We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Chapter

Molecular Defense Mechanisms in Plants to Tolerate Toxic Action of Heavy Metal Environmental Pollution

Istvan Jablonkai

Abstract

Toxic action of heavy metals on plants growing in contaminated soils intensified the research on detoxification and sequestering mechanisms existing in plants to understand and manipulate defense mechanisms that confer tolerance against metal ions. Increased biosynthesis of plant biomolecules to confer tolerance during toxic action of heavy metals is an intrinsic ability of plants. Induced formation of low-molecular weight amino acids, peptides or proteins as chelators such as proline (Pro), glutathione (GSH), phytochelatins (PCs) or metallothioneins (MTs) under heavy metal stress enhances metal binding and detoxification capability of plants. In addition, proline and GSH related enzymes such as GSH reductase, GSH peroxidases and glutathione S-transferases are also key components of the antioxidant defense system in the cells to scavenge reactive oxygen species (ROS). Protective action of oxidized fatty acids oxylipins at toxic levels of heavy metals is considered to activate detoxification processes as signaling molecules.

Keywords: heavy metals, stress, detoxification, glutathione, chelators

1. Introduction

Abiotic stress factors such as extreme temperatures, drought, salinity, heavy metals, xenobiotics have been considered as potential threats for agricultural productivity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses. Stress-tolerance of plants involves the activation of cascades of molecular networks leading to expression of stress-related enzymes.

The accumulation of heavy metals in the plant is accompanied by damage to the structural components and an imbalance of various metabolic processes in the cells, which leads to disruption of plant growth and development. Plants have evolved a number of mechanisms to adapt to increasing concentrations of metal ions. These include the immobilization, exclusion, chelation, and compartmentalisation of metal ions.

The toxic action of heavy metals can produce excessive reactive oxygen species (ROS). Glutathione and GSH related enzymes such as GSH reductase, GSH peroxidases and glutathione S-transferases are fundamental parts of the antioxidant
defense system in the cells to scavenge ROS and electrophilic organic molecules as well. The significance of plant thiols and glutathione S-transferases involved in plant response to almost all stress factors will be discussed with special attention to their overexpression, redox status and regulation that confer stress tolerance.

A number of metal-binding ligands have now been recognized in plants [1]. Polypeptide ligands include the metallothioneins (MTs), small, gene-encoded, cysteine-rich polypeptides, and the phytochelatins (PCs), which, in contrast, are enzymatically synthesized are effective metal binding ligands have been identified. Recent advances in understanding the role, biosynthesis and protective action of phytochelatins and metallothioneins as metal-binding ligands in heavy metal detoxification are reviewed.

Proteinogenic amino acid proline protects cell from environmental stress factors by several protective mechanisms such as osmoprotectant, acting as chemical chaperone by stabilization of proteins and antioxidant enzymes, chelation of metals, scavenging ROS, balancing the intracellular redox homeostasis (NADP+/NADPH ratio, GSH pool) and participating in cellular metabolic signaling. Proline accumulation has been observed in response to environmental stress in a variety of living organism including plants.

Reactive electrophile species oxylipins exhibit protective action under toxic concentration of pollutants by activation of detoxification processes. The less studied oxylipin signaling in plant stress response will be detailed as an important factor in plant adaptation to stress by heavy metal pollutants.

2. Role of glutathione (GSH) and glutathione related enzymes in protection of plants against toxic action of heavy metals

The accumulation of heavy metals in plant organs results in the damage of the structural components and disruption of cell metabolic processes leading to plant growth retardation. The toxic action of heavy metals can produce excessive reactive oxygen species (ROS). Glutathione and GSH related enzymes such as GSH reductase, GSH peroxidases and glutathione S-transferases are fundamental parts of the antioxidant defense system in the cells to scavenge ROS and electrophilic organic molecules as well (Figure 1).

2.1 Glutathione (GSH) and GSH/GSSG redox system

The most abundant biological thiol the tripeptide glutathione (GSH, γ-Glu-Cys-Gly) is a low molecular weight, water soluble compound that is ubiquitous in most plant tissues and has a key function in stress management. Besides the ROS detoxification, GSH also participates in detoxification of methylglyoxal and electrophilic xenobiotics [2]. As a component of the glutathione-ascorbate (AsA-GSH) cycle GSH has a key role in H₂O₂ detoxication. GSH is used as co-factor by a) various peroxidases in detoxication of peroxides formed in the reaction of oxygen radical with biological molecules and b) by glutathione S-transferases (GST) to conjugate GSH with endogenous substances and xenobiotics. Interaction of GSH with thioredoxin systems fine-tune photosynthetic and respiratory metabolism by modifying the sensitive protein Cys residues [3]. GSH is a substrate of phytochelatin synthase and oligomeric GSH products phytochelatins (PCs) can effectively sequester heavy metals by complexation alleviating metal stress of plants [4]. Interaction of GSH with known signaling molecules such as salicilic acid, jasmonic acid and ethylene can be important in treatment of plant biotic stress [5].
GSH biosynthesis is two-step pathway mediated by γ-glutamylcysteine synthase (γ-ECS) and glutathione synthase (GSHS) enzymes using 2 molecules of ATP. The first step occurs mainly in the chloroplast while the second step predominantly takes place in the cytosol [3, 6]. GSH produced in the cytosol can be transported directly to other cellular organelles by glutathione transporters [7].

The GSH redox state in plants, particularly in leaves is normally very stable but is extremely sensitive to oxidative stress. In the absence of stress, in leaf tissues measurable GSH: GSSG ratios typically around 20:1 [8]. Conversion of the reduced GSH into oxidized glutathione or glutathione disulfide (GSSG) can occur under stress conditions. Non-enzymatic reactions of GSH with ROS species such as \( \cdot O_2 \), \( O^\cdot \), \( OH \), \( H_2O_2 \) and \( O_2^\cdot \) convert the reduced GSH to the oxidized form (GSSG, glutathione disulfide) [9, 10]. Glutathione reductase (GR) and glutathione peroxidase (GPX) enzymes in conjunction with AsA-GSH cycle are responsible for the balanced state of GSH/GSSG and GSH homeostasis [11].

Plethora of information on alteration of plant GSH pool and GSH/GSSG redox system as a results of heavy metal stress are available. Elevated levels of GSH have been observed in various plant species with increasing Cd concentration. But depletion of GSH in roots of variety of species under cadmium and lead stress has also been reported [12]. In two genotypes of *Brassica juncea* treated with Cd increased levels of GSH and GSSG were detected. Higher increase in GSH content was found in Cd-tolerant genotype while in Cd-sensitive genotype the enhancement of level of the oxidized form, GSSH was more pronounced [13]. Remarkable decreases in \( O_2^\cdot \), \( H_2O_2 \), and MDA (malondialdehyde) accumulation were detected with exogenously applied GSH in rice seedlings treated with Cd as a result of modulation of the antioxidant system [14]. The oxidative stress induced by Cd in coontail, a free-floating freshwater macrophyte (*Ceratophyllum demersum*) was alleviated by exogenous Zn application. The application of Zn restored thiols and also inhibited oxidation of AsA and GSH, and sustained the redox state balance. Application of zinc enhanced the activities of AsA-GSH enzymes (APX, MDHAR, DHAR, and GR), GST, and GPX, conferring tolerance to Cd stress [15].
Toxic action of heavy metals seemed to induce the expression of genes encoding γ-glutamylcysteine (γ-ECS) and glutathione synthase (GSHS) enzymes by enhancing GSH levels. Moreover, GSH can efficiently influence coordination of metals to the active sites of affected enzymes preserving their activity [16].

2.2 Glutathione reductase

Glutathione reductase (GR) is a flavo-protein oxidoreductase mediates the reduction of GSSG to GSH using NADPH as an electron donor. GR is predominantly located in chloroplasts but some isoforms were also detected in mitochondria and cytosol [17]. GR activity secure the redox potential of cells at highly reduced GSH/GSSG and AsA/DHA ratios under regular and oxidative stress conditions. Biotic and abiotic stress factors including toxic metals were found to influence GR activities in plants [17, 18]. Under Cd stress GR activities were increased in sugarcane callus cultures in time- and concentration-dependent manner [19]. Elevated GR activities were detected in response to Cd-treatment in a variety of plant species such as *Solanum tuberosum*, *Raphanus sativus*, *Glycine max*, *Saccharum officinarum*, *Capsicum annuum*, *Arabidopsis thaliana*, *B. juncea*, *Brassica campestris*, *Vigna mung*, and *Pisum sativum* [12]. On the contrary significant reduction of GR activity was shown in *Ceratophylum demersum* treated with Cd [20]. A seedling age specific changes in GR activities were observed in *Oryza sativa* genotypes under Pb stress. After initial deacrease in GR activities in both roots and shoots up to 10 days a remarkable increase of enzyme activities was found after 15 days of treatment [21].

2.3 Glutathione peroxidases

Glutathione peroxidases (GPXs) are a large family of broad substrate spectrum multiple isozymes. Contrary to most of their counterparts in animal cells, plant GPXs contain cysteine instead of selenocysteine in their active site. Antioxidant GPX protecting cells from oxidative damage by reducing H$_2$O$_2$, organic and lipid hydroperoxides in association with the GSH pool [22]. Presence of GPXs were detected in cytosol, chloroplasts, mitochondria, peroxisome and apoplast.

Stress responses of GPXs are contradictory. Cd stress increased GPX activity in cultivars of *C. annuum* plants [23] but reduced activities were found in roots of Cd-exposed *P. sativum* plants [24] while no change was observed in GPX activities in maize roots exposed to Ni [25]. The activity of GPX activity was significantly enhanced in *Lolium perenne* shoots exposed to Se but chronic metal exposure decreased enzyme activity [26]. Externally supplied GSH to Cd treated barley genotypes was shown to counteract Cd-induced elevation of root GPX activity by reducing GPX activity to the control level [14].

2.4 Detoxification action of glutathione S-transferases

Glutathione S-transferases (glutathione sulfo-transferases, GSTs) are major phase II, GSH-dependent detoxication enzyme superfamily. GSTs catalyze the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds to form water soluble, non-toxic GSH conjugates [27, 28]. GSTs are divided into two distinct super-family members: the membrane-bound microsomal and cytosolic family members. Microsomal GSTs are structurally distinct from the cytosolic in that they homo- and heterotrimerize rather than dimerize to form a single active site [29]. In cytoplasm GSTs account for roughly 1% of soluble proteins
in maize leaves [30]. Various plant GST isozymes were shown to possess with GSTs/GPX activity mediating lipid hydroperoxide metabolism to non-toxic alcohols [31]. Elevated GST activities were found in leaves and roots of Cd exposed pea plants [24] and in roots of Phragmites australis plants by Iannelli et al. [32]. Cadmium- and mercury-induced root growth inhibition is strongly correlated with increased GST and GPX activity in barley [33] while in maize seedlings, Cd treatment strongly induced (20-50-fold increase) GST Bronze2 and GST III [34]. A detailed summary of literature studies on GST induction by heavy metals such as Cd, Pb, Cu, As(III) and As(V) has been published [12].

3. Phytochelatins (PCs) as heavy metal chelators

One of the detoxication mechanisms in plants to overcome heavy metal stress is the production of thiol-containing oligomer peptides from a precursor glutathione (GSH) by phytochelatin synthase (PCS, γ-glutamylcysteine dipeptidyltranspeptidase) (Figure 2).

PC synthase-deficient mutants of Arabidopsis and S. pombe exhibited high sensitivity towards Cd and arsenite providing strong evidence for the role of PCs in heavy metal detoxification [35]. Phytochelatin level of plant can serve as biomarkers for the initial detection of heavy metal stress. Since the immobilized metals are less toxic than the free ions, PCs are considered as part of the detoxication mechanism of higher plants, [36, 37]. Phytochelatins, with \((γ\text{-Glu-Cys})_n\text{-Gly}\) general formula (where \(n = 2-11\)), can act as chelators to bind heavy metals in the cytosol and the metal-phytochelatin complexes are compartmentalized to vacuoles. Varieties in structures of PCs include the replacement of glycine residue (Gly) with β-alanine (β-Ala), Ala, glutamine (Gln), serine (Ser) or glutamate (Glu) [38].

As a result of Cys residues PCs have high thiol (SH) contents and ability to strongly bind heavy metals exhibiting increased metal-binding capacity with increasing size [39]. PCs chain lengths varies with plant species and metal forms.

![Figure 2. General structure of (a) and biosynthesis (b) phytochelatins.](image-url)
The relative affinity of metals such as Cd, Pb, Hg, Cu to GSH and PCₙ oligomers increases with chain length (GSH < PC₁ < PC₃ < PC₄) [40]. After the formation, the high molecular weight metal-phytochelatin complexes are sequestered in the vacuoles by the involvement of ABC-type (ATP-binding cassette) transporters [41]. PC synthase was shown to be activated by heavy metal ions such as Cd²⁺, Cu²⁺, Ag⁺, Hg²⁺, Pb²⁺, Ni²⁺. Cd tolerance of Arabidopsis and tobacco was found to be mainly related to PC content. Contents of PCs and GSH in Arabidopsis were 3.5 and 3 times higher than in tobacco plants and the concentration of various PCs oligomers in the two species was different: PC₃ and PC₄ oligomers were prevalent in wild-type tobacco as compared to high concentration of PC₂ and PC₃ in Arabidopsis [42].

Cd²⁺ ions were found as the most effective stimulator of PCs biosynthesis and 4-6-fold higher induction of PCs were detected than with Cu²⁺ and Zn²⁺ compounds in cell cultures of Indian snakeroot (Rauwolfia serpentina) [43] and red spruce (Picea rubens Sarg) [44], respectively.

Biosynthesis of PCs takes place in the cytosol of root cells. PCs are produced from glutathione, homoglutathione, hydroxymethyl-gluthathione or glutamylcysteinyl-glutamate by a transpeptidase, the constitutive PC synthase enzyme [45]. In Solanum nigrum L., copper treatment enhanced the biosynthesis of PCs was shown followed by the immobilization of toxic Cu in the roots by inhibiting the translocation into the shoots [46]. Reduced transport of As from roots to shoots was found in rice cultivars subjected to the elevated levels of arsenic due to the stimulation of formation of phytochelatin-arsenic complexes [47]. Arsenite and arsenate anions are readily absorbed by plants and both anions were found to induce effectively the biosynthesis of PCs in vivo and in vitro [48]. A higher degree of production, accumulation, and transportation of PCs has a definite role in tolerance of plants to heavy metal stress. However, biosynthesis of PCs does not necessarily take place in roots. In hyperaccumulator Sedum alfredii plants PC synthesis and Cd accumulation were most abundant in the leaves followed by the stems but hardly detected in the roots [49]. In a Cd hyperaccumulator wheat plant, Cd accumulation was not only the sequestration of Cd-phytochelatin complexes in the roots but their translocation to shoots also takes place. The translocation of Cd from root to shoot is through the xylem appears to be the main process for shoot accumulation. At a relatively high Cd treatment (20 μM) phytochelatin biosynthesis was enhanced more evidently in shoots [50]. The phytochelatin independent mechanism of tolerance of higher plants to Cd toxicity also exists and can be attributed to the highly developed apoplastic transport systems [51].

PCs enzyme activities were not only also induced Cd stress. The principal mechanism of intracellular metal detoxification by complexing and transporting metals into the vacuole was also established for stress by various heavy metals such as mercury, copper, arsenic, silver, nickel, gold, and zinc [52–54].

Stoichiometry of complexes of Pb²⁺ formed with various PCₙ (n = 2–4) were examined in details. Mass spectrometry analysis of Pb–PC₂ revealed four different complexes corresponding to [Pb–PC₂], [Pb₂–PC₂], [Pb–(PC₂)₂], and [Pb₂–(PC₂)₂]. The coordination of Pb²⁺ with PC₂ was postulated via the thiol groups of cysteine residue of PC₂ and possibly by carboxylic groups. In case of PC₃ and PC₄, two complexes were detected for each metal such as Pb–PC₃, Pb₂–PC₃, Pb–PC₄, and Pb₂–PC₄ [55]. The metal-PCₙ complexes formed with Cd²⁺ differed from those of Pb²⁺. Higher metal/peptide molar ratios were estimated in Cd-PCₙ complexes than in Pb-PCₙ complexes (n = 3, 4), suggesting that phytochelatins of marine algae Phaeodactylum tricornutum are capable to sequester and detoxify more Cd²⁺ than Pb²⁺ ions forming complexes with a different structure and stoichiometry. As a confirmation 80% of Cd²⁺ was detected in a complex form, and only 40% of the absorbed Pb²⁺ was bound to phytochelatins in the cellular extract [56].
4. Metallothioneins (MTs), the metal-chelating proteins

Involvement of MTs has been reported in a number of physiological processes such as regulation of cell growth and proliferation, toxic metal protection and homeostasis, free radical scavenging or protection from oxidative stress, DNA damage repair [57]. For a long MTs was considered to be expressed only in mammals while in plants enzymatically formed PCs are the protective biomolecules against heavy metal toxicity. MTs is a cytosolic superfamily of cysteine-rich proteins capable to bind both physiological and xenobiotic heavy metals [58, 59]. While phytochelatins are formed enzymatically MTs are the products of mRNA translation [60, 61]. In the structure of MTs cysteine (C) residues representing about 30% of the constitutional amino acids. Primary structures of this low molecular weight protein family (M_w ranging from 5 to 10 kDa) are characteristically rich in highly conserved CC, CXC (where X is a general amino acid) and CXXC motifs that render a unique ability to bind mono- or divalent metal ions, such as Cu^{2+}, Zn^{2+} and Cd^{2+} [62]. While in animal MTs no aromatic acids occur histidine (His) residues can be found in a number of plant MT sequences. The replacement a part of the Cys residues by His would be an increased selectivity for Zn^{2+} over Cd^{2+}, and thus the function of the respective MT is more selective in maintaining Zn^{2+} homeostasis than heavy metal detoxification [63]. In addition, the thiol(ate)s in MTs can act as powerful antioxidants, and therefore MTs have definite roles in protection against oxidative stress [64].

Based on the arrangement of Cys residues four types of plant MTs exist [59]. Type 1 MTs are mainly expressed in roots, while the expression of type 2 MTs mostly occurs in shoots, type 3 MTs are induced in leaves and during fruit ripening, and type 4 MTs are abundant in the developing seeds [65]. Regarding high level of sequence diversity of plant metallothioneins, type 1-4 MTs is further subdivided to various isoforms. All four types of plant MTs and their isoforms are able to chelate heavy metals. In general, the primary structures of plant MTs in type 1, 2 and 3 have a similar cysteine topology. The two Cys-rich domains (α and β) are attached by a 30-40 amino acid long cysteine-poor linker depending on plant species. Cysteine topology of type 4 MTs different from that observed in MTs of type 1-3. In angiosperm species three Cys-rich regions linked by two Cys-poor linkers containing 15 and 40 amino acids [58, 60]. Experiments with Arabidopsis thaliana MTs expressed in copper and zinc sensitive yeast mutants provided evidences that MT1a, 2a, 2b and MT3 function as copper binding MTs. The seed-specific type-4 MTs were more effective than other Arabidopsis MTs in providing protection against Zn toxicity and enhancing Zn accumulation [66].

Studies on copper tolerance and expression MT1 and MT2 genes in several A. thaliana species revealed that MT1 was uniformly expressed in all ecotypes and MT2 was copper inducible. In cross-induction experiments, Ag^{+}, Cd^{2+}, Zn^{2+}, Ni^{2+} significantly enhanced the levels of MT2 genes [67]. A. thaliana plants knocked down for MT1a and b isoform expression exhibited increased cadmium sensitivity. These lines accumulated less As, Cd and Zn in the leaves than wild-types indicating that MT1 have a definitive role in Cd tolerance and possibly involved in Zn homeostasis [68]. Lack of MTs increased Cd and Cu sensitivity in PC-deficient Arabidopsis plants suggesting that PCs and MTs contribute to Cu and Cd tolerance and may overlap in their functions [66]. Experiments with A. thaliana MTs expressed in copper and zinc sensitive yeast mutants provided evidences that MT1a, 2a, 2b and MT3 function as copper binding MTs. The seed-specific type 4 MTs were more effective than other Arabidopsis MTs in confering protection against Zn toxicity and enhancing Zn accumulation [66].

MTs typically bind metal ions in characteristic metal–thiolate clusters that provide high thermodynamic stability coupled with kinetic lability [69]. The large
Plant Defense Mechanisms

diversity in the metal binding regions of plant MTs confers the ability to bind a greater range of metals than in animals possessing a greater range of function [59]. A model of cadmium binding to mammalian MTs showed that all cysteine residues participate in the coordination of 7 mol of Cd per mol of MT. Two polynuclear metal clusters formed during binding with 3 and 4 metal atoms on β- and α-domain, respectively.

In one metal cluster requires 9 cysteine SH group to bind 3 Cd in a six-membered ring while the four-metal-cluster forms a bicyclo[3.1.3] structure with the participation of 11 Cys-SH groups (Figure 3) [70].

Experimentally and predicted stoichiometries of metal-plant MTs are in agreement. Type 1 plant MTs with 12 Cys residues can bind 4-5 metal ions, type 2 with 14 Cys can coordinate 5 metal ions while type 3 MTs with only 10 Cys residues exhibit the lowest capacity for metal binding (4 metal ions) [58].

The key characteristics of metal-MT complexes are the high thermodynamic but low kinetic stability. Thermodynamically, MTs are the most stable zinc sites in eukaryotes but have appropriate kinetic lability for the protein to intermolecularly exchange zinc with proteins [72]. Metal binding affinities of MTs characterized by complex stability constants are hardly available in the literature. However, pH of half-displacement values (pH (1/2)) are available for several plant MTs indicating that the more stable protein-metal complex have lower pH(1/2) value [63]. pH(1/2) values for all MTs follow the order Cu(I) < Cd(II) < Zn(II). Accordingly, MTs will bind Cu(I) in vitro more strongly than Cd(II) or Zn(II), and Cd(II) will be bound more powerfully than Zn(II). Numerically The pH values at which MT1 of pea loses half of the initially bound metals are 5.6 for Zn(II), 4.0 for Cd(II), and 1.5 for Cu(I) [73].

Further studies on large and complex MT gene families in higher plants may exhibit beneficial metal binding and induction properties to enhance the phytoremediation capacity of plants used for heavy metal removal in soils. To understand the function and the mechanism of action of plant MTs requires further manipulations on the expression of this protein family.

Figure 3.
Proposed structures of the four-metal and three-metal clusters of rat liver metallothioneins based on 113Cd NMR data [71]. Adapted from Klaassen et al. [70].
5. Protective action of proline (pro) against heavy metals toxicity

Proline (Pro) is an essential proteinogenic amino acid that fulfill several developmental functions in plants and has a fundamental role in responses to biotic and abiotic stress. In plants proline can protect cells from environmental stress factors by several protective mechanisms such as acting as osmoprotectant, functioning as chemical chaperone stabilizing proteins and antioxidant enzymes, chelating metals, scavenging reactive oxygen species (ROS), balancing the intracellular redox homeostasis (NADP⁺/NADPH ratio, GSH pool) and participating in cellular metabolic signaling [74–76].

Protective mechanism of proline as ROS scavenger include direct reaction with ROS. Free and polypeptide-bound proline was demonstrated to react with hydrogen peroxide (H₂O₂) and hydroxy radicals (OH⁻) producing stable free radical adducts and hydroxyproline (Figure 4). However, the most important ROS-scavenging mechanism in the stress protection is the reaction with singlet oxygen (¹O₂). A direct reaction between H₂O₂ and proline plays a minor role in scavenging of cellular H₂O₂ and the formation of nitroxyl radical accumulate during is very sluggish as compared to that of proline and OH⁻. Nevertheless, proline effectively quenches ¹O₂ via a charge transfer mechanism to form the ground triplet oxygen (³O₂) as a ground state molecular oxygen. Due to its action as a ³O₂ quencher, proline may help stabilize proteins, DNA, and membranes [77].

Toxic action of lead, copper, and zinc on proline, malondialdehyde (MDA), and superoxide dismutase (SOD) has been studied in the cyanobacterium *Spirulina platensis*-S5. Parallel to growth reduction elevated MDA, SOD, and proline contents were found with increasing concentrations of metals. Elevated levels of MDA indicated the formation of free radicals in generated heavy metals stress while enhanced amounts of SOD and proline demonstrated the undergoing scavenging mechanism [78].

The molecular mechanism of proline protection of cells during stress may involve the effects on redox systems such as the glutathione (GSH) pool. Pro reduces heavy metal stress by detoxification of free radicals produced as a result of Cd poisoning. Pro may physically quench oxygen singlets or react directly with

![Figure 4](https://www.intechopen.com/books/molecular-defense-mechanisms-in-plants-to-tolerate-toxic-action-of-heavy-metals/9)

**Figure 4.**
Routes for scavenging reactive oxygen species (ROS) by proline. Adapted from Liang et al., 2013 [74].
hydroxyl radicals. These reactions result in reduced free radical damage (lower malondialdehyde levels) and a more reducing cellular environment (higher GSH levels). The high GSH levels in turn facilitate phytochelatin synthesis and sequestration of heavy metal phytochelatin conjugates in the vacuole. This enhanced sequestration of Cd-phytochelatin complexes in the vacuole accounts for the transiently increased Cd content of P5CS (Δ1-pyrroline-5-carboxylate synthetase)-expressing cells in transgenic algae (Figure 5) [76].

The chelation of metals is also considered as possible mechanism responsible for the protective effect of proline in cells against stress. Reversal on Cd- and Zn-induced inhibition of glucose-6-phosphate dehydrogenase and nitrate reductase enzymes by proline supported the function of proline as a metal chelator by forming proline-metal complexes [79]. Nevertheless, the stability of metal-proline complexes was found to be relatively low [80], to effectively influence the inhibitory

---

**Figure 5.**

Participation of proline in reducing cadmium stress by detoxification of free radicals generated by Cd toxicity. In wild type algae (Figure 5A), Cd²⁺ induces the production of reactive oxygen species that rapidly oxidize GSH to GSSG. In transgenic algae (Figure 5B) the resulting decreased GSH are reduced with free pro leading to increased GSH levels and therefore enhanced phytochelatin synthesis to coordinate and sequester Cd. Adapted from Siripornadulsil et al., 2002 [76].
concentration of the metal ions in the assay mixtures. A copper–proline complex was found in the roots of copper-tolerant sea thrift (Armeria maritima) [75].

Accumulation of free proline plants in response to abiotic stresses has been demonstrated by Delauney and Verma [81]. In Arabidopsis, accumulation of Pro occurs after NaCl stress and can reach 20% of the total free amino acid pool [82]. The metal-tolerant plant species such as Armeria maritima, Deschampsia cespitosa, and Silene vulgaris were reported to contain substantially higher levels of constitutive proline than non-tolerant plants [46]. In hyperaccumulator artichoke (Cynara scolymus L.), Pro elevation in cells correlated with increasing lead concentration [83]. Enhancement of Pro concentration in root of rape seed (Brassica napus L.) was more significant than in shoot tissues as a result of increasing concentrations of Pb2+ [84]. Root specific accumulation of the proline was detected in Indian mustard (Brassica juncea L.) under lead and cadmium stress [85]. Similarly elevated proline levels were detected in roots of a variety of plants such as black nightshade (Solanum nigrum L.) exposed to copper [4], in wheat stressed by cadmium [86], and mercury and cadmium stressed lemongrass (Cymbopogon flexuosus Stapf) [87]. In hybrid poplar (Populus trichocarpa × deltoides) Pro accumulation in roots was almost double than in leaf tissues due to highly toxic Cd2+ exposure [88]. In the roots of pepper plants (Capsicum annuum L.) elevated proline level was detected with increasing Cr concentration, while Pro decreased in leaves [89]. On the contrary, shoot specific accumulation of Pro was detected in sunflower (Helianthus annuus L.) seedlings as a result of copper stress [90]. In metal non-tolerant bladder campion (S. vulgaris) Cu was most effective inducer of the proline accumulation followed by Cd and Zn, respectively [46]. Treatment of cauliflower seedlings (Brassica oleracea var. botrytis) with Cd, Hg, and Zn resulted in the concentration dependent accumulation of Pro up to double. Among these heavy metals Hg was the most effective inducer of Pro biosynthesis [91]. Treatment of sal seedlings (Shorea robusta) with heavy metals increased the proline accumulation in plants in the order of Cd2+ > Pb2+ > As3+ [92]. In wheat seedlings stimulation of Pro levels was more pronounced by copper than zinc [93]. Additionally, induction of Pro contents in shoots of black gram (Vigna mungo L.) seedlings treated with heavy metals changed in the order of highest to lowest as Hg > Pb > Co > Zn and followed the toxicities of heavy metals. The results suggest that heavy metal stress induced proline accumulation is strongly dependent on the concentration and type of heavy metal and plant species [94].

It seems that two mechanisms are responsible by which proline provides protection against heavy metal stressors. One of these, up-regulation of proline biosynthesis to accumulate proline to serve as an osmolyte, a chemical chaperone, and a direct scavenger of hydroxyl radical and singlet oxygen. Secondly, linkage of proline metabolic flux to metabolic pathways to maintain the intracellular redox homeostasis (NADP+/NADPH ratio, GSH pool).

6. Oxylipin signaling under heavy metal stress

Oxidized fatty acids, oxylipins, are an important class of signaling molecule in plants in responses to abiotic stresses [95, 96]. Oxylipins regulate growth, development, and responses to environmental stimuli of organisms [97]. Lipoxygenases, allene oxide synthase and a series of cytochromes P450 related oxygenasesare involved in oxylipin biosynthesis. Enzymatically synthesized oxylipin, the jasmonic acid (JA) and a precursor molecule 12-oxo-phytodienoic acid (OPDA) were shown to accumulate in response to pathogen infection [98]. Jasmonate-dependent tolerance to heavy metals is
mediated by defensins, small cysteine-rich proteins present in plant cells [99, 100]. Oxylipins are involved in stress signal transduction, regulate stress-induced gene expression, and interact with other signaling pathways in plant cells, including signaling pathways of the plant hormones auxin, gibberellin, ethylene, and abscisic acid (ABA) [101, 102]. However, the role of oxylipins in plant adaptation and defense mechanism to abiotic stress is less studied. Protective action of oxylipins at toxic levels of heavy metals is considered to activate detoxification processes. Non-enzymatic reaction of reactive oxygen species with lipidic substances results in hydroxy fatty acids which are biologically active oxylipins and play important role in protective action of heavy metal stress [103]. Spontaneously formed oxylipins are called phytoprostanes. Pretreatment of tobacco plants with phytoprostanes results in reduction of cell death in response to copper sulfate stress [104]. In roots of tobacco seedlings Al-induced accumulation of 2-alkenals formed from fatty acid hydroperoxides was detected [105]. ROS generated during aluminum stress formed toxic aldehydes in reaction with fatty acids and caused severe root growth inhibition. Removal of 2-alkenals from tissue through overexpression of 2-alkenal reductase reduced Al phytotoxicity. Stimulation the expression of a number of stress responsive by phytoprostanes [106] makes oxylipins promising tools for improving stress tolerance of plants to heavy metals.

7. Conclusion

Heavy metal stress on plants in contaminated soils due to increased anthropo-genetic activities led to an intensive research on detoxification and sequestering mechanisms of plants to understand and manipulate defense mechanisms developed in plants to tolerate stress by metal ions. The accumulation of heavy metals in plant organs results in the damage of the structural components and disruption of cell metabolic processes leading to plant growth retardation. Increased biosynthesis of various plant biomolecules to confer tolerance during toxic action of heavy metals is an intrinsic ability of plants. Induced formation of low-molecular weight amino acids, peptides or proteines as chelators such as proline (Pro), glutathione (GSH), phytochelatins (PCs) or metallothioneins (MTs) under heavy metal stress enhances metal binding and detoxification capability of plants. In addition, proline and GSH related enzymes such as GSH reductase, GSH peroxidases and glutathione S-transferases are also key components of the antioxidant defense system in the cells to scavenge reactive oxygen species (ROS). Protective action of oxidized fatty acids oxylipins at toxic levels of heavy metals is considered to activate detoxification processes as signaling molecules.

Exploring and manipulating genes induced under heavy metal stress make possible to develop transgenic plants with enhanced detoxication properties for phytoremediation technologies in polluted soils. The use of hyperaccumulator plants [107] which can accumulate heavy metals in the leaves at 100-fold higher concentration than normal plant species can be the alternative solution to clean-up polluted soils.
Plant Defense Mechanisms

References


[34] Marrs KA, Walbot V. Expression and RNA splicing of the maize glutathione S-transferase of wheat bronze2 gene is regulated by cadmium and other stresses. Plant Physiology. 1997;47:127-158


[45] Cobbett SS. Heavy metal detoxification in plants: phytochelatin biosynthesis and function. IUBMB Life. 2001;51:183-188. DOI: 10.1080/152165401753544250


[56] Scarano G, Morelli E. Characterization of cadmium- and
Plant Defense Mechanisms


[68] Zimeri AM, Dhankher OP, McCaig B, Meagher RB. The plant MT1 metallothioneins are stabilized by binding cadmium and are required for cadmium tolerance and accumulation. Plant Molecular Biology. 2005;58:839-855. DOI: 10.1007/s11103-005-8268-3


[86] Lesko K, Simon-Sarkadi L. Effect of cadmium stress on amino acid and


[90] Zengin FK, Kirbag S. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (Helianthus annuus L.) seedlings. Journal of Environmental Biology. 2007;28:561-566


[101] Lopez MA, Vicente J, Kulasekaran S, Vellosillo T, Martinez M,


