and scavenging radicals, thereby protecting the plants under stress conditions.[121,122] It also plays a significant role in growth and development of plants, including flowering[123] and cell division.[124] The two processes involved in the formation of GSH require ATP during a step where γ-glutamylcysteine synthetase (GSH1) acts as catalysts in the chemical reaction involving peptide bond formation between -COOH groups of glutamate with -$NH_2$ group of cysteine giving γ-glutamylcysteine (γ-EC) as a product. This step holds a central role during high production of glutathione.[125] Furthermore, in the second process, glutathione synthetase (GSH2) joins a glycine amino acid with γ-EC to give GSH as end product.

Under the condition when a heavy metal such as cadmium enters the cytoplasm, glutathione (GSH) forms a complex with the metal ion (Cd-$GS_2$) (Figure 3). This complex has stronger affinity to PC synthase than free heavy metal ions and it functions as enzyme activator by causing the folding of the protein. Then, the heavy metal may bind with PC synthase and facilitate a conformational change for its activation as discussed in section 4.1. It was first identified by Grill et al.[126] that glutathione is involved as a substrate for the synthesis of PC when PC synthase is activated by heavy metals such as Cd.[126]

b. Chaperones

The accumulation of metals in crops is a serious concern, as they can be consumed by humans and act as carcinogens[127] or favour neurological disorders.[128] In plants, metals like mercury, lead and cadmium may interact with proteins[129,130] and DNA thereby damaging nucleic acids as well as inhibiting protein folding. Hence, these proteins become non-functional under stress conditions. To overcome these stress conditions, plants activate chaperones. Chaperones repair and protect the proteins from unfolding under metal toxicity.[131-133] Most of the chaperones are heat shock proteins.

## Concluding remarks and future prospects

Among the different pollutants in the environment, a major role is played by metals (heavy metals, light metals and metalloids). These metal ions are discharged through chemical and leather industries, pesticides, etc. In due course of

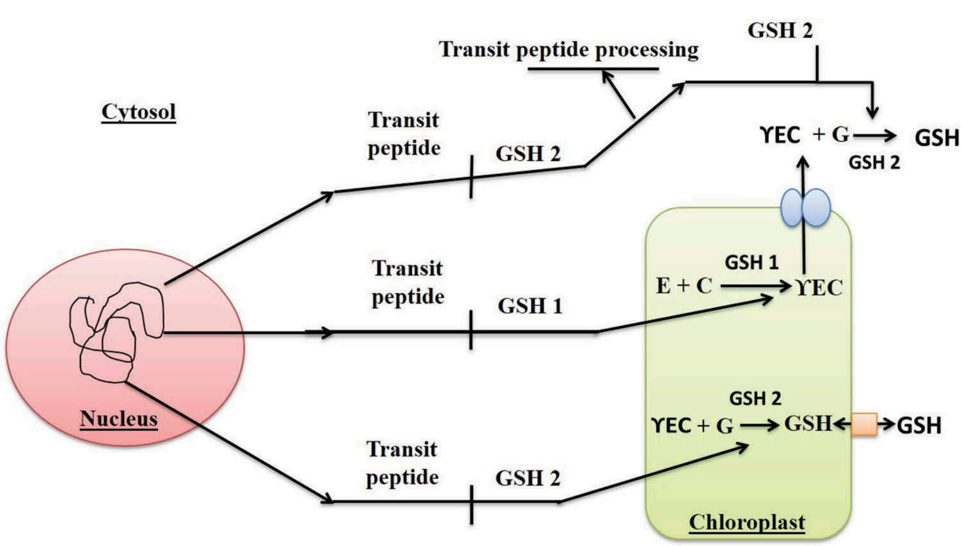


**Figure 4.** Biosynthesis of Glutathione (GSH) in chloroplast and cytosol in plant cellγ-glutamylcysteine (γ-EC), γ-glutamylcysteinesynthetase (GSH1) glutathione synthetase (GSH2) E – carboxylic part of glutamate and C – amino group of cysteine, G – glycine amino acid. (modified from Xiang and Oliver [93]).

time, the high accumulation of toxic metal ions in plants retards the growth and development and ultimately leads to death of the plant tissues. Eventually, these plants are consumed by humans, thereby causing major health concerns. Plants have innate mechanisms to tolerate toxicity by metal ions, notably via the up-regulation of some genes which provide resistance and acclimatize plants under abiotic stress conditions, via the release of inhibitors, the increase of antioxidants to scavenge free radicals, etc. Proteins involved in plant metal stress response act as initiators or precursors and continue the signalling pathway; for example, calmodulin binding to the calcium ions released under abiotic stress, catalase and superoxide dismutase act as antioxidants by lowering the levels of free radicals or ROS released under stress conditions. As described above, some of the proteins act as transporters, like ABC and NRAMP transporters, which help in the translocation of toxic metal ions like cadmium, copper, zinc and release them out from the plants. Proteins also have chelating properties, like PCs and MTs, which chelate the toxic ions by breaking down complex compounds. Some of the other types of proteins are glutathione, which maintains the redox potential of plant cell during metal toxicity. Due to the presence of toxic metal ions like Ni, Cd in plants, the proteins unfold and become non-functional. In such cases, plant chaperones facilitate the correct folding of proteins. Hence, these plant proteins hold a central role in providing protection during abiotic stress by metals.

By analysing those plant proteins involved in the tolerance under metal stress, we can devise biotechnological strategies improving the tolerance of plants to metals. Biochemical and -omics analyses can provide in-depth study which will be necessary to exploit the great potential of the stress-responsive proteins. Finally, highly tolerant plant species can be studied and some of their gene transferred to other plants, like crops, to improve their resistance against metals.

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## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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