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REVIEW

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# Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants

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## Abstract

Uptake, translocation, detoxification, and sequestration of heavy metals (HMs) are key processes in plants to deal with excess amounts of HM. Under natural conditions, plant roots often establish ecto- and/or arbuscular-mycorrhizae with their fungal partners, thereby altering HM accumulation in host plants. This review considers the progress in understanding the physiological and molecular mechanisms involved in HM accumulation in nonmycorrhizal versus mycorrhizal plants. In nonmycorrhizal plants, HM ions in the cells can be detoxified with the aid of several chelators. Furthermore, HMs can be sequestered in cell walls, vacuoles, and the Golgi apparatus of plants. The uptake and translocation of HMs are mediated by members of ZIPs, NRAMPs, and HMAs, and HM detoxification and sequestration are mainly modulated by members of ABCs and MTPs in nonmycorrhizal plants. Mycorrhizal-induced changes in HM accumulation in plants are mainly due to HM sequestration by fungal partners and improvements in the nutritional and antioxidative status of host plants. Furthermore, mycorrhizal fungi can trigger the differential expression of genes involved in HM accumulation in both partners. Understanding the molecular mechanisms that underlie HM accumulation in mycorrhizal plants is crucial for the utilization of fungi and their host plants to remediate HM-contaminated soils.

**KEYWORDS**

ATP-binding cassette transporter, heavy metal ATPase, phytoremediation, transcriptional regulation, transporter

## 1 | INTRODUCTION

Heavy metals (HMs) are naturally occurring metals with atomic numbers (Z) greater than 20 and an elemental density greater than 5 g $cm^{-3}$ (Ali & Khan, 2017). Some HMs, such as zinc (Zn), copper (Cu), and molybdenum (Mo), are essential nutrients for normal plant growth and development because they are involved in enzymatic and redox reactions, electron transport, and the biosynthesis of biomolecules (Zenk, 1996). These HMs are essential and beneficial for plants at low concentrations, but they are toxic to plants when present in excess. In contrast, other HMs, including cadmium (Cd), lead (Pb), and mercury (Hg), are nonessential and highly toxic to plants (Ding et al., 2017; Ma et al., 2018; Shi et al., 2019). To complete their life cycle, plants must absorb essential HMs and other elements, such as calcium (Ca), magnesium (Mg), and nitrogen (N). In addition to essential HMs, plants can also absorb nonessential and toxic HMs from the soil

**Abbreviations:** ABC, ATP-binding cassette transporter; AM, arbuscular mycorrhizae; Asc, ascorbate; CDF, cation diffusion facilitator; EM, ectomycorrhizae; GSH, glutathione; $H_2O_2$, hydrogen peroxide; His, histidine; HM, heavy metal; HMA, heavy metal ATPase; IRT1, iron-regulated transporter 1; MT, metallothionein; MTP, metal tolerance protein; NA, nicotianamine; NRAMP, natural resistance-associated macrophage protein; PC, phytochelatin; PCS, phytochelatin synthase; ROS, reactive oxygen species; ZIP, zinc-iron permease; ZNT1, zinc transporter 1

  

solution through their uptake system because some transporters can take up several similar HM ions. For instance, root transporters for $Zn^{2+}$ and $Ca^{2+}$ can also take up $Cd^{2+}$ (Rogers, Eide, & Guerinot, 2000; Williams, 2011), and the transporters for phosphate can also absorb arsenate ($AsO_3^-$; Ditusa et al., 2016). As a result, these nonessential and toxic HMs can be absorbed by plants including crops, eventually entering the food chain. Nonessential and toxic HMs are mutagenic, teratogenic, and carcinogenic to humans (Alshatwi et al., 2014; Koedrith, Kim, Weon, & Seo, 2013). The entry of these HMs into human food poses a serious risk to human health. Thus, to control the accumulation of HMs in plants, scientists need a better understanding of the physiological and molecular mechanisms involved in HM uptake, translocation, detoxification, and accumulation.

Under natural conditions, plant roots are usually colonized by soil microorganisms, including ecto- and arbuscular-mycorrhizal fungi, which can significantly affect HM accumulation in host plants (Coninx, Martinova, & Rineau, 2017; Ho-Man et al., 2013; Leyval, Turnau, & Haselwandter, 1997; Luo et al., 2014). The associations between the roots of vascular plants and ectomycorrhizal fungi belonging to Asco- and Basidiomycetes are called ectomycorrhizae (EM). The symbioses between host roots and arbuscular-mycorrhizal fungi of the phylum Glomeromycota are referred to as arbuscular mycorrhizae (AM). EM are characterized by the mantle and Hartig net (Smith & Read, 2008). The mantle is formed of fungal hyphae that ensheath the root tip. Inside the mantle, the hyphae can penetrate the cell walls of epidermal and cortical cells of the root tips and grow in the intercellular space to form the Hartig net (Smith & Read, 2008). In contrast to EM, where the fungal hyphae never enter the lumina of the root cells, AM are characterized by the presence of arbuscules inside root cells (Bonfante & Genre, 2010). The Hartig net and arbuscules are interfaces that allow the exchange of nutrients, water, and other substances between both partners. Obviously, the accumulation of HMs in mycorrhizal plants is complex because it involves fungal partners and the plants (Coninx, Martinova, & Rineau, 2017). However, there are some higher plant families that are unable to form mycorrhizae, such as species belonging to the *Chenopodiaceae* and *Brassicaceae* families (Smith & Read, 2008). It is important for scientists to understand the physiological and molecular mechanisms underlying HM accumulation in these nonmycorrhizal versus mycorrhizal plant species.

In recent years, great progress has been made in our understanding of the physiological and molecular mechanisms that underlie the uptake, translocation, detoxification, and accumulation of HMs in nonmycorrhizal plants (Clemens & Ma, 2016; Fasani, Manara, Martini, Furini, & Dalcorso, 2018; Luo, He, Polle, & Rennenberg, 2016; Shao, Yamaji, Shen, & Ma, 2017), as well as in mycorrhizal plants (Ho-Man et al., 2013; Luo et al., 2014). In this review, we first describe recent progress in elucidating the physiological and molecular mechanisms of HM accumulation in nonmycorrhizal plants, mainly in *Arabidopsis thaliana* (L.) Heynh. and *Noccaea caerulescens* (J.Presl & C.Presl) F.K. Mey. (formerly known as *Thlaspi caerulescens* [J.Presl & C.Presl] F.K. Mey.), which are unable to be colonized by mycorrhizal fungi. We then discuss the physiological and molecular regulation of HM accumulation in ecto- and arbuscular-mycorrhizal fungi, as well as in ecto- and arbuscular-mycorrhizal host plants. Finally, we briefly describe recent progress in the utilization of ecto- and arbuscular-mycorrhizal plants to remediate HM-polluted soils.

## 2 | PHYSIOLOGICAL AND MOLECULAR MECHANISMS OF HM ACCUMULATION IN NONMYCORRHIZAL PLANTS

HMs are often present as HM ions in the soil solution where they can be accessed by the roots of plants. HM ions in the soil solution can enter the roots and can be loaded into the xylem of the vascular system where they are further translocated to the aerial parts along the transpiration stream. The uptake, translocation, detoxification, and accumulation of HMs are key processes for HM accumulation in plants. These processes are controlled by physiological and molecular regulatory mechanisms.

### 2.1 | Physiological mechanisms underlying HM accumulation

#### 2.1.1 | Uptake and transport of HMs via apoplastic and symplastic pathways

HM ions in the soil solution move according to their respective concentration gradients. The chemical and physical properties of the soil, such as the concentrations of other metal ions, the pH of the soil solution, and soil organic matter contents can markedly affect the mobility and availability of HM ions to plant roots (Miransari, 2011). The centripetal movements of HM ions in the roots (i.e., the movements of HM ions on the root surface to the vascular cylinder) follow apoplastic and symplastic pathways. HM ions that pass through the apoplastic pathway can also reach the stele in the root apical region where the endodermal suberin lamellae are not well developed (Lux, Martinka, Vaculã, & White, 2011; Schreiber, 2010; Tao et al., 2017). Due to variations in the anatomical and biochemical properties of different functional regions (i.e., root cap, meristematic zone, and elongation and maturation zones) of the root tips, the radial movement rates of HM ions can differ significantly among root tip regions (He et al., 2011; Ma et al., 2014). Both the apoplastic and symplastic pathways play pivotal roles in the mobilization of HM ions toward the vascular cylinder, but most studies have focused on the contribution of the symplastic path to HM uptake into plant roots (Courbot et al., 2007; Yin et al., 2015). Thus, little information is available about the contribution of the apoplastic route to HM absorption by the roots. Recently, it has been found that the contribution of the apoplastic pathway to $Cd^{2+}$ movements toward the vascular xylem is related to the concentration of $Cd^{2+}$ applied in the hyperaccumulating ecotype of *Sedum alfredii* Hance. This pathway contributed up to 37% at the highest $Cd^{2+}$ concentration applied (Tao et al., 2017). Obviously, more studies are needed to quantify the contribution of the apoplastic route to HM movements toward the stele of the root before any general conclusions can be drawn.

After HM ions in the root cells are centripetally transported to the vascular cylinder, they are loaded into the conduits of the xylem where they are further translocated to the aerial parts of plants,

including the stems, leaves, flowers, fruits, and seeds (Clemens & Ma, 2016). In the aerial parts, HM ions in the xylem conduits can also be radially translocated to cells of the phloem and other tissues. In the aerial parts of plants, HM translocation can also occur through apoplastic and symplastic routes. Several studies have demonstrated that HMs can be accumulated along their transport pathways in plants (Clemens & Ma, 2016; He et al., 2013, 2015; Wang et al., 2015).

#### 2.1.2 | HM detoxification by chelators

After uptake and translocation of HMs, their detoxification is essential for HM accumulation in plants. Several compounds function as chelators to detoxify HMs in plant cells, including thiols such as reduced glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs) and nonthiol ligands such as histidine (His), nicotianamine (NA), oxalate, and organic acids (Alvarez-Fernández, Díaz-Benito, Abadía, López-Millán, & Abadía, 2014). GSH can act as a low-affinity ligand for HMs in plants (Seth et al., 2012; Zlobin, Kartashov, & Shpakovski, 2017). HM-induced higher GSH levels are often observed in various plants (Drzewiecka et al., 2017; He et al., 2015; Shi et al., 2015). Moreover, GSH is the precursor for the biosynthesis of PCs, which are key players in chelating HM ions in plant cells (Pal & Rai, 2010). Decreased levels of free PCs are often found in HM-treated plants due to the formation of HM-PC complexes (Pal & Rai, 2010).

In recent years, many complexes with HM-chelators have been identified and characterized in various plants (Alvarez-Fernández et al., 2014; Leitenmaier & Küpper, 2013). For instance, complexes of Cd-PCs have been detected in the xylem sap of *Brassica juncea* (L.) Czern (Wei et al., 2007). Complexes of Cd-PCs and Cd-GSH have also been proposed to exist in *Brassica napus* L. based on the high concentration of PCs relative to that of Cd, as well as the contents of GSH relative to Cd in the phloem sap (Mendoza-Cózatl et al., 2008). Cd is mainly chelated with thiol groups in the roots of *Zygophyllum fabago* L., which is a Cd- and Zn-tolerant plant (Lefèvre, Vogel-Mikuš, Arčon, & Lutts, 2016). In *Carpobrotus rossii* (Haw.) Schwantes, most of the Cd is bound to sulphur-containing chelators in different tissues, except for the xylem sap (Cheng et al., 2016). Among the nonthiol chelators, HM-chelators have also been detected in several plants. For example, NA binds to Cu and Zn in the Cd/Zn hyperaccumulator *N. caerulescens* (Mijovilovich et al., 2009; Trampczynska, Küpper, Meyer-Klaucke, Schmidt, & Clemen, 2010). Furthermore, oxalate can bind Cu in *N. caerulescens* (Mijovilovich et al., 2009). These findings strongly suggest that thiol and nonthiol ligands play crucial roles in chelating HM ions to reduce their toxicity in plant cells, resulting in enhanced HM accumulation.

#### 2.1.3 | HM sequestration in cell walls, vacuoles, and the Golgi apparatus

The sites for HM sequestration in plant cells mainly include cell walls, vacuoles, and the Golgi apparatus. Cell walls are the first barrier against HM entry into plant cells. HM ions can bind to the functional groups of cell wall components, such as cellulose, hemicellulose, lignin, and pectin (Chen, Liu, Wang, Zhang, & Owens, 2013; Parrotta, Guerriero, Sergeant, Cai, & Hausman, 2015). Some plants can enhance their lignin biosynthesis in response to HM exposure, thereby suggesting that lignin in plant cell walls plays a role in sequestering HMs (Cheng et al., 2014; Elobeid, Gobel, Feussner, & Polle, 2012). By HM sequestration in root cell walls, the entry of HMs into the protoplasts can be reduced, thereby alleviating the toxicity of HMs in plant cells. Inside the cells, toxic HMs are often transported to subcellular organelles such as vacuoles and the Golgi apparatus, where the HMs can also be sequestered (Peiter et al., 2007; Sharma, Dietz, & Mimura, 2016). For instance, Cd is mainly sequestered in vacuoles of parenchyma cells in the leaf mesophyll, stem pith, and cortex in the shoots of the Zn/Cd hyperaccumulator *S. alfredii* (Tian et al., 2017). Vacuolar sequestration of HMs can reduce concentrations of HM ions in the cytosol and thereby alleviate HM toxicity to enzymes involved in cytosolic biochemical reactions. The Golgi apparatus is a part of the endomembrane system in the cytoplasm and packages proteins into membrane-bound vesicles inside the cell before the vesicles are sent to their destination. The Golgi apparatus plays a pivotal role in Mn accumulation via vesicular trafficking and exocytosis to reduce excessive cytosolic $Mn^{2+}$ levels, likely alleviating Mn toxicity in plants (Erbasol, Bozdag, Koc, Pedas, & Karakaya, 2013; Peiter et al., 2007).

### 2.2 | Molecular mechanisms underlying HM accumulation

#### 2.2.1 | Transporters involved in HM uptake and transport

Plant roots take up nonessential and toxic HM ions via transporters for essential metal ions, such as $Mn^{2+}$, $Fe^{2+}$, $Cu^{2+}$, and $Zn^{2+}$. Thus, identifying and functionally characterizing the transporters for these essential metal ions has helped to elucidate the transporters involved in the uptake and translocation of nonessential and toxic HM ions. The roles of many transporters in HM absorption and transport have been characterized in model plants and some hyperaccumulators (Migeon et al., 2010; Shao et al., 2017). These HM transporters include members of the zinc-iron permease (ZIP) family, the natural resistance-associated macrophage protein (NRAMP) family, and the heavy metal ATPases (HMA) family. These transporters play pivotal roles in HM accumulation in plants.

ZIP family members are involved in the uptake and translocation of HMs in plants. *A. thaliana* possesses 18 ZIP members (Migeon et al., 2010). AtZIP1 functions as a vacuolar $Mn^{2+}$ exporter, whereas AtZIP2 is a plasma membrane-localized transporter, which is involved in $Mn^{2+}/Zn^{2+}$ uptake into root stellar cells (Milner, Seamon, Craft, & Kochian, 2013). In addition, the transcript levels of *AtZIP4* and *AtZIP9* increase significantly in *Arabidopsis* roots and shoots after Zn starvation or Cd exposure (Jain, Sinilal, Dhandapani, Meagher, & Sahi, 2013). In the hyperaccumulator *N. caerulescens*, NcZNT1 (a homolog of AtZIP4) modulates the uptake of $Zn^{2+}$ and $Cd^{2+}$ (Pence et al., 2000). Recently, it has been demonstrated that NcZNT1 is a plasma membrane-localized $Zn^{2+}/Cd^{2+}$ transporter and that the *NcZNT1* promoter is mainly active in cells of the cortex, endodermis, and pericycle of *N. caerulescens* roots (Lin, Hassan, Talukdar, Schat, & Aarts, 2016). In addition, iron-regulated transporter 1 (IRT1), a ZIP family member, is a plasma membrane-localized transporter that can absorb $Fe^{2+}$/

$Fe^{3+}$, $Zn^{2+}$, and $Cd^{2+}$, and its transcriptional upregulation has been observed in *N. caerulescens* in response to Fe deficiency (Lombi, Tearall, Howarth, & Zhao, 2002).

NRAMP family members are involved in the transport of bivalent HM ions in plants. Six NRAMP family members have been identified in *Arabidopsis*. AtNRAMP1 is a plasma membrane-localized transporter that is responsible for $Mn^{2+}$ uptake into root cells (Cailliatte, Schikora, Briat, Mari, & Curie, 2010). AtNRAMP3 and AtNRAMP4 are tonoplast membrane-localized transporters and function redundantly by exporting vacuolar $Fe^{2+}$/$Mn^{2+}$ into the cytosol (Lanquar et al., 2005, 2010; Thomine, Wang, Ward, Crawford, & Schroeder, 2000). In the Zn/Cd hyperaccumulator *N. caerulescens*, the NcNRAMP1 protein is implicated in $Cd^{2+}$ uptake into endodermal cells and probably plays a pivotal role in $Cd^{2+}$ movement towards the stele (Milner et al., 2014).

The P1B-type ATPases, also known as HMAs, play key roles in transporting monovalent and divalent HM ions in plants (Williams & Mills, 2005). There are eight HMA members in *A. thaliana* (Migeon et al., 2010). AtHMA1 to AtHMA4 are responsible for the transport of divalent HM ions including $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Pb^{2+}$, and other AtHMAs are involved in the transport of monovalent HM ions such as $Cu^{+}$ and $Ag^{+}$ (Cobbett & Haydon, 2003). AtHMA1 is a chloroplast-localized transporter that is responsible for exporting excessive $Zn^{2+}$ from the chloroplast to the cytosol (Kim et al., 2009). AtHMA2 and AtHMA4 are plasma-membrane-localized transporters that are responsible for the export of cytosolic $Zn^{2+}$ and $Cd^{2+}$ into the vascular cylinder to facilitate the movements of these HM ions from the roots to the shoots (Hussain et al., 2004; Mills, Krijger, Baccarini, Hall, & Williams, 2003). Overexpression of *AtHMA4* in *Arabidopsis* has led to the extraction of greater amounts of Zn and Cd from HM-contaminated soil (Cun et al., 2014). Moreover, HMA proteins have major roles in HM accumulation in hyperaccumulators such as *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz, *N. caerulescens*, and *Sedum plumbizincicola* L. (Craciun et al., 2012; Hanikenne et al., 2008; Liu et al., 2017). For instance, a higher expression level of *AhHMA4*, due to higher number of gene copies in the roots of *A. halleri*, contributes to the high rate of $Zn^{2+}$ translocation from the roots to the shoots (Hanikenne et al., 2008; Talke, Hanikenne, & Krämer, 2006).

### 2.2.2 | Genes involved in HM detoxification and sequestration

A number of genes have been functionally characterized for their essential roles in HM detoxification and sequestration of plant cells, leading to enhanced HM accumulation in plants. Some of these genes belong to the *NRAMP* family, *HMA* family, *ATP-binding cassette* (*ABC*) family, and the *cation diffusion facilitator* (*CDF*) family. For instance, AtNRAMP6, a member of NRAMP family in *A. thaliana*, is localized at a vesicular-shaped endomembrane system, where it is involved in the intracellular distribution of Cd (Cailliatte, Lapeyre, Briat, Mari, & Curie, 2009). AtHMA3 resides in the tonoplast where it transports cytosolic $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Pb^{2+}$ into vacuoles (Morel et al., 2009). In the Cd and Zn hyperaccumulator *S. plumbizincicola*, SpHMA3 is a tonoplast-localized transporter and plays a pivotal role in the vacuolar storage of HMs (Liu et al., 2017). AtABCC1 and AtABCC2 are responsible for the transport of Cd-PCs and Hg-PCs into vacuoles for HM storage in *A. thaliana* (Park et al., 2012).

CDF family members are known as metal tolerance proteins (MTPs). CDF family members function as transporters for HM ions, including $Mn^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Ni^{2+}$, from the cytosol to the outside of the cell or into intracellular compartments (Podar et al., 2012; Ricachenevsky, Menguer, Sperotto, Williams, & Fett, 2013). There are 12 MTPs in *Arabidopsis* (Gustin, Zanis, & Salt, 2011; Migeon et al., 2010). AtMTP1 is a vacuolar transporter that is responsible for mobilizing excessive $Zn^{2+}$ in the cytosol into vacuoles to maintain Zn homeostasis in the cytoplasm (Kobae et al., 2004; Zaal et al., 1999). The MTP1 homolog in *A. halleri* contributes to the Zn hyperaccumulation trait and is also involved in the hyperaccumulation of Zn, Cd, Co, and Ni in *Noccaea goesingense* (Halacsy) F.K.Mey. (formerly known as *Thlaspi goesingense* [Halacsy] F.K.Mey.; Dräger et al., 2004; Persans, Nieman, & Salt, 2001). AtMTP8 is a vacuolar $Mn^{2+}$ transporter involved in the interference of Mn on Fe nutrition (Eroglu, Meier, Von, & Peiter, 2016). AtMTP11 is a Golgi-based $Mn^{2+}$ transporter with a possible role in Mn accumulation via vesicular trafficking and exocytosis (Peiter et al., 2007). Thus, MTPs play crucial roles in the transport of HM ions into cellular organelles or out of cells to the vascular stele to facilitate root-to-shoot mobilization in plants.

Based on the above-mentioned studies, a schematic model of the physiological and molecular mechanisms underlying HM accumulation in nonmycorrhizal plants is presented in Figure 1. Some functionally characterized genes involved in HM accumulation in nonmycorrhizal plants are listed in Table 1.

## 3 | PHYSIOLOGICAL AND MOLECULAR MECHANISMS OF HM ACCUMULATION IN MYCORRHIZAL PLANTS

Ecto- and arbuscular-mycorrhizal plants have altered physiological and molecular characteristics in comparison with nonmycorrhizal plants. Thus, mycorrhizal plants often exhibit increased or reduced HM accumulation. For instance, the leaves of ectomycorrhizal plants of *Populus tremula* L. grown on Zn-contaminated soil contain much higher Zn concentrations compared with those of nonmycorrhizal poplars (Langer et al., 2012). Experimental studies found higher concentrations of Zn in the stem and of Cu in the roots of *Salix* × *dasyclados* Wimm. when inoculated with *Paxillus involutus* (Batsch) Fr. and grown on HM-polluted soil (Baum, Hrynkiewicz, Leinweber, & Meissner, 2006), and greater accumulation of Mn in the needles of ectomycorrhizal Douglas fir (*Pseudotsuga menziesii* variety *glauca* [Mayr] Franco) exposed to excess Mn (Ducic, Parlade, & Polle, 2008) in comparison with that in nonmycorrhizal plants. Similarly, higher concentrations of Cu and Zn and a greater plant biomass were found in arbuscular *Populus alba* L. when grown on Cu- and Zn-polluted soil compared with nonmycorrhizal plants (Cicatelli et al., 2010). Thus, both EM and AM can lead to higher HM concentrations in the host plants and even stimulate plant growth, thereby suggesting that mycorrhizal plants possess enhanced HM accumulation.

However, some ecto- and arbuscular-mycorrhizal plants were found to have lower HM concentrations in their shoots than

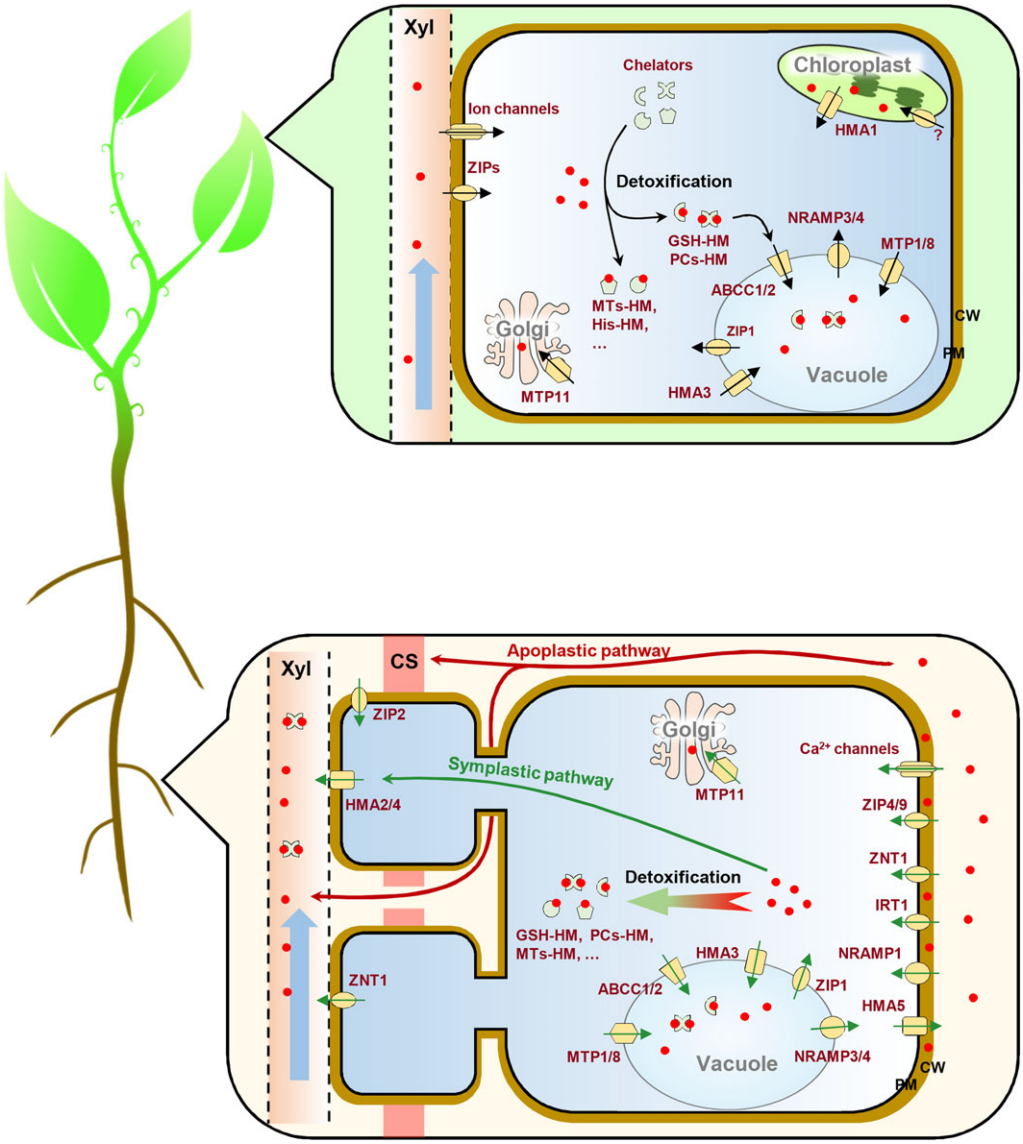


**FIGURE 1** A schematic model for the uptake, translocation, detoxification, and accumulation of HMs in vascular plants. HMs in soil solution are absorbed by roots. In the roots, these HMs are detoxified, sequestered, or transported along the symplastic and/or apoplastic pathways towards the vascular cylinder, where they are loaded into the conduits in the xylem and further translocated to the aerial parts along the transpiration stream. After translocation to the leaves, HMs are unloaded and enter foliar cells for detoxification and storage. HMs access to root cells through plasma membrane transporters or channels, including ZIPs, NRAMPs, and $Ca^{2+}$ channels. HMs in the root and foliar cells can be detoxified by chelators such as metallothioneins (MTs), histidine (His), glutathione (GSH), and phytochelatins (PCs). The chelated- and free-HM ions can be sequestered in the vacuoles through ABCC1/2, MTP1/8, and HMA3 and in Golgi apparatus via MTP11. The HMs sequestered in the vacuoles can also be released back to the cytosol through ZIP1 and NRAMP3/4. In the vascular cylinder of roots, loading of HMs mainly depends on transporters, such as ZNT1 and HMA2/4. In the leaves, HMs enter the cytosol through plasma-membrane-located transporters and ion channels. In the chloroplast, HMs can be transported to the cytosol through HMA1. CW: cell wall; CS: casparian strip; Xyl: xylem; HM: heavy metal; NRAMP: natural resistance-associated macrophage protein; ZIP: zinc-iron permease [Colour figure can be viewed at wileyonlinelibrary.com]

nonmycorrhizal plants (Gu et al., 2017; Sousa, Ramos, Marques, & Castro, 2014; Zhou et al., 2017; Zong et al., 2015). For instance, in ectomycorrhizal *Eucalyptus globulus* Labill. inoculated with *Pisolithus albus* (Cooke & Massee) Priest, the concentrations of HMs, including Co, Cr, Fe, Mn, and Ni, were lower than those in nonmycorrhizal plants (Jourand et al., 2014). Similarly, a Cu-sensitive maize genotype (cv. Orense) inoculated with the arbuscular-mycorrhizal fungus *Rhizophagus irregularis* C. Walker & A. Schuessler had lower Cu concentrations in the shoots compared with nonmycorrhizal plants (Merlos, Zitka, Vojtech, Azcón-Aguilar, & Ferrol, 2016). These results indicate that fungal partners can function as physical barriers to prevent HM entry into host plants, thereby facilitating mycorrhizal-induced resistance to HMs in host plants.These experimental results indicate that HM accumulation in mycorrhizal plants is determined not only by the fungal partners but also by the host plants (Colpaert & Assche, 1992; Coninx, Martinova, & Rineau, 2017; Galli, Meier, & Brunold, 2010; Schützendübel & Polle, 2002). Although some studies have showed that HM accumulation remains unaltered in mycorrhizal plants (Baum et al., 2006; Krpata, Fitz, Peintner, Langer, & Schweiger, 2009; Sell, Kayser, Schulin, & Brunner, 2005), here, we only discuss

**TABLE 1** Some genes involved in HM accumulation of nonmycorrhizal and mycorrhizal plants

| Gene names | Species | Functions related to HM accumulation | References |
|---|---|---|---|
| Nonmycorrhizal plants | | | |
| *ZNT1* | *Noccaea caerulescens* J. Presl & C. Presl | Transporting apoplastic $Zn^{2+}$ and $Cd^{2+}$ into the cytosol | (Lin et al., 2016) |
| *IRT1* | *N. caerulescens* | Transporting apoplastic $Mn^{2+}$, $Fe^{2+}$/$Fe^{3+}$, $Zn^{2+}$, and $Cd^{2+}$ into the cytosol | (Lombi et al., 2002) |
| *NRAMP1* | *N. caerulescens* | Implicating in $Cd^{2+}$ uptake in endodermal cells | (Milner et al., 2014) |
| *NRAMP3/4* | *Arabidopsis thaliana* (L.) Heynh. | Exporting vacuolar $Fe^{2+}$/$Mn^{2+}$ into the cytosol | (Lanquar et al., 2005) |
| *HMA3* | *Sedum plumbizincicola* L. | Transporting cytosolic $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Pb^{2+}$ into vacuoles | (Liu et al., 2017) |
| *HMA4* | *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz | Pumping cytosolic $Zn^{2+}$ and $Cd^{2+}$ into the vascular cylinder | (Hanikenne et al., 2008) |
| *ABCC1/2* | *A. thaliana* | Transporting cytosolic Cd-PCs and Hg-PCs to vacuoles | (Park et al., 2012) |
| *MTP1* | *Noccaea goesingense* (Halacsy) F.K. Mey. | Mobilizing excessive $Zn^{2+}$ from the cytosol into vacuoles | (Dräger et al., 2004) |
| Mycorrhizal fungi | | | |
| *ZRT1* | *Suillus luteus* (L.) Roussel | Involving in $Zn^{2+}$ uptake | (Coninx et al., 2017) |
| *mte1* | *Tricholoma vaccinum* (Schaeff.) P. Kumm. | Exporting HM ions outside of the cell or transporting $Cu^{2+}$/$Ni^{2+}$ into vacuoles | (Schlunk, Krause, Wirth, & Kothe, 2015) |
| *MTs* | *Laccaria bicolor* (Maire) P.D. Orton | Chelating $Cu^{2+}$ and $Cd^{2+}$ in the cytosol | (Reddy, Prasanna, Marmeisse, & Fraissinet-Tachet, 2014) |
| *ZnT1* | *S. luteus* | Transporting excessive $Zn^{2+}$ from the cytosol into vacuoles | (Ruytinx et al., 2017) |
| *ABC1* | *Rhizophagus intraradices* N.C. Schenck & G.S. Sm. | Transporting excessive $Cu^{2+}$ and $Zn^{2+}$ from the cytosol into vacuoles | (González-Guerrero, Benabdellah, Valderas, Azcón-Aguilar, & Ferrol, 2010) |
| Mycorrhizal host plants | | | |
| *ZIP2* | *Populus* × *canescens* (Aiton) Sm. | Putatively transporting apoplastic $Zn^{2+}$ and $Cd^{2+}$ into the cytosol | (Ma et al., 2014) |
| *ABCC1* | *P.* × *canescens* | Putatively transporting cytosolic Cd-PCs and Hg-PCs into vacuoles | (Ma et al., 2014) |
| *CAT* | *Betula pubescens* Ehrh. | Scavenging HM-triggered ROS | (Fernández-Fuego, Keunen, Cuypers, Bertrand, & González, 2017) |
| *NRAMP* | *Solanum lycopersicum* L. | Implicating in $Cd^{2+}$ uptake in endodermal cells | (Fuentes, Almonacid, Ocampo, & Arriagada, 2016) |
| *GS* | *Populus alba* L. AL35 | Catalyzing GSH biosynthesis | (Cicatelli et al., 2012) |
| *PCS* | *P. alba* | Catalyzing PCs biosynthesis | (Pallara, Todeschini, Lingua, Camussi, & Racchi, 2013) |
| *ABC* | *Schedonorus arundinaceus* Schreb | Involving in Ni detoxification | (Shabani, Sabzalian, & Mostafavi, 2016) |
| *MTs* | *S. lycopersicum* | Chelating $Cu^{2+}$ and $Cd^{2+}$ in the cytosol | (Fuentes et al., 2016) |

*Note*. GSH: glutathione; HM: heavy metal; PC: phytochelatin; ROS: reactive oxygen species.

physiological and molecular mechanisms underlying ecto- and arbuscular-mycorrhizal-induced changes in HM accumulation in fungi as well as their host plants.

## 3.1 | Physiological and molecular mechanisms underlying HM accumulation in mycorrhizal fungi

### 3.1.1 | HM detoxification by the exudates of mycorrhizal fungi

The exudates of mycorrhizal fungi can contain HM chelators such as oxalic acid, formic acid, malic acid, and succinic acid, which play important roles in HM detoxification (Colpaert, Wevers, Krznaric, & Adriaensen, 2011; Meharg, 2003; Ray & Adholeya, 2009). There are also positive correlations between the levels of these exudates and the concentrations of HMs, including Cd, Pb, and Ni, inside the hyphae of mycorrhizal fungi (Ray & Adholeya, 2009). When the ectomycorrhizal fungus *Russula atropurpurea* (Krombh.) Britzelm. was exposed to excess Zn, the exudates were rich in cysteine-containing peptides, which can bind up to 80% of the Zn extracted from the sporocarps (Leonhardt, Sácký, Šimek, Šantrůček, & Kotrba, 2014). Moreover, the exudates of arbuscular fungi contain glomalin and HMs ($Cu^{2+}$, $Pb^{2+}$, and $Cd^{2+}$) that can be extracted from the chelates of glomalin-HM complexes (González-

Chávez, Carrillo-González, Wright, & Nichols, 2004). Clearly, the exudates of mycorrhizal fungi can chelate HM ions and reduce their toxicity to the host plants, resulting in elevated HM accumulation in mycorrhizal plants.

### 3.1.2 | Cell walls and vacuoles of mycorrhizal fungi as biological barriers against HM entry into hosts

In addition to HM detoxification via exudates of mycorrhizal fungi, HM ions can be bound by cell walls of mycorrhizal fungi or sequestered in vacuoles. Under these conditions, fungal cell walls and vacuoles may function as biological barriers to prevent HM entry into host plants (Blaudez, Botton, & Chalot, 2000; Frey, Zierold, & Brunner, 2000; Tichelen, Colpaert, & Vangronsveld, 2001; Turnau, Kottke, & Dexheimer, 1996). The components of mycorrhizal fungal cell walls, such as chitin and extracellular slime, can bind HM ions (Meharg, 2003). Cd is mainly restricted to the cell walls of the fungus in the extraradicle hyphae of AM between *Lotus japonicus* L. and *R. irregularis* (Nayuki, Chen, Ohtomo, & Kuga, 2013). In addition, the vacuoles of mycorrhizal fungi can sequester large amounts of HM ions (Blaudez et al., 2000; González-Guerrero et al., 2008; Nayuki et al., 2013). Several HM ions, including $Cu^{2+}$, $Zn^{2+}$, and $Cd^{2+}$, are mainly localized in the vacuoles in the extraradicle hyphae of the arbuscular-mycorrhizal fungus *Rhizophagus intraradices* N.C. Schenck & G.S. Sm. (previously known as *Glomus intraradices*; González-Guerrero et al., 2008). Thus, the cell walls and vacuoles of mycorrhizal fungi play key roles in HM sequestration, thereby decreasing the amount of HM ions that enter the host plants, leading to enhanced HM accumulation in the fungal partners of mycorrhizae and reducing toxic effects of HMs to host plants.

### 3.1.3 | Transcriptional regulation of genes involved in HM accumulation in mycorrhizal fungi

In EM and AM, the fungal partner often functions as the first biological filter against HMs in the soil solution. At the molecular level, ecto- and arbuscular-mycorrhizal fungi can regulate the expression levels of a number of genes related to the absorption, transport, detoxification, and sequestration of HMs in fungal cells. In the ectomycorrhizal fungus *Suillus luteus* (L.) Roussel, transcripts of a member of the *ZIP* family involved in Zn uptake, *SlZRT1*, are accumulated immediately when grown in medium without Zn, but the mRNA level of *SlZRT1* is reduced upon exposure to elevated concentrations of Zn (Coninx, Thoonen, et al., 2017). In the ectomycorrhizal fungus *Tricholoma vaccinum* (Schaeff.) P. Kumm., mte1, a multidrug and toxic compound extrusion protein, is localized on the plasma membrane and/or the vacuolar membrane where it can export HM ions outside the cell or transport $Cu^{2+}$/$Ni^{2+}$ into vacuoles depending on its localization, thereby facilitating Cu and Ni detoxification in fungal cells (Schlunk et al., 2015). In the ectomycorrhizal fungus *Tuber melanosporum* Vittadini, the transcript levels of genes encoding metal transporters related to Cu and Zn trafficking were found to be increased in mycorrhizae, indicating active translocation of both metals to root cells and to fungal metalloenzymes (Bolchi et al., 2011). In mycorrhizal fungal cells, MTs play critical roles in chelating HM ions. Several MT genes were characterized, and their expression levels were assessed in fungal cells in response to HMs. In *S. luteus*, SlMTa and SlMTb function as chelators for $Cu^{2+}$ and the transcript abundance of both genes is increased by exposure to excess $Cu^{2+}$ (Nguyen et al., 2017). Similarly, the transcriptional levels of the MT genes from *Laccaria bicolor* (Maire) P.D. Orton, *LbMT1* and *LbMT2*, are upregulated upon excess Cu exposure, and the mRNA level of *LbMT1* also responds to Cd treatment (Reddy et al., 2014). In the ectomycorrhizal fungus *P. albus*, increased transcript levels of *PaMT1* were observed in both the extraradicle hyphae and the mycorrhizae with the host *Eucalyptus tereticornis* Sm. in response to Cd and excess Cu (Reddy et al., 2016). Thus, transcriptional induction of fungal MT genes probably contributes to HM detoxification in ectomycorrhizal fungi. Recently, *SlZnT1* and *SlZnT2* from *S. luteus*, encoding CDF family transporters have been characterized. SlZnT1 was found to be localized in the tonoplast and plays a role in transporting excess Zn into the vacuole (Ruytinx et al., 2017). Both *SlZnT* genes are constitutively expressed, irrespective of the external Zn concentrations (Ruytinx et al., 2017).

In addition, the efflux of HMs is a valuable strategy employed by mycorrhizal fungi to reduce the accumulation and toxicity of HMs. This is indicated by transcriptional induction of genes involved in the efflux of HMs from fungal cells in response to HM exposure. Three genes that encode a P-type ATPase, an ABC transporter, and a major facilitator superfamily permease, respectively, which are involved in the efflux of HMs from fungal cells, were characterized in the Ni-tolerant ectomycorrhizal fungus *P. albus*, where transcriptional overexpression of these genes was observed in mycelia following exposure to Ni (Majorel, Hannibal, Ducousso, Lebrun, & Jourand, 2014). Therefore, these genes seem to play key roles in the export of Ni from *P. albus* cells into the soil solution.

Similar to ectomycorrhizal fungi, genes involved in the transport, detoxification, and sequestration of HMs were characterized in arbuscular-mycorrhizal fungi. *RintABC1* was isolated from *R. intraradices* as a putative transporter involved in the detoxification of Cu and Zn in fungal cells (González-Guerrero et al., 2010). MTs in arbuscular-mycorrhizal fungi can chelate HMs by binding HM ions via the thiol group of the cysteine residues (Latef et al., 2016). Two fungal genes, *GrosMT1* and *RintMT1*, have been reported to be involved in HM detoxification. *GrosMT1* was found in *Gigaspora rosea* T.H. Nicolson & N.C. Schenck BEG9 (Stommel, Mann, & Franken, 2001). *RintMT1* was isolated from *R. intraradices* and is involved in maintaining reactive oxygen species (ROS) homeostasis under HM stress (González-Guerrero, Cano, Azcón-Aguilar, & Ferrol, 2007). *RintZnT1* was also isolated from *R. intraradices* as a putative transporter implicated in vacuolar Zn sequestration (González-Guerrero et al., 2005). The transcriptional upregulation of several genes encoding a Zn transporter, an MT, a 90-kDa heat shock protein, and a glutathione S-transferase was found in the intraradicle and extraradicle mycelia of *R. intraradices* in response to Cd and excess Cu or Zn (Hildebrandt, Regvar, & Bothe, 2007).

Overall, these results suggest that molecular regulation of genes involved in the absorption, transport, detoxification, and sequestration of HMs has key roles in HM accumulation in mycorrhizal fungal cells, which can further affect the mobilization of HMs towards host plants.

## 3.2 | Physiological and molecular mechanisms underlying HM accumulation in mycorrhizal host plants

### 3.2.1 | Mycorrhizal-improved nutritional and water status of hosts conferring HM accumulation

Many studies have demonstrated that the nutritional and water status of ectomycorrhizal- and arbuscular-mycorrhizal plants are often superior to nonmycorrhizal plants (Coninx, Martinova, & Rineau, 2017; Godbold, Jentschke, Winter, & Marschner, 1998; Jamal, Ayub, Usman, & Khan, 2002; Luo et al., 2014). The improved nutritional and water status of mycorrhizal plants is mainly ascribed to the enhanced uptake of nutrients and water from the soil by mycorrhizal hyphae. It can stimulate growth and alter the biosynthesis of numerous metabolites, such as free amino acids, phytohormones, and fatty acids, to deal with various stresses including HM stress (Luo et al., 2009; Luo et al., 2011; Luo, Ke, Jiang, & Polle, 2009; Ma et al., 2014). Ectomycorrhizal *Populus* × *canescens* (Aiton) Sm. plants possess larger surface area of fine roots, higher concentrations of nutrients, including phosphorus (P), Ca, Fe, and Zn, elevated photosynthetic rates, and increased soluble carbohydrate contents compared with nonmycorrhizal poplars, and exhibit faster $Cd^{2+}$ uptake and greater Cd accumulation in the hosts (Ma et al., 2014). In arbuscular-mycorrhizal pigeonpea (*Cajanus cajan* L. Millsp.) plants, AM improve the nutritional (P, N, Mg, and Fe) and water status, and they stimulate proline biosynthesis in the host, which enhances the accumulation of Cd and Zn (Garg & Singh, 2018).

### 3.2.2 | Mycorrhizal-induced antioxidative system of hosts for scavenging HM-triggered ROS

During the establishment of EM and/or AM, higher concentrations of antioxidants are often detected in host plants in comparison with those of uninoculated plants (Coninx, Martinova, & Rineau, 2017; Garg & Bhandari, 2014). The mycorrhizal-induced antioxidative system in hosts can scavenge excess ROS produced by HM exposure (Emamverdian, Ding, Mokhberdoran, & Xie, 2015). The antioxidative system includes enzymatic and nonenzymatic antioxidants. Higher antioxidative enzyme activities have been frequently reported in mycorrhizal plants and may contribute to the scavenging of HM-induced ROS (Canton et al., 2016; Rozpądek et al., 2014). Recently, it was showed that the activities of superoxide dismutase, ascorbate peroxidases, and glutathione peroxidase are elevated in arbuscular-mycorrhizal *Robinia pseudoacacia* L., which probably contributes to the alleviation of Pb-induced toxicity (Yang et al., 2015). In addition, nonenzymatic antioxidants, such as Asc, GSH, proline, and phenolic compounds, play key roles in ROS homeostasis in mycorrhizal plants exposed to HMs (Coninx, Martinova, & Rineau, 2017; Luo et al., 2014). In ectomycorrhizal poplars, higher concentrations of Asc and/or GSH were found in the root, wood, bark, and leaf tissues compared with those in nonmycorrhizal poplars. Elevated antioxidant levels may contribute to the scavenging of Cd-induced ROS in the hosts (Ma et al., 2014). Therefore, the mycorrhizal-induced antioxidative system can contribute to ROS homeostasis in HM-exposed plants, thereby improving HM accumulation in mycorrhizal plants.

### 3.2.3 | Mycorrhizal-fungal-induced differential expression of host genes implicated in HM uptake, transport, detoxification and sequestration

Mycorrhizal fungi can induce the transcriptional regulation of host genes implicated in HM accumulation in response to HM exposure. In the EM of *P.* × *canescens* colonized with *P. involutus*, transcriptional overexpression of several genes implicated in HM uptake, translocation, detoxification, and sequestration, including *ZIP2*, *NRAMP*, *HMA4*, *PCS*, and *ABCC1*, leads to enhanced Cd accumulation (Ma et al., 2014). Furthermore, EM-induced transcript levels of *HA2.1* and *AHA10.1* genes that encode plasma membrane $H^+$-ATPases were detected in the host *P.* × *canescens* (Ma et al., 2014). Recently, it was demonstrated that activated plasma membrane $H^+$-ATPases can stimulate $Cd^{2+}$ uptake through hyperpolarization-activated $Ca^{2+}$ channels in ectomycorrhizal roots of this poplar species (Zhang et al., 2017). In addition, differential expression of a number of genes involved in HM transport, detoxification, and stress signalling has also been reported in host plants inoculated with arbuscular-mycorrhizal fungi. Inoculation with arbuscular-mycorrhizal fungi leads to the transcriptional upregulation of *MT1*, *MT2*, *MT3*, and *PCS* in the leaves of poplar plants grown on HM-contaminated soil (Cicatelli et al., 2010, 2012; Pallara et al., 2013). The transcriptional induction of genes involved in HM accumulation was also found in herbaceous plants, including ABC transporter and MT genes in Ni-tolerant tall fescue plants inoculated with *Funneliformis mosseae* (formerly known as *Glomus mosseae*; Nicol. & Gerd.) Gerd. & Trappe under Ni exposure (Shabani et al., 2016) and *NRAMP*, *MT2b*, and *PCS* in *Solanum lycopersicum* L. inoculated with *R. irregularis* grown on HM-polluted soil (Fuentes et al., 2016). These findings indicate that mycorrhizal-induced transcriptional overexpression of host genes involved in HM transport, detoxification, and sequestration can lead to enhanced HM accumulation in mycorrhizal plants.

### 3.2.4 | Mycorrhizal-fungi-activated defence-related genes of hosts enhance HM accumulation

Plants inoculated with mycorrhizal fungi can enhance their defence systems to cope with HM-induced stress. Activation of defence systems has been documented in plants colonized by mycorrhizal fungi (Fernández-Fuego et al., 2017; Lingua et al., 2012; Luo, Janz, et al., 2009). For instance, the formation of EM between *P.* × *canescens* and *P. involutus* activates defence-related genes and signalling cascades in host plants, thereby priming related pathways to enhance salt tolerance (Luo, Janz, et al., 2009). Similarly, EM-induced defence-related genes may lead to increased accumulation of HMs. In particular, transcriptional upregulation of genes encoding catalase (CAT) and APX, as well as higher enzymatic activities of CAT and APX, were detected in ectomycorrhizal birch plants (*Betula pubescens* Ehrh.) grown on HM-polluted soil compared with nonmycorrhizal plants (Fernández-Fuego et al., 2017). The defence system may also be improved in plants inoculated with arbuscular-mycorrhizal fungi. Upregulation of defence-related genes, including genes encoding thaumatin-like protein, glutathione synthase, remorin, and peroxidase was found in the leaves of poplar plants inoculated with arbuscular-

mycorrhizal fungi and grown on HM-polluted soil (Cicatelli et al., 2012, 2014). These findings suggest that mycorrhizal fungi can enhance the defence systems of host plants to cope with HM stress.

The physiological and molecular mechanisms related to mycorrhizal-induced HM accumulation in host plants have prompted us to propose the model that is illustrated in Figure 2. Some genes involved in HM accumulation in mycorrhizal plants are presented in Table 1.

## 4 | PHYTOREMEDIATION BY MYCORRHIZAL PLANTS

At present, it is estimated that 3.5 million sites in the European Union and 600,000 sites in the USA have been polluted by HMs (Coninx, Martinova, & Rineau, 2017). In China, national soil surveys showed that ~16% of soil samples were polluted with HMs (equivalent to ~$1.0 \times 10^6$ $km^2$ if we assume that the area is proportional to the number of samples; Zhao, Ma, Zhu, Tang, & Mcgrath, 2015). Throughout the world, contamination with HMs is of great concern due to their effects on human health and ecosystem sustainability. Therefore, HM-polluted soils must be remediated, and phytoremediation of HM-contaminated soil has been proposed due to its low cost, environmental friendliness, and good performance (Krämer, 2005; Meagher, 2000; Peuke & Rennenberg, 2005). Phytoremediation involves the utilization of plants and associated microorganisms to absorb HMs from soil solutions before finally eliminating the HMs from soils by harvesting the plants (Luo et al., 2014; Raskin, Smith, & Salt, 1997). As discussed in the previous sections, ecto- and arbuscular-mycorrhizal fungi mediate HM accumulation in their host plants. Thus, using mycorrhizal plants to remediate HM-polluted soils is a valuable strategy.

The establishment of mycorrhizae can facilitate the modulation of HM accumulation in host plants. Therefore, it is important to select appropriate fungal and host plant partners to form mycorrhizae for the use in remediation of HM-polluted soils. Furthermore, the accumulation of HMs can be enhanced, reduced, or not affected in host plants after inoculation with ecto- or arbuscular-mycorrhizal fungi. To remediate HM-polluted soil, it is desirable to use mycorrhizal fungi that can stimulate uptake and accumulation of HMs in host plants. Thus, several ecto- and arbuscular-mycorrhizal fungi as well as their host plant partners have been explored to assess their capacity for remediating HM-contaminated soils (Aggangan & Aggangan, 2012; Ho-Man et al., 2013; Luo et al., 2014). For instance, EM between *P.* × *canscens* and *P. involutus* stimulate the uptake and accumulation of $Cd^{2+}$ in aerial plant parts (Ma et al., 2014). Similarly, arbuscular-mycorrhizal fungi can stimulate the accumulation of HMs in their host plants. The rates of Pb accumulation are higher in the shoots of vetiver grass (*Vetiveria zizanoides*) inoculated with *R. intraradices* exposed to Pb compared with nonmycorrhizal plants (Bahraminia, Zarei, Ronaghi, & Ghasemi-Fasaei, 2016). Sunflower plants inoculated with *R. irregularis* or *F. mosseae* accumulate more Cd or Zn in their aerial parts than nonmycorrhizal plants (Hassan, Hijri, & Starnaud, 2013). These studies indicate that selecting appropriate mycorrhizal fungi can enhance the accumulation of HMs in host plants and increase the efficiency of phytoremediation. However, it has to be pointed out that some mycorrhizal fungi can lead to less HM accumulation in host plants, which may decrease the efficiency of phytoremediation in the field (Luo et al., 2014; Sousa et al., 2014). For instance, lower Cu contents were found in the shoots of a maize genotype (cv. Orense) inoculated with *R. irregularis* in comparison with nonmycorrhizal plants (Merlos et al., 2016). Thus, it should be noted that the effects of mycorrhizal-assisted phytoremediation on the accumulation of HMs by host plants depend largely on the fungal isolates, the specific HMs, and other factors (Schneider, Bundschuh, Rangel, & Guilherme, 2017).

Selected fungal isolates, host plants, HMs, and the effects of mycorrhizae on host plant growth and accumulation of HMs in host plants are summarized in Table 2.

## 5 | CONCLUSIONS AND OUTLOOK

In nonmycorrhizal plants, nonessential and toxic HM ions enter plants through both apoplastic and symplastic pathways. HM ions in the cells can be detoxified with the aid of several chelators, such as GSH, PCs, MTs, His, and NA. Subsequently, HMs can be sequestered in cell walls, vacuoles, the Golgi apparatus, and other subcellular organelles in plants. A number of HM transporters are involved in HM accumulation in nonmycorrhizal plants. The uptake and translocation of HMs are mediated by members of the ZIPs, NRAMPs and HMAs, and HM detoxification and sequestration are mainly modulated by members of the ABCs and MTPs in nonmycorrhizal plants.

Plant roots often form ecto- and/or arbuscular-mycorrhizae with soil mycorrhizal fungi under natural conditions. Mycorrhizal plants have modified physiological characteristics and molecular processes compared with nonmycorrhizal plants, thereby often leading to enhanced HM accumulation in host plants. The enhanced HM accumulation in mycorrhizal plants is mainly related to the following physiological and molecular mechanisms in the fungal partners: (a) HM detoxification by the exudates of mycorrhizal fungi in the rhizosphere, (b) HM sequestration in the cell walls and vacuoles of mycorrhizal fungi, and (c) transcriptional regulation of genes involved in HM accumulation in mycorrhizal fungi and to the following molecular and physiological adjustments in the host plants: (a) mycorrhizal-improved nutritional and water status, (b) mycorrhizal-induced antioxidative systems for scavenging HM-triggered ROS, (c) mycorrhizal-fungi-induced differential expression of host genes implicated in HM accumulation, and (d) mycorrhizal-fungi-activated defence-related genes. Therefore, it is desirable to select mycorrhizal fungi to stimulate the accumulation of HMs in host plants to remediate HM-contaminated soils.

The physiological and molecular mechanisms that underlie HM accumulation have been studied extensively in nonmycorrhizal and mycorrhizal plants, but the identification and functional characterization of genes involved in HM accumulation require further research. First, many genes involved in uptake, translocation, detoxification, and accumulation of HMs were discovered and studied in *A. thaliana* and *N. caerulescens* that are unable to be colonized by ecto- and/or arbuscular-mycorrhizal fungi. Therefore, the functions and transcriptional regulation of these genes must be verified in plants that are able

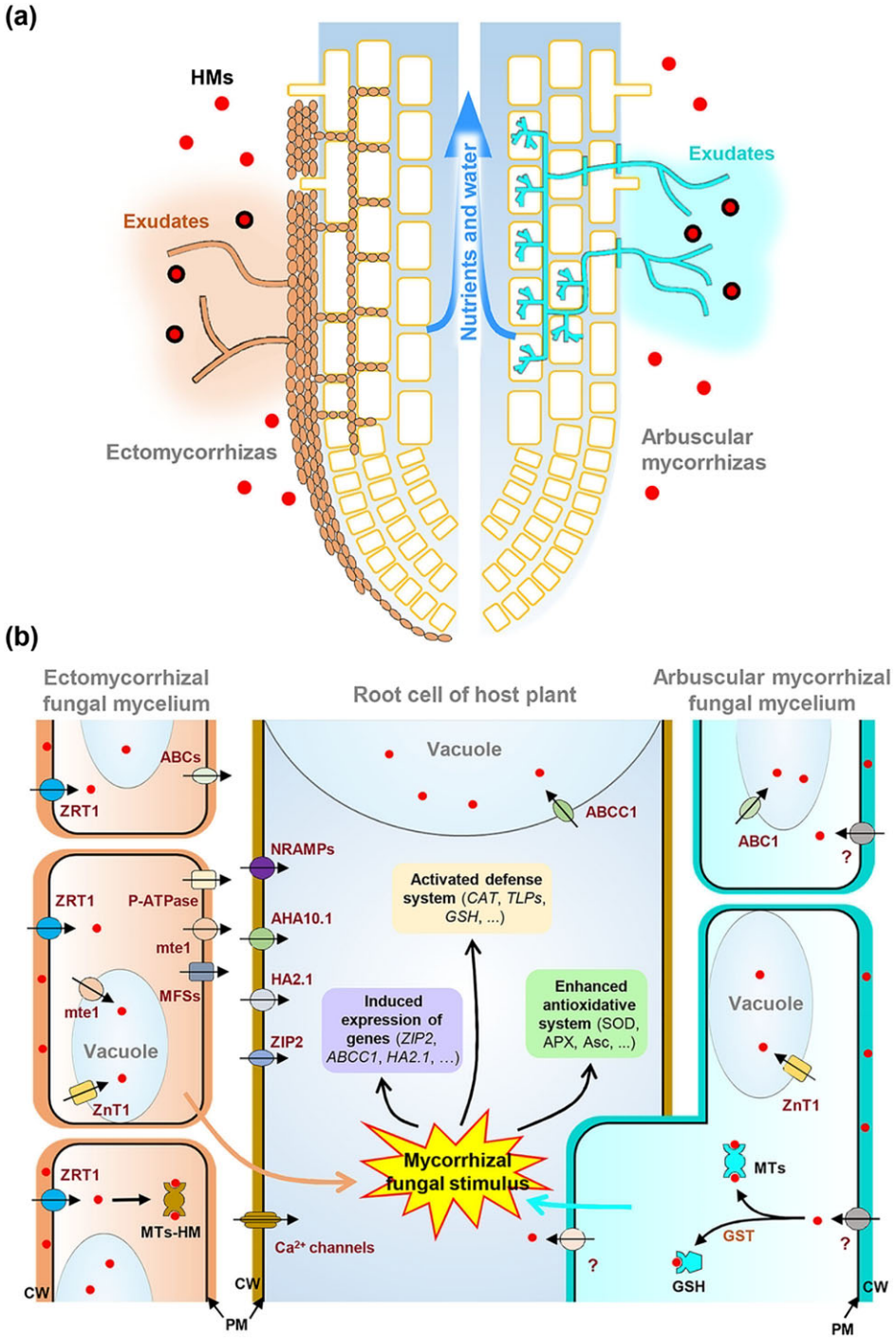


**FIGURE 2** Schematic representation of ecto- and arbuscular-mycorrhizal-induced HM accumulation of host plants. (a) A tangential-section view of EM and AM, and physiological mechanisms underlying mycorrhizal-mediated HM accumulation in host plant. (b) The cellular and molecular processes of mycorrhizal-modulated HM accumulation in host plant. The physiological processes of mycorrhizal-mediated HM accumulation mainly include (i) binding HMs by exudates of mycorrhizal fungi and (ii) enhanced uptake of mineral nutrients and water. The cellular processes for HM detoxification mainly include (i) reduced HM movement to host plants through cell wall chelating and vacuolar storage of mycorrhizal fungi, (ii) pumping HM out of cytosol, (iii) chelation of HM in cytosol, and (iv) enhanced antioxidative system in mycorrhizal plants. The molecular processes for mycorrhizal-mediated HM accumulation mainly include (i) upregulated expression of genes involved in HM uptake, translocation, detoxification, and sequestration in fungal cells, including *P-ATPase*, *ABCs*, *met1*, *MFSs* (*major facilitator superfamily permease*), *ABC1*, *ZnT1*, *MTs*, and *GST*; (ii) triggered the differential transcriptional regulation of the host genes involved in HM accumulation in plants, including *ZIP2*, *NRAMPs*, *HA2.1*, *AHA10.1*, *$Ca^{2+}$ channels*, and *ABCC1*; and (iii) induced transcriptional levels of genes related to the defence system of host plants, including *GSH*, *PCS*, *TLPs*, *MTs*, and genes encoding antioxidative enzymes. Fungal and plant cells do not have the same size as drawn. CW: cell wall; PM: plasma membrane; AM: arbuscular mycorrhizae; EM: ectomycorrhizae; HM: heavy metal [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** The selected ecto- and arbuscular-mycorrhizae mediate HM accumulation of host plants

| Microorganisms | Plants | Metals | Biomass increment (%)[a] | Metal con. increment (%)[b] | References |
|---|---|---|---|---|---|
| Ectomycorrhizae | | | | | |
| *Agaricus bisporus* (J.E.Lange) Imbach | *Pistacia vera* L. | Zn | NA | −48 ↓ | (Mohammadhasani, Ahmadimoghadam, Asrar, & Mohammadi, 2017) |
| *Pisolithus arrhizus* (Scop.) Rauschert *Cenococcum geophilum* Fr. | *Pinus tabulaeformis* Carr. | Cu | 73 | ~50 | (Wen et al., 2016) |
| *Paxillus involutus* (Batsch) Fr. | *Populus* × *canescens* (Aiton) Sm. | Cd | 27 | ~22 | (Ma et al., 2014) |
| *Rhizopogon roseolus* (Corda) Th.Fr. | *Pinus pinaster* Aiton | Cd | 30 | 36 | (Sousa, Ramos, Marques, & Castro, 2012) |
| *Hebeloma mesophaeum* (Pers.) Quél. | *Salix viminalis* L. | Cd/Zn | 51 | ~12 (Cd), 19 (Zn) | (Hrynkiewicz, Dabrowska, Baum, Niedojadlo, & Leinweber, 2012) |
| *Pisolithus* sp. (from Eucalyptus coded internationally as H6394) | *Eucalyptus urophylla* S.T.Blake | Cu | 347 | 303 | (Aggangan & Aggangan, 2012) |
| *H. mesophaeum* | *Populus nigra* L. | Cd Zn/ Pb | 20 | −50 (Cd), −45 (Pb), −30 (Zn) ↓ | (Mrnka et al., 2012) |
| *P. arrhizus* (Pers.) Coker and Couch | *Populus alba* × *Populus tremula* var. *glandulosa* | Cd | 25 | 70 | (Han, Kim, & Lee, 2011) |
| *Pisolithus albus* (Cooke and Massee) Priest | *Eucalyptus globulus* Labill. | Ni | 500 | −95 ↓ | (Jourand et al., 2012) |
| Arbuscular mycorrhizae | | | | | |
| *Funneliformis coronatum* Giovannetti | *Tagetes patula* L. | Cu | ~50 | ~−73 ↓ | (Zhou et al., 2017) |
| *Gigaspora margarita* Becker & Hall | *Miscanthus sacchariflorus* (Maxim.) Franch. | Zn | ~67 | 250 | (Sarkar, Asaeda, Wang, Kaneko, & Rashid, 2017) |
| *Rhizophagus intraradices* N.C. Schenck & G.S. Sm. | *Capsicum annuum* L. | Cu | ~27 | ~400 | (Ruscitti, Arango, & Beltrano, 2017) |
| *Rhizophagus irregularis* C. Walker & A. Schuessler | *Medicago sativa* L. | Cd/Ni | 185 (Cd), 49 (Ni) | 295 (Cd), 155 (Ni) | (Mnasri, Janoušková, Rydlová, Abdelly, & Ghnaya, 2016) |
| *R. intraradices* | *Populus deltoides* Bartr. | Cd | 8 (male), 6 (female) | ~100 (male), ~−44 (female) | (Chen et al., 2016) |
| *Diversispora versiformis* (P. Karsten) S.M. Berch | *Solanum nigrum* L. | Cd | 310 | 259 | (Liu et al., 2015) |
| *R. intraradices* | *Linum usitatissimum* L. | Cd/Cr | 17 (Cd), 19 (Cr) | 27 (Cd), 47 (Cr) | (Amna et al., 2015) |
| *Funneliformis mosseae* (Nicol. & Gerd.) Gerd. & Trappe | *Populus cathayana* Rehd. | Pb | 15 | 25 | (Chen et al., 2015) |
| *R. roseolus* | *Pinus pinaster* Aiton | Cd | 14 | 36 | (Sousa et al., 2014) |
| *F. mosseae* | *Zea mays* L. | Cd | 13 | 350 | (Liu, Gong, Zhang, & Li, 2014) |
| *F. mosseae* | *Helianthus annuus* L. | Pb | 13 | 316 | (Jarrah et al., 2014) |
| *F. mosseae* | *P. alba* L. "AL35" | Cu/Zn | 464 | Cu (58), Zn (38) | (Pallara et al., 2013) |

*Note*. HM: heavy metal; NA: no data available; ~: approximately.

[a]Biomass increment (%) = (Leaf or shoot biomass of mycorrhizal plant − Leaf or shoot biomass of nonmycorrhizal plant)/Leaf or shoot biomass of nonmycorrhizal plant × 100.

[b]Metal con. increment (%) = (Metal concentrations in leaf or shoot of mycorrhizal plant − Metal concentrations in leaf or shoot of nonmycorrhizal plant)/Metal concentrations in leaf or shoot of nonmycorrhizal plant × 100.

to establish ecto- and arbuscular-mycorrhizae in response to HMs. Second, in future studies, additional key genes involved in the uptake, translocation, detoxification, and sequestration of HMs should be functionally characterized in mycorrhizal plants to further increase accumulation of HMs. Third, some mycorrhizal plants can increase the accumulation of HMs, but the underlying molecular mechanisms still need to be elucidated to create genetically modified mycorrhizal plants that facilitate more efficient phytoremediation of HM-polluted soils. Finally, large numbers of nonmycorrhizal and mycorrhizal plants have been examined to assess their HM accumulation under laboratory conditions, but only a limited number of these plants have been applied in HM-polluted field sites (Gerhardt, Gerwing, & Greenberg, 2016; Peuke & Rennenberg, 2005). Thus, it is important to investigate these plants and their fungal partners to assess their performance in HM-contaminated soils under field conditions.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Z. B. L. and S. L. C. planned and designed the outline of this review. Z. B. L., W. G. S., and Y. H. Z. prepared the figures and tables. Z. B. L., W. G. S., Y. H. Z., S. L. C., A. P., and H. R. wrote the manuscript.

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