Impact of heavy metal stress on plants and the role of various defense elements

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Abstract

Heavy metal (HMs) pollution is currently one of the serious issues for the environment and agriculture as it has a direct impact on the production yield. This situation has gained a rapid momentum in the present era due to the population pressure, industrialization, and various anthropogenic activities which in turn lead to oxidative stress in plants and thus disturbs the redox homeostasis and ultimately affects the crop yield. However, plants possess a different regulatory system that work in a synergetic manner to combat stress and thus adapt themselves in such contaminated soils. These act as sinks to neutralize the toxic effects of these heavy metals by chelation, sequestration, and intensification of enzyme system. Excessive stress induces a cascade, MAPK (mitogen-activated protein kinase) pathway and biosynthesis of metal detoxifying ligands. The understanding of detoxification mechanism of metal ions through MAPK cascade and biosynthesis of metal-detoxifying ligands is an interesting way for searching insights and outlook in the field of plant biotechnology. Here, we discuss the impact of heavy metals on plants and the role of different regulatory elements that play a prominent role in metal detoxification.

Keywords: heavy metals; ROS; MAPK pathway; metallothioneins; transcription factors

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Introduction

The ever increasing population of the world is projected to reach 10.9 billion by 2050 and would lead to food crises in near future. Presently, agriculture sector is confronted with immense challenges to provide adequate food supply while maintaining high productivity and quality standards. Current population of India is approximately 1.27 billion, thus feeding 1.27 billion mouths would indeed be a huge challenge because of degradation of soil quality due to various factors viz., mal-agricultural practices, anthropogenic activities, and natural calamities that in turn affect the agro-industry. These mal-practices disturb the soil microflora by incorporating toxic heavy metals which causes shunted growth and ultimately affects the crop yield. Heavy metal toxicity is indeed a great threat to agriculture sector as it leads to oxidative stress in plants and cause deleterious effects resulting in the decline of production yield. From chemical point of view, metals with atomic density greater

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than 5 g/cm^3 or more, greater than water and an atomic number 20 are categorized as heavy metals (Hawkes, 1997). But, biologists occasionally use this term for a wide range of metalloids and metals that are lethal to plants like molybdenum (Mo), zinc (Zn), magnesium (Mg), manganese (Mn), iron (Fe), aluminium (Al), lead (Pb), mercury (Hg), and copper (Cu) etc. However, many of these heavy metals such as cobalt (Co), sodium (Na), Fe, Zn, and Mg at lower concentrations are considered as micronutrients indispensable for various physiological processes in plants (metallo-proteins and ion-dependent enzymes which include peroxidase, nitrate reductase, superoxide dismutase, cytochrome catalase, ascorbate oxidase etc) (Hansch and Mendel, 2009). But, at higher concentrations these metal ions can become inimical to plants by blocking enzyme action either by binding with functional groups, expelling or replacing with crucial components, or causing membrane disruption. Generation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet -}$) within distinct organelles is the foremost response when plants are confronted with heavy metal toxicity (La Rocca et al., 2009; Das and Roychoudhury, 2014). Excessive ROS production disturbs the chemical equilibrium in plants and thus causes various oxidative damages like degradation of polyunsaturated fatty acids, leakage of ions, DNA damage, and apoptosis (Pirzadah et al., 2018). ROS were previously considered as lethal molecules whose concentration should be maintained for the proper functioning of the cell. But, this perception has been changed as they are essential signalling molecules and are also being involved in various developmental processes such as lignin biosynthesis (Schutzendubel and Polle, 2002). Nature has engineered the plants to combat distinct biotic and abiotic stresses and thus acts as sinks for obnoxious chemicals. Plants act as green livers to neutralize the toxicity of the heavy metals either inside the plant matrix or in the rhizosphere. The detoxification of heavy metals by plants involves a co-ordinated approach at the physiological, biochemical, or genomic level (Dalcorso et al., 2010). Generally, counteraction to heavy metal toxicity can be either accomplished by “evasion” when plants are capable to hamper metal uptake or by ‘tolerance’ when plants sustain under huge metal concentration. The process of evasion involves the chelation or precipitation of metals in the rhizosphere and thus preventing its entry and translocation to the above ground part. In later situation, heavy metals are intra-cellularly chelated by oozing out organic acids or other metal-binding ligands (phytochelatins and metallothioneins) (Seth et al., 2012). The metal binding ligands such as MTs and PCs help the plant to absorb HM ions and sequester them inside vacuoles. Signaling cascades that involves perception and transmission of signals to the nucleus and regulate defense metabolites/genes is an important pathway to combat HM stress.

The various receptors well documented in plants in response to HM stress include flagellin sensitive 2 (FLS2), receptor like protein kinases (RLKs), and EF-Tu receptor (EFR) (Jalmi and Sinha, 2015). Plants possess distinct signaling pathways such as hormone signaling, calcium signaling, and MAPK signaling that gets stimulated in response to abiotic stress (HM stress) and plays a leading role in plant-HM interactions (Vitti et al., 2013; Steinhorst and Kudla, 2014; Chen et al., 2014). Among the three distinct pathways, MAPK-pathway is more predominant to provide tolerance against abiotic as well as biotic stress (Hamel et al., 2006; Sinha et al., 2011). It has been reported that plant growth under HM stress results in the formation of signaling molecules and stress-induced proteins/metabolites which cause chelation and sequestration of toxic HM (Mendoza-Cozatl et al., 2011). Besides, the transportation of HMs via xylem/phloem requires metal transporters localized inside parenchyma cells (xylem) and companion cells (phloem). The prominent metal transporters viz., HM transport ATPase, Zn-Fe permease (ZIP), cation diffusion facilitator (CDF), ATP-binding cassette (ABC), etc (Singh et al., 2015). Despite metal transporters and chelating ligands, vacuole sequestration capacity is another important factor in metal allocation and is regulated in response to changing environment (Peng and Gong, 2014). The fate of the toxicity of HMs is determined by the regulation of vacuole sequestration capacity and thus it is very important to unravel its mechanism and impact transport and sequestration. This ability of plants to extract toxic metals has nowadays been exploited to rejuvenate the soil health contaminated with various obnoxious HMs through phytoremediation process. Besides, the ores of economically important metals can be extracted by means of phytomining. In this review, we will emphasize on the impact and detoxification mechanisms of HMs and role of different metal chelators.

Heavy metals and their lethal effects in plants

Heavy metal pollution is currently a great threat to agricultural sector as it directly affects the production yield by interrupting the crop physiology. Generally, plants exhibit numerous symptoms in response to heavy metal stress which include shunted growth and root ultra structure (Sharma and Dubey, 2007; Pirzadah et al., 2018), leaf necrosis, chlorosis, turgor loss, decrease in photosynthetic activity, reduction in germination rate and percentage, apoptosis, and finally death of the plant (Dalcorso et al., 2010; Wang et al., 2018). Besides, the toxicity of metal ions also disturbs the homeostasis in plants such as water uptake, transpiration, nutrient metabolism (Poschenrieder and Barcelo, 2004) and also interferes with gated ion channels (Demidchik, 2018).

Lead (Pb) is not considered as an essential element and plants does not possess lead uptake channels. However, this toxic element gets entrapped by organic acids (carboxylic group of mucilage uronic acid) on root surfaces (Sharma and Dubey, 2005). But how lead gets incorporated into the root cells is still unknown. Pb in association with other salts (sulphate and phosphate) gets precipitated and thus possesses low solubility and availability to plants. As per several reports, maximum concentration of lead gets accumulated inside root cells, thus it creates a primary obstruction for the Pb translocation to the above-ground parts of the plant (Blaylock and Huang, 2000), acting like an innate barrier. Accumulation of Pb in plants has several lethal effects on their physiology and biochemical parameters (growth, morphology, and photosynthesis). The enzymes get inactivated under high Pb concentration by binding with their sulfhydryl (-SH) groups. However, the tolerance of plants to Pb concentration varies depending upon the species and the nature of mechanism. Some plants are prone to Pb concentration while some others are tolerant and have the capability to accumulate high Pb concentration and are considered as hyperaccumulators. Buckwheat has tremendous potential to tolerate and accumulate high Pb concentration in its leaves without showing any significant damage (Horbowicz et al., 2013). On dry weight basis buckwheat accumulates approximately about 1000 mg/Kg of Pb in its shoots and therefore, it is regarded as an excellent hyperaccumulator (Pirzadah et al., 2014). Reports also revealed that buckwheat accumulates high Pb concentration in various parts of the plant such as in leaves (8000 mg/Kg), stem (2000 mg/Kg), and roots (3300 mg/Kg) on dry weight basis without showing any lethal damage (Tamura et al., 2005). In another study, Pb was involved in inhibiting the elongation of stem and root and expansion of leaf as observed in *Allium* species (Gruenhage and Jager 1985), *Raphanussativus* and barley (Juwarkar and Shende, 1986) but the intensity of elongation depends on various parameters such as concentration gradient, ionic composition as well as pH of the medium (Pourrut et al., 2011).

Aluminium (Al) which constitutes about 7% of the earth's crusts is ranked the third most abundant element after oxygen and silicon. Major portion of the Al occurs as oxides and alumino-silicates which are harmless compounds; however, when soil becomes acidic due to anthropogenic activities, Al gets solubilized into the lethal form (Al^{3+}). The toxicity of Al inhibits plant growth and thus affects the agricultural yield (Smirnov et al., 2014). Al not only causes shunted growth and development of plants but also inhibits root and shoot elongation thus declines the biomass production. The immediate adverse effects of Al-toxicity is root inhibition and elongation by destroying the root apex (Zheng, 2010). It therefore interferes with water and mineral uptake. Moreover, Al is also involved in halting the action of proton pumps on endosomes which in turn traps transferrin-bound iron inside these vesicles and acts as a barrier in stimulation of ferritin synthesis. In addition to this, Al also causes an oxidative stress even though it is not a redox metal (Liu et al., 2008). Besides, Al possesses the capacity to form electrostatic bonds preferably with oxygen donor ligands (viz. phosphates), cell wall pectin, and outer surface of the plasma membrane (Yamamoto et al., 2001). Some reports also revealed that binding of Al to bio-membranes results in rigidification (Jones et al., 2006) which in turn facilitates the radicle chain reaction by iron ions and thus boosts the peroxidation of lipid as is reported in various species like barley (Guo et al., 2004), triticale (Liu et al., 2008), wheat (Hossain et al., 2005), and green gram (Panda et al., 2003). Higher Al concentration also leads to calcium and magnesium deficiency (Jones et al., 1998). The level of Al toxicity varies from species to species; some plants are very prone to Al-induced stress even at micro-molar (µM) concentration while some species exhibits high resistance. Among various plant species, buckwheat, tea, mangroves, and certain other grass species have the ability to develop symplastic tolerance mechanism and thus accumulate higher Al concentration in the above-ground parts. Buckwheat has the large tendency to accumulate approximately about 1500 mg/Kg Al-concentration mainly in the form of Al-oxalates in the leaves without any lethal effect (Ma et al., 1997; Zhang et al., 1998).

Mercury (Hg), another most toxic HM, has devastating effect on the agricultural production. It generally occurs in different forms such as mercury sulphide (HgS), Hg^{2+}, Hg^0, and methyl-mercury (CH_3Hg^+), but the predominant form of Hg in the agricultural land is ionic form i.e., Hg^{2+} (Pirzadah et al., 2018). Hg possesses the unique property to remain in solid phase by binding onto the clay particles, sulphides, or other organic matter. This toxic metal has the tendency to get accumulated in various plant parts especially higher plant species (Israr et al., 2006), where it has deleterious effects on the plant cells and tissues. For instance, closure of stomata and blockade of water flow in plants gets binding with water channel proteins (Zhang and Tyerman, 1999). Besides, Hg toxicity also hampers the mitochondrial activity which is usually considered as a power house of the cell and induces the oxidative stress which in turn leads to the generation of ROS, thus enhances the lipid peroxidation and ultimately disrupts the integrity of bio-membrane (Cargnelutti et al., 2006; Pirzadah et al., 2018).

Signal perception in response to HM stress

HMs interacts with metabolic pathways and generates signals which are recognized by the cell. To date, less information is available regarding signaling networks in plants that involve the primary recognition (i.e, via receptors). Metal ions are perceived by receptors (viz. plasma membrane proteins) that are involved in metal acquisition like reductase and transporters. Besides, other receptors also come into play due to physio-chemical changes in cellular structures upon exposure to metal stress. Once the metal is recognized by sensors present in the cells, it is further taken over by the elements of the signal transduction pathways viz., calcium fluxes. One of the prominent secondary signaling molecules is calcium (Xiong and Zhu, 2002). Nielsen et al. (2003) reported that different concentrations of copper (Cu) treatment of *Fucusserratus* (brown algae) resulted in the inhibition or induction of cytoplasmic calcium fluxes which in turn, initiated calcium dependent protein kinases and thus conjugated this universal inorganic signal to specific protein phosphorylation cascades like MAP kinase activities (Yeh et al., 2007). The other inorganic signal involves the proton that enables fast concentration shifts (not as fast as calcium) and these proton fluxes resulted in so-called “pH-signatures” in cytoplasm (Roos et al., 1998) and thus generated instinct micro-areas in order to increase metal solubility. Santi and Schmidt (2009) reported that lack of Fe causes a proton outflow directed by a P-type proton ATPase (AHA7) thus acidifying the rhizosphere that produces a pH-shift in the near neutral cytoplasm.

Mechanism of action of toxic metals in plant cells

Plants have developed various ways to detoxify HMs toxicity when accumulated at huge concentration levels. Usually, these heavy metals are categorized into two types which include redox active (Co, Cr, Cr, Cu, Fe) and redox inactive (Mn, Pb, Cd, Hg As, Ni, etc). The redox active heavy metals play a direct role in generating ROS through various chemical reactions like Fenton and Haber-Weiss reactions (Schutzendubel and Polle, 2002; Cuypers et al., 2016). The second category of heavy metals plays an indirect role to induce

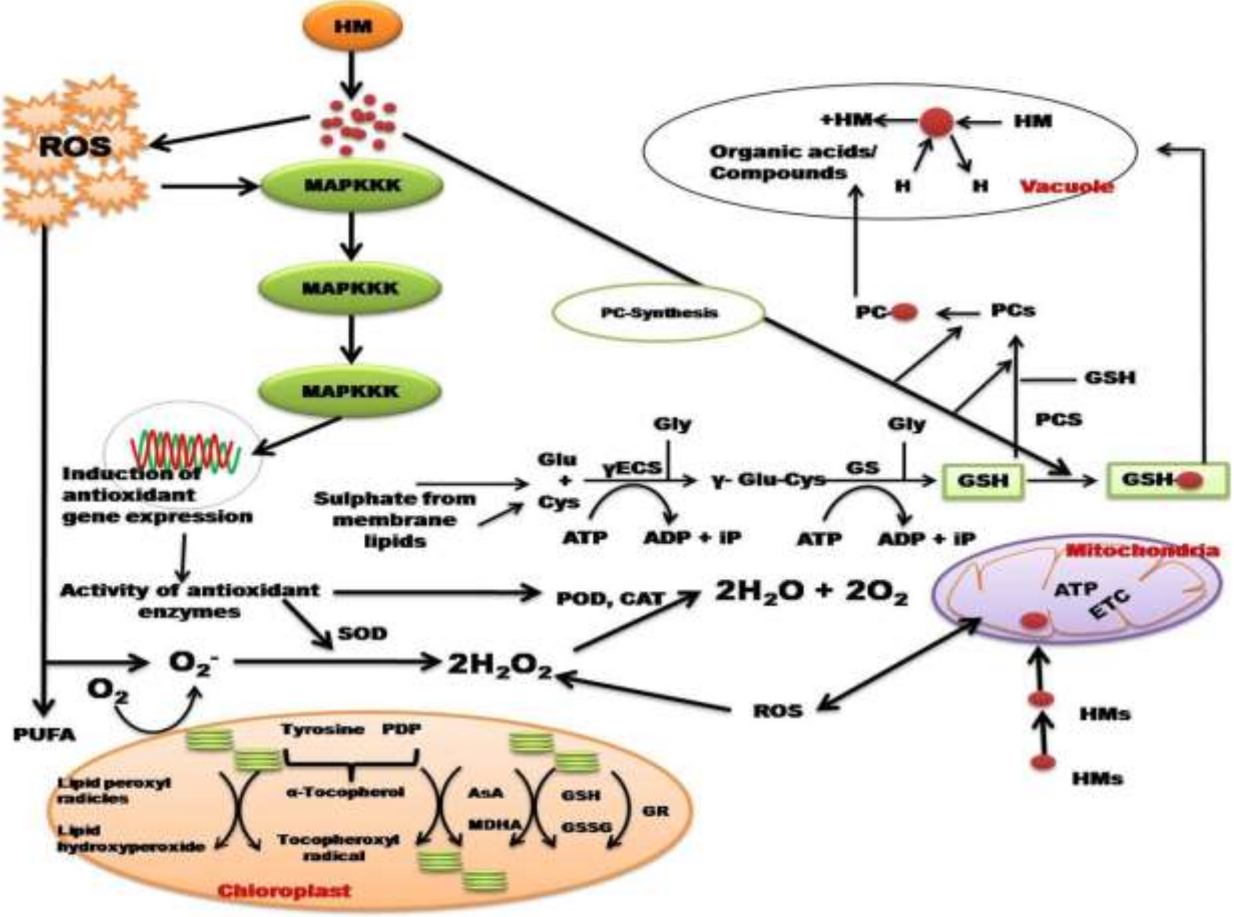

Fig. I. Overview of heavy metal stress and its detoxification mechanism in plant cell; ADP: adenosine dinucleotide phosphate; AsA: ascorbic acid; ATP: adenosine triphosphate phosphate; CAT: catalase; Cys: cysteine; γ-ECS: γ-glutamylcysteine synthetase; ETC↓: electron transport chain damage; Glu: glutamine; Gly: glycine; GR: glutathione reductase; GS: glutathione synthetase; GSH: glutathione (reduced); GSSG: glutathione (oxidized); H: hydrogen molecule; HMs: heavy metals; H_2O_2: hydrogen peroxide; MAPK: mitogen-activated protein kinase; MAPKK MAPK: kinase; MAPKKK: MAPK kinase kinase; MDHA: monodehydroascorbate; iP: inorganic phosphate; O_2: oxygen molecule; $O_2^{\bullet -}$: superoxide radicals; PCs: phytochelatins; PCS: phytochelatins; PDP: phytol diphosphate; POD: peroxidases; PUFA: polyunsaturated fatty acids; ROS: reactive oxygen species; SOD: superoxide dismutase

oxidative stress like interference with enzyme defense system, disruption of the electron transport chain, and lipid peroxidation initiation due to enhanced lipoxygenase activity. Moreover, heavy metals also possess the capability to bind firmly with atoms such as nitrogen, sulphur, and oxygen that is associated with free enthalpy of the formation of the product of the HM and ligand and reduces their solubility. Besides, these HMs also inactivate the enzymes by binding their thiol-group, for instance, binding of heavy metals like cadmium (Cd) to sulfhydryl group of structural enzymes and proteins either results in misfolding and arrest of enzyme action or impede with redox enzymatic regulation (Hossain et al., 2012; Gill, 2014). Most of the enzymes need cofactors which are regarded as helper molecules to aid in biological activity. These cofactors may be either of inorganic (Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}) or organic (biotin, FAD, NAD) origin and replacement of any cofactor halts the enzyme action. For instance, displacement of magnesium (Mg^{2+}) in ribulose-1,5-bisphosphate-carboxylase / oxygenase (RuBisCO) by Co^{2+}, Zn^{2+}, and Ni^{2+} thus halts the activity of enzymes (Sharma and Dubey, 2005; Gill, 2014). Moreover, these HMs are also responsible for disruption of bio-membranes by enhancing the lipid peroxidation, oxidation of thiol-group of proteins, and blockage of basic membrane proteins (H^+-ATPase) (Meharg, 1993; Hossain et al., 2012). Furthermore, these toxic heavy metals also lead to the initiation, production, and accumulation of most lethal compounds like methyl glyoxal-a cytotoxic chemical due to disruption of glyoxalase system that finally elicits oxidative stress by diminishing the glutathione content (Hossain and Fujita, 2010; Hossain and Hasanuzzaman, 2011).

Cross-talk among ROS signaling and MAPK cascades under HMs stress

A wide range of changes occurs in plants at the cellular and genomic level when exposed to HM toxicity and these include dynamic changes in gene expression and biosynthesis of some low molecular weight proteins. Mitogen-activated protein kinase (MAPK) is one the main signaling modules that is involved in transmitting information from sensors to cellular responses and is considered as an evolutionary conserved signaling mechanism in eukaryotes (MAPK Group, 2002). Generation of ROS in response to HM stress in plants and its function to stimulate MAPK pathway is well studied. Different authors have reported well characterized MAPK cascades (MEKK1-MKK4/5-MPK3/6 and MEKK1-MKK2-MPK4/6) that play an important role in down-regulation of ROS and are operated under both biotic and abiotic stress signaling (Asai et al., 2002; Jalmi and Sinha, 2015). Besides, there exists a positive feedback regulation among MAPK cascades and ROS generation viz., ROS activated cascade of OXI1-MPK6 (Asai et al., 2008). Under stress conditions, MAPK cascades acts as converging points in the signaling pathways (Nakagami et al., 2005). Generally, MAPK cascades engage the stimulus-triggered activation of MAPK kinase kinase (MAPKKK) which further undergoes phosphorylation reactions. Plants generate a huge number of MAPK components as reported by MAPK group in 2002 when subjected to HM (Cd, Cu, As) stress (Smeets et al., 2013). For example, in *Medicago sativa* four different MAPKs gets activated in response to Cd and Cu stress. Differential kinetics of MAPK activation revealed that Cu^{2+} induces the MAPKs viz., SIMK, MMK2, MMK3, and SAMK rapidly whereas Cd^{2+} induces delayed MAPK activation (Jonak et al., 2004; Dubey, 2011). Stimulation of MAPK3 and MAPK6 via accretion of ROS in *Arabidopsis* has been demonstrated by Liu et al. (2010) and these cascades end up by phosphorylation of transcription factors interacting with gene promoters and thus inducing gene expressions (Fig. I).

Role of aquaporins under heavy metal (HM) stress

Aquaporins (AQPs) are considered as transmembrane proteins (26-34kDa) belonging to the major intrinsic protein (MIP) super family that play an essential role in regulating the water dynamics in plants. Besides, these are also responsible to conduct non-polar solutes viz. CO2, glycerol, and boron, through biological membranes (Tao et al., 2014; Hu et al., 2015). Reports revealed that about 75-95% of water is mediated by AQPs (Henzler et al., 2004; Ye et al., 2005). In case of plant species, usually these AQPs are categorized into four major groups depending upon the sequence homology of amino acids as well as localization of subcellular protein (Johanson et al., 2001). These involve nodulin 26-like intrinsic proteins (NIPs) (Weaver et al., 1991); plasma membrane intrinsic proteins (PIPs) (Kammerloher et al., 1994), tonoplast intrinsic proteins (TIPs) (Karlsson et al., 2000), and small basic intrinsic proteins (SIPs) (Chaumont et al., 2001). Recently, three more subfamilies have been reported that include GlpF-like intrinsic proteins (GIPs), the hybrid intrinsic proteins (HIPs), and uncategorized members designated X intrinsic proteins (XIPs) in the nonvascular moss *Physcomitrella patens* (Danielson and Johanson, 2008). However, XIPs have been reported in other organisms viz., fungi, protozoa, and a few terrestrial plant species (*Populus trichocarpa*, *Nicotiana tabacum*, *Solanum lycopersicon*, and *Solanum tuberosum*)

(Lopez et al., 2012). Regulation of AQPs is controlled by numerous abiotic factors like pH, temperature, humidity, and heavy metals (Levin et al., 2009). To date, little information is available regarding the consequences of HMs on water dynamics in plants. Gunse et al. (1997) reported the impact of aluminum (Al^{2+}) ions on the water permeability in maize roots. Similarly, Henzler et al. (2004) reported restriction of AQPs by mercuric (Hg^{2+}) salts. Beaudette et al. (2007) revealed that AQPs expression gets up-regulated in *Pisum sativum* when subjected to mercury stress, probably to compensate for the blocked AQPs. It

Table 1
An update on metallothionein genes and their function

Genes	Species	Function	Reference
AtPCS1/CePCS1	Tobacco	As tolerance and accumulation	Gielen et al., (2017)
CdPCS1	Tobacco	Accumulation of As and Cd	Das et al., (2017)
SpMTl	*Sedum plumbizincicola*	Elevated SpMTL transcript level might contribute to the trait of Cd hyperaccumulation and hypertolerance	Peng et al. (2017)
NnPCS1	*Arabidopsis*	Accumulation of Cd	De Araújo et al., (2017)
TaPCS1	Poplar	Accumulation of Pb and Zn	Gong et al., (2017)
PtPCS1	Poplar	Zn accumulation	Chen et al., (2017)
TaPCS1	Rice	Cd hypersensitivity	Mayerová et al., (2017)
TcPCS1	Tobacco	Accumulation of Cd	Zou et al., (2017)
CAT1 and CAT2	*Arabidopsis*	Low level of H_2O_2 and enhanced stress tolerance	González-Guerrero et al., (2016)
DHAR/GR/GST	Tobacco	Enhanced metal tolerance overexpression due to redox homeostasis of ascorbate and glutathione pool	Quintela-Sabarís et al., (2017)
GST	Tobacco	Enhanced Cd tolerance vis-a-vis no Cd accumulation, high activity of antioxidant enzymes	Zanella et al., (2016)
Sulfite oxidase (SO)	Tobacco	High tolerance against S as a result of efficient H_2O_2 scavenging by CAT	Sharma et al., (2016)
AtPCS1	*Arabidopsis*	Cd tolerance and accumulation	Soda et al., (2016)
TaMT3	*Tamarix androssowii*	Cd resistance in transgenic tobacco	Zhou et al., 2014; Ashraf et al., 2017
BcMT1	*Brassica campestris*	Enhanced tolerance to Cd and Cu and increased Cu concentration in the shoots; decreased Production of Cd- and Cu-induced ROS, thereby protecting plants from oxidative damage	Lu et al. (2015)
MT2-like protein	*Solanum lycopersicum*	Hg-induced	Nagata, 2014
PjMT1	*Prosopis juliflora*	Enhance Cd stress	Usha et al., 2014
SaMT2	*Sedum alfredii*	Enhance Cd and Zn tolerance	Zhang et al., 2014
ScMT2-1-3	*Saccharum spp.*	Enhances tolerance to Cd/ Cu/PEG and NaCl	Guo et al., 2013
KcMT2	*Kandelia candel*	Enhances tolerance to Zn/Cu/Pb and Cd	Huang et al., 2012
PsMTa	*Pisum sativum*	Cu homeostasis in *Populus alba*, *A.thaliana*	Turchi et al., 2012
OsMT1	*Oryza sativa*	Cd tolerance	Yang and Chu, 2011; Soda et al., 2016
PcMT3	*Porteresia coarctata*	Cd/Cu/Zn induced	Usha et al., 2011
ThMT3	*Tamarix hispida*	Enhances Cd/Zn/Cu/NaCl tolerance in transgenic yeast	Yang et al., 2011
BgMT2	*Bruguiera gymnorrhiza*	Enhance Cd/Zn/Pb and Cu tolerance	Huang et al., 2012
ccmt1	*Cajanus cajan*	Enhances Cu andCd tolerance in *A. thaliana*	Sekhar et al., 2011
FeMT3	*Fagopyrum esculentum*	Enhances drought and oxidative tolerance in buckwheat	Samardzic et al., 2010
AmMT2	*Avicennia marina*	Enhances Zn, Cd, Cu, Pb tolerance in transgenic A. marina	Huang and Wang, 2010
AtMT4α	*Arabidopsis thaliana*	Enhances Cu/Zn tolerance in *A. thaliana*	Rodriguez et al., 2010
GhMT3a	*Gossypium hirsutum*	Enhance tolerance against high salinity, drought, low temperature, heavy metals, effective ROS scavenger in transgenic tobacco	Xue et al., 2009
OsMT2b	*O. sativa*	Plays roles in root development and zygotic embryo germination of rice	Yuan et al., 2008
MT1	*Populus alba L*	Zn induced	Castiglione et al., 2007
BjMT2	*Brassica juncea*	Enhances Cu and Cd tolerance in E. coli and A. thaliana	An et al., 2006; Soda et al., 2016
MT3	*Thlaspi caerulescens*	Cu homeostasis function in *T.caerulescens*	Roosens et al., 2004
AtMT3	*A. thanliana*	Enhances resistance to Cd in *Vicia faba* guard cells	Lee et al., 2004
tymt	*Typha latifolia*	Enhances Cu and Cd tolerance in A. thaliana	Zhang et al., 2004
PdtMT1a *PdtMT2a*	*Populus trichocarpas× deltoide*	Zn induce expression in root Enhances Cd tolerance in yeast and Zn induced expression in root, leaves and stem	Kohler et al., 2004
AtMT	*A. thanliana*	Tissue specific expression and induction during senescence and in response to Cu	Guo et al., 2003

has been reported that AQPs gene expression was down-regulated by about 50% in root and leaf cells of *Mesembryanthemum crystallinum* (ice plants) when subjected to copper (Cu) stress (25-50 µM after 24h) (Kholodova et al., 2011). The results revealed that leaf expression of the *Mc TIP2; 2* genes, which encode tonoplast AQP was suppressed more than two fold, when subjected to long term heavy metal stress; however, the magnitude of stress depends upon the type of metal and the degree of its toxicity (Przedpelska-Wasowicz and Wierzbicka, 2011). AQPs such as TIPs, PIPs, SIPs, and NIP exhibit significant down-regulation when subjected to cadmium (Cd) toxicity, because the down-regulation of AQP genes due to HM stress makes unavailability of AQPs that in turn halt the transport of water and solutes across the membranes (Hirotaka et al., 2010). Recently, AQP gene cDNA *BjPIP1* was isolated from *Brassica juncea* that encodes 286-residue proteins. *BjPIP1* aquaporin gene shows up-regulation in response to heavy metals (HM), drought, and salt stress. When *BjPIP1* gene was introduced into the tobacco by *Agrobacterium* mediated transformation, the transgenic tobacco exhibited least water loss and declined transpiration rate and stomatal conductance as compared to non-transgenic species. Besides, overexpression of *BjPIP1* gene enhances Cd resistance thus suggesting that *BjPIP1* gene is involved in enhancing HM tolerance by maintaining water dynamics in transgenic tobacco (Zhang et al., 2008). Savage and Stroud (2007) reported that Hg has been extensively studied to provide evidences regarding the involvement of AQPs in water transport especially in plant cells. Hg results in conformational changes in plasma membrane AQPs by binding with sulphydryl groups; thus it hampers the water channels that results in declining their hydraulic conductivity (Barone et al., 1997; Tyerman et al., 1999). It has been revealed that some HMs inhibit the AQP action more strongly than others; this is because of the differences in their atomic structure as well as chemical properties.

Role of metallothioneins (MT)

MTs are metal binding low molecular weight cysteine rich proteins that are generally synthesized during mRNA translation (Kagi, 1991). These metalloproteins have got immense potential to sequester numerous HMs such as Cd, Pb, Hg, Cu, Zn etc. by binding via thiol group (-SH) of cysteine residues; thus, it is involved in the homeostasis and detoxification of heavy metal toxicity (Pirzadah et al., 2014; Tripathi et al., 2015). The structure of MT revealed that it possesses two metal binding domains which are assembled from cysteine clusters. These include α-domain (N-terminal) which bears three binding sites for divalent ion and β-domain (C-terminal) which has the capability to bind with four divalent ions of heavy metals. In plants, the genes encoding these metalloproteins are influenced by stress conditions viz., HMs, cold stress, drought stress etc. (Berta et al., 2009; Jia et al., 2012). These MTs are usually regarded as major transition metal ion binding proteins in cells because they mainly form the complex compounds with Cu and Zn (essential metals) but bear less capacity to bind with Cd, Hg, Pb etc. Besides, some researchers also reported that these metallo-proteins also act as a shielding agent to protect plants from oxidative stress by removing free radicles (Jia et al., 2012). Nowadays, researchers are very interested in determining the ROS scavenging activity of these MT besides developing databases containing gene sequences of MT that act as a repository to generate HM resistant plants (Leszczyszyn et al., 2013). However, the transaction between metal binding and ROS scavenging is still unknown. It has been reported that during scavenging of ROS, metal ions would be released when free radicles get bound to cysteine residues of these metalloproteins. A few authors suggested that the released metals might be involved in signaling cascade. Various MT-types have been reported from plants like rice, oil palm, hybrid poplar, buckwheat, and lichens. Moreover, many cDNA encoding MT-genes have been isolated (Backor and Loppi, 2009) (Table 1). It has been reported that buckwheat cDNA clone (PBM290) which encodes MT-like proteins was isolated from cDNA library and the reduced amino acid sequence revealed its maximal homology with MT3-like protein isolated from *Arabidopsis thaliana*. Upon expression analysis it is found that buckwheat

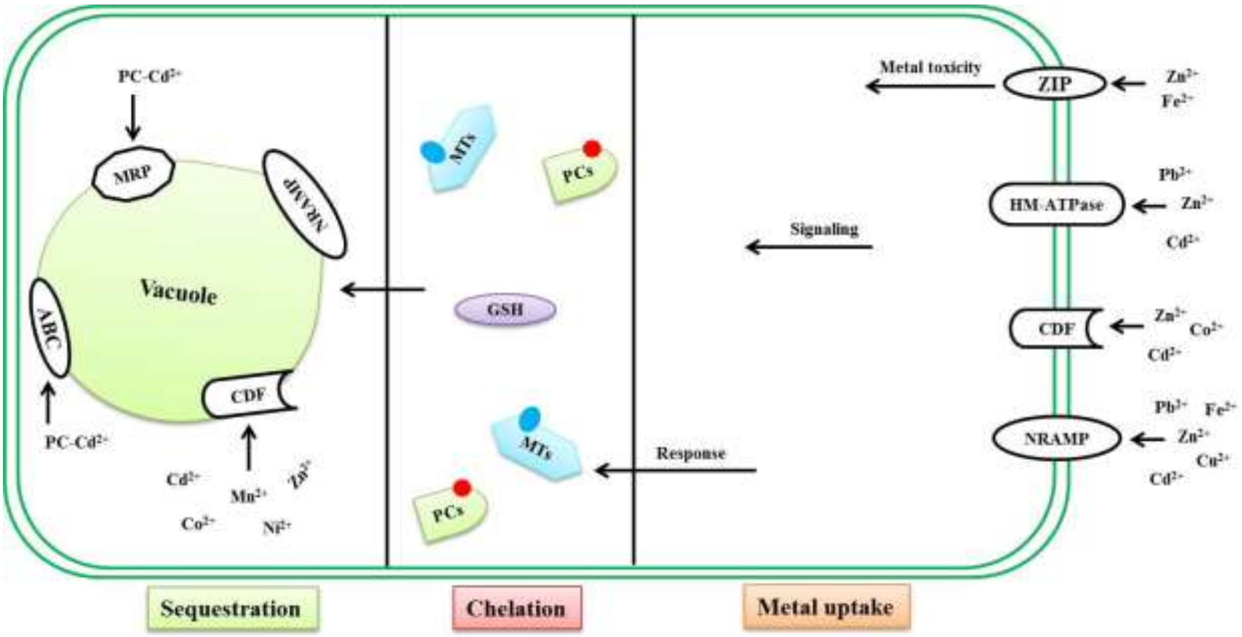

Fig. II. Schematic illustrations of various cellular mechanisms (detection, signaling and sequestration) for mitigating heavy metal (HM) stress

MT3 mRNAs seem to be present in tissues of root and leaf during development of seed under normal circumstances and its expression is greatly altered by HM stress (Pirzadah et al., 2014).

Role of transcription factors (TFs)

Hyperaccumulating plant species contain several metal TFs that are involved in various regulatory pathways to detoxify HMs and some have been already identified in various plant species that impart HM tolerance and these TFs belong to different families viz., WRKY, basic leucine zipper (bzip), ethylene-responsive factor (ERF), and Myeloblastosis protein (Myb) (Fusco et al., 2005; Van De Mortel et al., 2008). Van De Mortel et al. (2006) reported that under Zn-sufficient conditions, 131 TFs showed 5-times more expression in *T. caerulescens* compared to *A. thaliana*. Similarly, Ban et al. (2011) reported that Cd and Cu cause either up-regulation or down-regulation of dehydration responsive element-binding protein (DREB). Another important metal transporter known as NRAMP (natural resistance associated macrophage protein) is found in fungi, bacteria, animals, and plants, which is responsible to transport a wide variety of metal ions viz.,Cd^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, and Mn^{2+}across membranes (Nevo and Nelson, 2006). In case of plants the type of transporter is usually expressed in roots and shoots and is engaged in transportation of metal ions through plasma membrane and tonoplast (Schmidtet al., 2007). It has been reported that NRAMPs in *Arabidopsis thaliana* play a significant role in transporting iron (Fe) and Cadmium (Cd); however, NRAMP1 exhibits Fe transport specificity and homeostasis (Thomine et al., 2000). Other transporters involved in sequestration of HMs include ATP-binding cassette (ABC) transporters, cation diffusion facilitator (CDF) transporters, heavy metal ATPases (HMA) transporters etc. These are actually intracellular transporters that are involved in carrying out the transport of xenobiotics and toxic heavy metals into the vacuole. Multidrug resistance-associated proteins (MRP) and pleiotropic drug resistance (PDR) transporters are two sub-families involved in sequestration of chelated heavy metals (Lee et al., 2005). In case of plants, vacuoles are considered as dominant sites for accumulation and storage of phytochelatins-Cadmium (PC-Cd) complexes. Usually cytosol is the major site where these complexes are generated and are then translocated by means of ABC-transporters (Yazaki, 2006). HMT1 is the first vacuolar transporter present in the tonoplast and is responsible to transport PC-Cd complexes inside the vacuole in a magnesium-adenosine triphosphate-dependent (Mg-ATP) manner (Yazaki, 2006). Salt and Rauser (1995) reported similar homolog protein (HMT1) in oat roots. Magnesium proton exchanger (MHX) and (Cation exchangers) CAX-transporters are the members of calcium-calmodulin (CaCA) families and are responsible for metal homeostasis. MHX was first identified in vacuolar vesicles of rubber tree (*Hevea brasiliensis*) and is magnesium (Mg^{2+}) and zinc (Zn^{2+})/ H^+ antiport dominantly expressed in xylem-associated cells. However, overexpression of these transporters enhances sensitivity to Mg and Zn although the titer of the metals in shoots is unchanged (Shaul et al., 1999). Besides, CAX family are considered as Ca^{2+}/H^+ antiports and help to recognize Cd^{2+}-ions, thus they are involved in Cd-sequestration inside the vacuoles (Salt and Wagner, 1993). AtCAX2 and AtCAX4 are two CAX proteins reported in *A. thaliana* and possess an essential role in accumulation of Cd inside the vacuoles. Korenkov et al. (2007) reported that overexpression of AtCAX2 and AtCAX4 leads the accumulation of large concentration of Cd inside the vacuoles. Moreover, AtCAX4 is usually expressed in primordia and the root tip and it is induced by nickel (Ni) and manganese (Mn) (Fig. II).

Conclusion and further perspective

HMs contamination is increasing at an alarming rate due to numerous anthropogenic activities which in turn affects plant health and thus declines the production yield. Even though several species possess the capacity to hyper-accumulate these HMs and resist the oxidative stress, some others are prone to HM stress. Generally, plants have the innate ability to detoxify HMs by means of several ways like exclusion, chelation, and sequestration. Nowadays, research must be focused on different signaling pathways induced by HMs as they can utilize common signal elements that can also be elicited by other environmental stresses to better understand the metal homeostasis. In future, multiple stress factors will be investigated as it happens in real environmental conditions. An interdisciplinary approach is necessary to unravel the molecular mechanisms involved in HM-stress. Besides, it provides a concrete understanding in the metalloproteome synergistic action. In addition to above, plant-metal interaction is also indispensible for numerous purposes such as;

(1) Prognosis health hazards caused by metal bioaccumulation in crops failing visible manifestations of phytotoxicity.

(2) Bio-fortification i.e., designing plants in such a way that they can accumulate metals indispensable for human health.

(3) Rejuvenation of soil health (phytoremediation) and excavation of rare metals through green route (phytomining).

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