

# Water relations in plants subjected to heavy metal stresses

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**Abstract** Concentrations of heavy metals in soil seldom reach a level sufficient to cause osmotic disturbances in plants. It is likely that water entry to the roots is indirectly governed by other factors which are themselves affected by metals. Decreased elongation of the primary root, impaired secondary growth, increased root dieback, or reduced root hair caused by toxic ions all exert a deleterious effect on the root-absorbing area and water uptake. Moreover, metals are able to decelerate short-distance water transfer both in symplast and apoplast, which in turn reduce the movement of water into the vascular system and affect water supply to the shoot. Long-distance transport is limited also due to decreased hydraulic conductivity in the root, stem and leaf midrib caused by a reduction in the size of vessels and tracheids, and partial blockage of xylem elements by cellular debris or gums. Heavy metals influence water delivery to the shoot due to inhibition of transpiration as they decrease the size of the leaves and the thickness of the lamina, reduce intercellular spaces, affect the density of stomata and decrease their aperture. Stomata closure is induced by direct interaction of toxic metals with guard cells and/or as a consequence of the early effects of metal toxicity on roots and stems. In metal-stressed plants, root-derived ABA or ABA-induced signals might play a role in stomatal movement. Disturbances in water relations trigger differential regulation of aquaporin gene expression, which may contribute to further reductions in water loss.

**Keywords** Water transport · Dehydration · Aquaporin · Abscisic acid (ABA) · Cytokinin

## Introduction

Heavy metals as a general collective term refers to metals and metalloids with an atomic number above 20 and a relatively high density greater than 4 g/cm<sup>3</sup>, or 5 times or more, greater than water (Barceló and Poschenrieder 1990; Nagajyoti et al. 2010). Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), Cu (copper), iron (Fe), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag), mercury (Hg) and elements in the platinum group (Nagajyoti et al. 2010).

An excess of both essential and non-essential metals induces ion stress in plants and causes multiple direct or indirect effects, which concern practically all physiological functions. This review provides a broad overview of the data for an influence of metals on plant water relations at the cellular level as well as at organism level as a whole. It has been reported that water content decreased in various organs of plants grown in solutions containing toxic levels of metal ions (Table 1). Therefore, the attempt was undertaken to demonstrate some common effects of metal-induced water deficiency, mechanisms engaged in maintaining plant water balance or those providing for tolerance to its disturbance. It appears evident that specific mechanisms are employed for specific metals in particular species but this consideration introduces a further layer of complexity that is beyond the scope of this review.

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**Table 1** Selected reports describing heavy metal-induced dehydration in plants

Heavy metal	Organ	Plant specie	References
Cd	Leaf	<i>Phaseolus vulgaris</i>	Barceló et al. (1986a)
	Leaf	<i>Phaseolus vulgaris</i>	Becerril et al. (1989)
	Leaf	<i>Medicago sativa</i>	Becerril et al. (1989)
	Leaf	<i>Triforium pratense</i>	Poschenrieder et al. (1989)
	Leaf	<i>Helianthus annuus</i>	Kastori et al. (1992)
	Root, shoot	<i>Oryza sativa</i>	Moya et al. (1993)
	Shoot	<i>Lactuca sativa</i>	Costa and Morel (1994)
	Shoot	<i>Lupinus albus</i>	Costa and Morel (1994)
	Shoot	<i>Pisum sativum</i>	Hernández et al. (1997)
	Leaf	<i>Silene vulgaris</i>	Schat et al. (1997)
	Leaf	<i>Hordeum vulgare</i>	Vassilev et al. (1997, 1998)
	Root, leaf	<i>Mesembr. cryst.</i>	Shevyakova et al. (2003)
	Co	Leaf	<i>Brassica oleracea</i>
Cr	Leaf	<i>Phaseolus vulgaris</i>	Barceló et al. (1986b)
Cu	Leaf	<i>Helianthus annuus</i>	Kastori et al. (1992)
	Leaf	<i>Silene vulagris</i>	Schat et al. (1997)
	Shoot	<i>Oryza sativa</i>	Chen et al. (2004)
Ni	Leaves	<i>Mesembr. cryst.</i>	Kholodova et al. (2005, 2011)
	Root, shoot	<i>Oryza sativa</i>	Moya et al. (1993)
	Root, shoot	<i>Pisum sativum</i>	Gabbrielli et al. (1999)
	Leaf	<i>Brassica oleracea</i>	Pandey and Sharma (2002)
	Shoot	<i>Triticum aestivum</i>	Gajewska et al. (2006)
Pb	Root, shoot	<i>Oryza sativa</i>	Llamas et al. (2008)
	Root, stem, leaf	<i>Psidium guajava</i>	Bazihizina et al. (2015)
	Leaf	<i>Medicago sativa</i>	Becerril et al. (1989)
	Leaf	<i>Triforium pratense</i>	Becerril et al. (1989)
	Leaf	<i>Helianthus annuus</i>	Kastori et al. (1992)
	Shoot	<i>Brassica juncea</i>	Vassil et al. (1998)
Zn	Leaf	<i>Carthamus tinctorius</i>	Sayed (1999)
	Leaf	<i>Helianthus annuus</i>	Kastori et al. (1992)
	Leaf	<i>Silene vulagris</i>	Schat et al. (1997)
	Root < stem < leaf	<i>Phaseolus vulgaris</i>	Kasim et al. (2007)
	Leaf	<i>Mesembr. cryst.</i>	Kholodova et al. (2005, 2011)
	Root	<i>Nicotiana tabacum</i>	Bazihizina et al. (2014)
	Root, shoot	<i>Beta vulgaris</i>	Sagardoy et al. (2010)

*Mesembr. cryst.* *Mesembryanthemum crystallinum*

## Effects of heavy metals on water uptake

The main functions of plant roots are the absorption of water and inorganic nutrients, supporting the plant body and anchoring it to the ground, storage of food and nutrients, and vegetative reproduction. These organs are also the first-contact sites for heavy metal ions and significantly higher amounts of metal usually accumulate in them than in aboveground plant parts (Barceló et al. 1988; Becerril et al. 1989; Burkhead et al. 2009; Chen et al. 2004; Feleafel and Mirdad 2013; Kastori et al. 1992; Małeczka et al. 2008; Seregin and Ivanov 2001). An excess of metal ions in

tissues may affect water absorption from the soil, and in turn decrease water content in the root. It has been experimentally proved that a water deficit in this organ occurs as a result of exposure to Cd, Ni and Zn (Table 1).

The impact of heavy metals on plant water relations has to be distinguished from their effects on water availability in soils, on root growth, limiting water uptake, as well as other phytotoxic effects. If soils are high in soluble salts (including heavy metal salts), the osmotic potential in the soil solution might be lower than the potential of the cell sap in root. Under these circumstances, the soil solution would severely restrict the rate of water uptake by plants

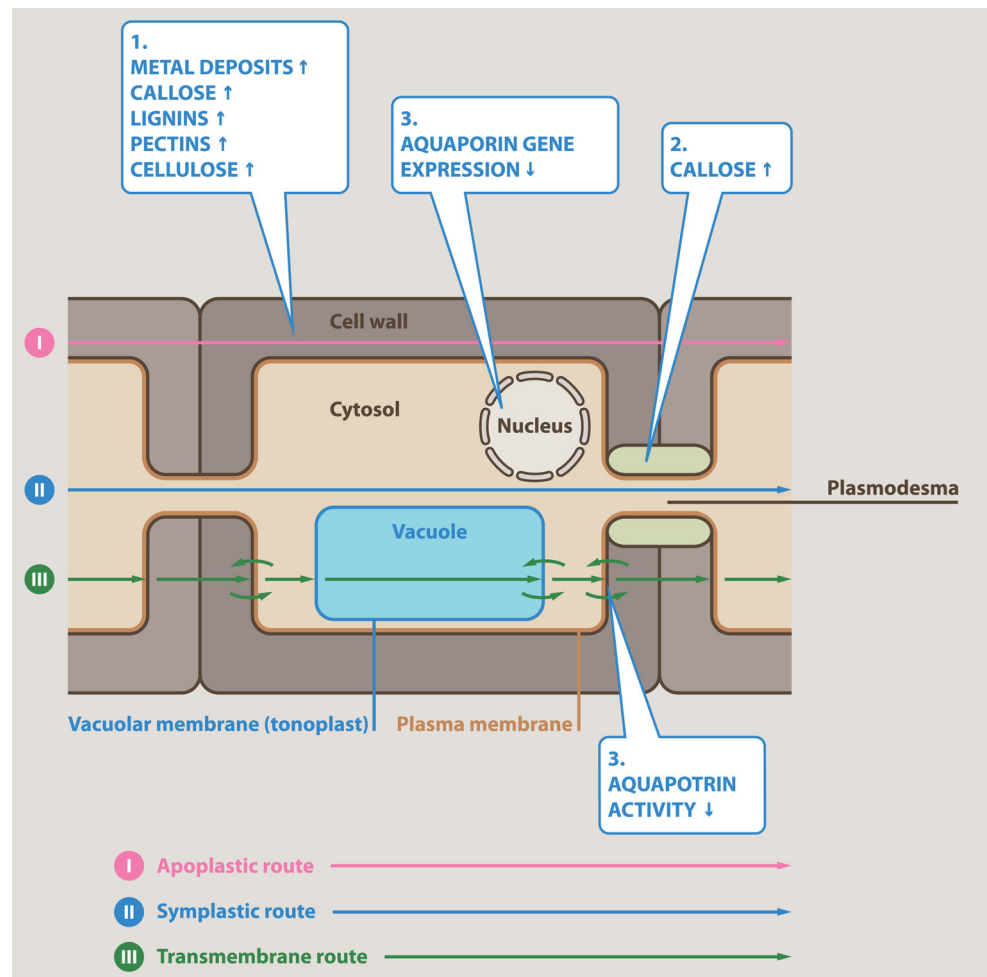
and lead to osmotic stress. It is thought that osmotic stress takes place when the concentration of salt exceeds the threshold value of  $10^{-3}$  M (Levitt 1972). Heavy metal ions seldom reach solution concentrations sufficient to cause osmotic disturbances in plants without previous symptoms of lethal toxicity. It is more likely that water absorption to the plant is indirectly regulated by changes in endogenous factors, e.g., root anatomy and/or morphology. In *Salix caprea*, a marked decrease in total root area was observed after exposure to Zn, Cd or a combination of both metals (Vaculík et al. 2012). Abnormalities in metal-stressed plants frequently described in the literature include decreased elongation of the primary root, impaired secondary growth, increased root dieback and reduced root hair surface: Ag (Anjum et al. 2013), As (Garg and Singla 2011; Panda et al. 2010; Sharma 2012), Cd (Gallego et al. 2012; Lux et al. 2011), Co (Collins and Kinsela 2011), Cr (Chandra and Kulshreshtha 2004; Hayat et al. 2012b; Shanker et al. 2005), Cu (Burkhead et al. 2009), Hg (Chen and Yang 2012), Ni (Sreekanth et al. 2013), Pb (Fahr et al. 2013; Feleafel and Mirdad 2013; Sharma and Dubey 2005), Zn (Broadley et al. 2007; Ivanov et al. 2003). Metal-induced structural changes lead to inadequate root–soil contact and lower the capacity of plants to exploit the water from the soil.

Water absorbed from the ground by the roots is transported through the xylem network to the leaves. When transpiration occurs, water is not taken up actively by the roots, but instead moves passively through them in response to a water potential gradient established by transpiration. An excess of heavy metals influences the efficiency of the water flow by lowering the transpiration rate and/or through changes in stomal resistance in leaves (Barceló and Poschenrieder 1990). These issues will be discussed in more detail in the next part of this paper. In the absence of transpiration, e.g., when stomata are closed, water movement is driven by the active pumping of solutes in roots (Javot and Maurel 2002; Stuedle 2000). Water transport through the roots is important because hydraulic conductivity ( $L_p$ ) influences the water supply for the whole plant. It has been proven that a pressure-induced water flux from *Lycopersicon esculentum* (Maggio and Joly 1995) and *Triticum sativum* (Carvajal et al. 1996) roots was reduced in response to Hg. Strong suppression of de-topped root exudation was also detected in the roots of *Mesembriantemum crystallinum* and *Oryza sativa* exposed to Cu and Zn (Kholodova et al. 2011). In Pb-treated *Cucumis sativus* plant exudation was inhibited, suggesting that root resistance to water transport was increased by metal (Cseh et al. 2000). To gain better insight into the direct effect of metal ions on water status and radial transport in the root, some experiments were carried out on young seedlings of *Lupinus luteus* at the stage which preceded the development of

leaves (Rucińska-Sobkowiak et al. 2013). Two concentrations of Pb were investigated: lower, which inhibited root growth by about 50 % (medium stress intensity), and higher, which almost entirely suppressed root elongation (severe stress intensity). It can be assumed that the osmotic potential of the second solution (about  $10^{-3}$  M) was low enough to cause some osmotic stress in roots (Levitt 1972). In spite of this, relative water content (RWC) in metal-treated roots was similar to that estimated in the control. Previously, it was found that Pb increased the degree of vacuolisation in the meristem cells (Przymusiński and Woźny 1985) and cortex parenchyma (Gzyl et al. 1997) of *L. luteus* roots, which may imply that the water status of these cells was not affected by the metal. It seems that enhanced volume fraction in the vacuoles is a common response of root cells to the toxic effect of heavy metals. This phenomenon was described for meristemic cells of Zn-treated *Festuca rubra* (Davies et al. 1991) and Cu-treated *Zea mays* (Doncheva 1998), as well as for epidermis and cortex cells of *Psidium guajava* roots exposed to Ni (Bazihizina et al. 2015). In the case of Cr, the roots of *Brassica juncea* showed an increase in the size of vacuoles, which was accompanied by the formation of electron-dense depositions along the walls of the xylem and phloem (Han et al. 2004). Pb-induced vacuolisation in *L. luteus* was correlated with high values of RWC, suggesting that water might be stored in the vacuoles in response to metal stress (Rucińska-Sobkowiak et al. 2013). These water reserves can minimize the cytosolic volume change in root cells, protecting them from significant perturbations in metabolite concentrations. However, this mechanism seems to be confined to those cells which were not already severely damaged by metal ions. It was demonstrated that at a higher Pb concentration, cell vacuolization is limited to the central region of the meristem only (Rucińska-Sobkowiak et al. 2013). This is in line with the observation that the frequency of Pb deposits, as well as the degree of cell destruction, decreases towards the root axis (Gzyl et al. 1997). In older roots, where the endodermis is properly formed, cortex cells generally contain higher amounts of metal, and are usually damaged earlier and more intensively by metal ions than the cells in the stele. For this reason, the decreased turgor and even plasmolysis which appeared in epidermal and cortex cells of *Phaseolus vulgaris* roots exposed to Cr can be attributed to cell injury (Barceló and Poschenrieder, 1990; Vázquez et al. 1987). It can also be supposed that extensive tissue damage was involved in the dehydration of roots evoked by Cd and Ni in *O. sativa* or *P. sativum* (Barceló and Poschenrieder 1990; Gabrielli et al. 1999; Hernández et al. 1997; Llamas et al. 2008; Moya et al. 1993).

Short-distance water transport in roots plays a central role in controlling the movement of solutes and water in

**Fig. 1** Short-distance water transport in plants exposed to heavy metal stress. Heavy metals decelerate short-distance water transport via (I) apoplastic, (II) symplastic and (III) transmembrane rout. 1 Cell wall thickening caused by metal deposits and/or incrustation of substances on the cell walls increase apoplast resistance to water flow. 2 Deposition of callose in cell walls reduces the rate of water movement through apoplast and the vacuolar continuum. 3 Diminished transmembrane water transport results from the inhibition of aquaporin activity and/or changes in the gene expression of these proteins



and out of the vascular system via the tissues that surround the conducting cells, and may influence the rate of long-distance water transport to aerial parts of the plant (Fig. 1). The flow of water across the root to the xylem can occur through different pathways: the apoplastic pathway around the protoplasts and cell-to-cell (protoplasmic) transport that can be divided into symplastic (via plasmodesmata) and transcellular (across cell membranes) paths. According to the composite membrane model of the root, these pathways are arranged in parallel rather than in a serial manner (Heinen et al. 2009; Steudle 2000, 2001). It has been shown that diffusive (short-distance) water transport was decelerated in *L. luteus* roots treated with Pb ions due to a reduction of the water transfer rate across the membranes and vacuoles (together comprising the intercellular endoplasm system), as well as water diffusion along the root apoplast (Rucińska-Sobkowiak et al. 2013). Transmembrane water flow passes predominantly (75 or 95 %) through water channel proteins, aquaporins (Henzler et al. 2004; Ye et al. 2005). It was demonstrated, using epidermal cells of *Allium cepa* as a model system, that Zn, Pb, Cd and Hg can change aquaporin (AQP) conductivity, leading to a

decrease in membrane water permeability (Przedpelska-Wasowicz and Wierzbicka 2011). Since Pb suppress water flow through aquaporins in *A. cepa* (Przedpelska-Wasowicz and Wierzbicka 2011), it might be assumed that diminished transmembrane water transfer in *L. luteus* roots (Rucińska-Sobkowiak et al. 2013) is connected with reduced permeability of water channels. It was documented that in Pb-treated roots, the level of  $\alpha$ -tocopherol is increased and degree of lipid peroxidation is diminished. It suggests that  $\alpha$ -tocopherol effectively alleviates Pb-induced lipid degradation in lupine roots. Therefore, inhibition of water movement is rather not related to changes in the permeability of the lipid bilayer (Rucińska-Sobkowiak and Pukacki 2006).

Diffusive water transport in *L. luteus* roots treated with Pb ions was also slowed because of a reduction in water movement throughout the intercellular endoplasm system (Rucińska-Sobkowiak et al. 2013). A reduction in water flow along the vacuolar continuum of the symplast (endoplasm) may result from breakage of the endoplasm, e.g., the rupture of intercellular contacts (plasmodesmata) or conformational changes in them (Ionenko and Anisimov

2001; Volobueva et al. 2004). It is thought that the permeability of plasmodesmata is actively regulated by the ATP-dependent sphincter, located in the neck regions of the plasmodesmata and/or by rapid reversible depositions of callose in the cell wall around the plasmodesmata (Radford and White 1998). It has been found that Pb induces the accumulation of callose within the plasmodesmata in *Lemna minor* roots (Samardakiewicz et al. 2012). The presence of callose deposits observed in the cell walls of Pb-treated *L. luteus* roots may lead to the assumption that intercellular water transport through the plasmodesmata is limited, which in turn would favor water accumulation in the vacuoles (Rucińska-Sobkowiak et al. 2013).

The rate of apoplast transport in the roots of metal-exposed plants can be decreased due to a reduction in the conductivity of cell walls for water (Le Gall et al. 2015; Rucińska-Sobkowiak et al. 2013). The presence of metal deposits, e.g., Pb in *L. luteus* (Gzyl et al. 1997) or Zn in *P. vulgaris* (Kasim 2007), occluding intercellular spaces and impregnating middle lamellae, may hamper apoplastic water flow. It was reported that Pb is involved in the formation of cell wall thickening (Krzyszowska 2011; Krzyszowska et al. 2009), as well as in increases in lignin content in *Glycine max* (Pawlak-Sprada et al. 2011) and *Pisum sativum* roots (Pb and As) (Päivöke 1983), callose in *L. minor* roots (Samardakiewicz et al. 1996, 2012) and in pectins, callose, cellulose, and sometimes the sudanophilic lipid compound in the protonemata of *Funaria hygrometrica* (Krzyszowska et al. 2009; Krzyszowska and Woźny 2000). The formation of characteristic callose deposits was also observed as a general response of plants to many other metals: Ni, Co, Cr, Mn, Pb, Sr, As, Cd and Al (Piršelová and Matušíková 2013 and references therein). The existence of wall thickening and the deposition of callose were confirmed in Pb-treated roots of *L. luteus* (Rucińska-Sobkowiak et al. 2013). Callose formed “patches” in parenchyma cortex cells placed lengthwise to the vascular cylinder and was localized in cell walls and/or in the vicinity of them. These results imply that Pb-induced wall thickening and callose deposits may be responsible for the inhibition of the water diffusion in the apoplast of the tissues that surround the conducting cells in roots. Exposure of *S. caprea* to Zn or Cd, as well as a combined treatment, indicated the development of apoplastic barriers related to the presence of these metals (Vaculík et al. 2012). The casparian strips and suberin lamellae that appeared in both the exodermis and the endodermis might stop water radial movement in *S. caprea* roots (Vaculík et al. 2012).

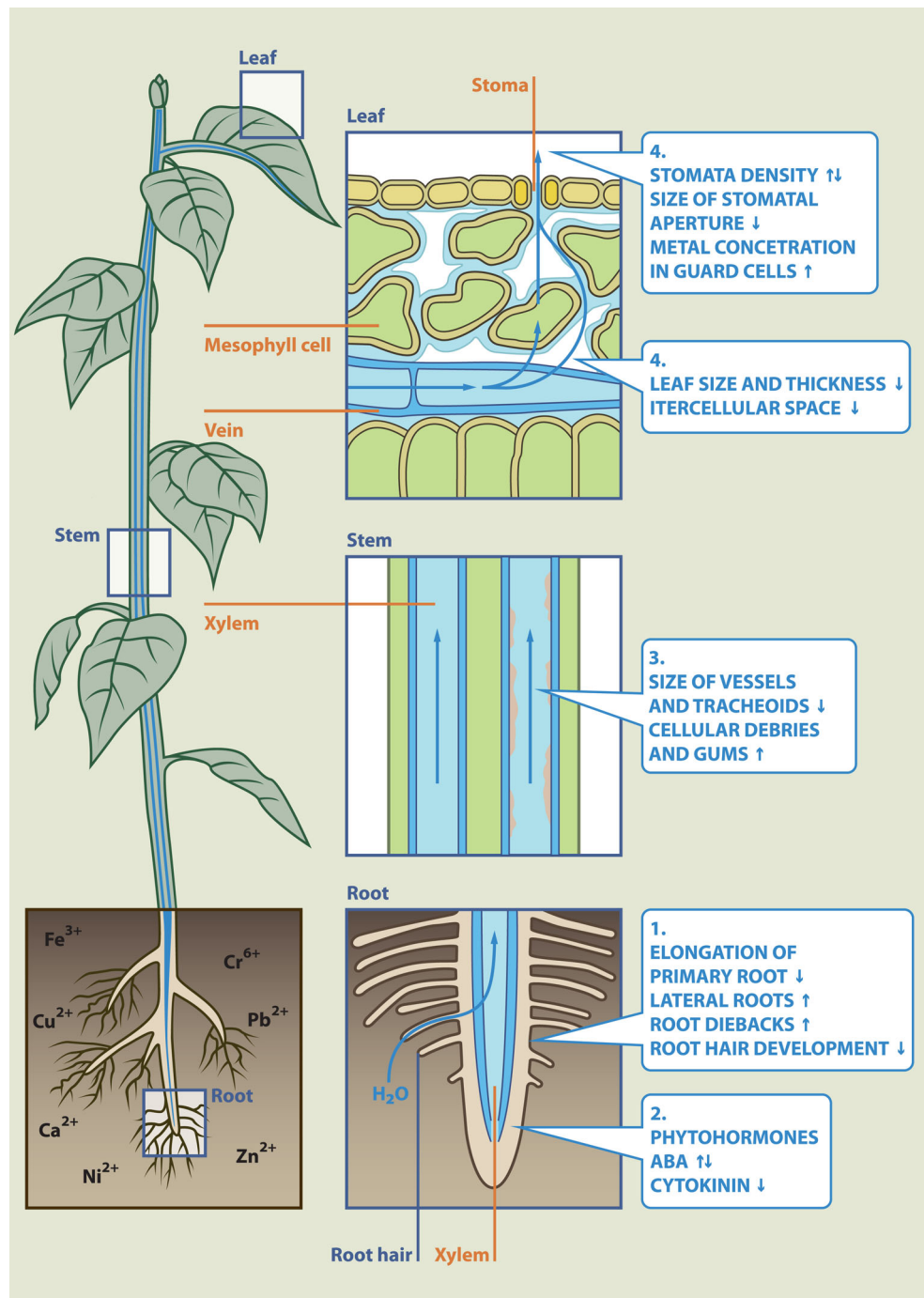
It seems that the root dehydration might be ameliorated by an increase in the volume fraction of the vacuoles developed in response to metal exposure (Rucińska-Sobkowiak et al. 2013). However, this effect would appear to

be a consequence of metal toxicity rather than a result of root adjustment to water deficiency. It was demonstrated that in *S. caprea* originating from polluted provenances, the area of the vascular bundles in roots remained constant while the area of xylem elements slightly increased with Zn or Cd treatment (Vaculík et al. 2012). Such adaptive traits might improve water supply to the plants, and help them to increase their tolerance to heavy metal.

### Effects of heavy metals on longitudinal water movement

The magnitude of the pressure gradient along the capillary xylem system is mainly determined by the transpiration rate or, when stomata are closed, by root pressure. Both factors are influenced by ion toxicity (Fig. 2). Moreover, xylem conductivity could be reduced by a metal-induced decrease of the cross-sectional area available for water transport. It has been shown that Cd stress is able to reduce plant hydraulic conductivity in excised stems of *Ace saccharinum* (Lamoreaux and Chaney 1977). This decline was caused by a progressive decrease in the proportion of xylem tissue available for water conduction, a reduction in the size of vessels and tracheids, and partial blockage of xylem elements by cellular debris or gums (Lamoreaux and Chaney 1977). In the roots of *P. sativum*, Pb and As affected the anatomy of the vascular tissues due to irregular lignification, observed in conducting elements (Päivöke 1983). The narrowing of metaxylem vessels was highly significant in the root, stem and leaf midrib of *Sorghum bicolor* treated with Cd and Cu and in the leaf midrib of *P. vulgaris* treated with Zn (Kasim 2006, 2007). In *S. caprea*, a marked decrease in the area of vascular bundles and the area of xylem elements in the root became evident after exposure to Zn or Cd or a combination of both metals (Vaculík et al. 2012). The effects of heavy metal stress on the hydraulic architecture were also investigated in *Acer rubrum* grown on soil contaminated with Co, Cr, Cu, Ni, Pb and Zn (De Silva et al. 2012). The metals had no significant effect on root depth, but reduced the cross-sectional area and xylem tissue area in roots. Maximal tracheary element size was decreased, and this response is likely to be adaptive in nature, as a decrease in conduit diameter has been shown to reduce vulnerability to embolisms (De Silva et al. 2012). Xylem responses in *A. rubrum* differed between the roots and shoots with respect to conduit density. In the roots, metal stress increased conduit density as a result of its stronger effects on root cross-sectional area than on conduit number. In shoots, metal reduced conduit density due to an increase in parenchyma cells in the xylem (De Silva et al. 2012). Decreased hydraulic conductance brought about by

**Fig. 2** Long-distance water transport in plants exposed to heavy metal stress. Metal-induced disturbances in short-distance transport (Fig. 1) slow the movement of water into the vascular system and affect water supply to the shoot. 1 Decreased elongation of the primary root, increased root diebacks or reduced root hair exerts a deleterious effect on the root-absorbing area and water uptake. Development of lateral roots partially compensates for limited growth in the primary root. 2 Inhibited root growth affects hormone synthesis and transport, as well as stomatal movement. 3 A reduction in the size of vessels and tracheids and partial blockage of xylem elements by cellular debris or gums decrease hydraulic conductivity in the root, stem and leaf midrib. 4 A decrease in leaf size and the lamina's thickness, intercellular spaces, density of stomata, and the sizes of the stomatal aperture reduces the rate of transpiration. Stomata closure is induced directly by heavy metals and/or is a consequence of the early effects of metal toxicity in roots and stems



changes in the xylem structure in metal-treated *A. rubrum* was found in both stems and roots (De Silva et al. 2012). It can be expected that the movement of water from root to shoot through the xylem might be diminished due to clear structural responses to the metal treatment. However, the lack of metal-induced effects on RWC in leaves of *A. rubrum* indicated that the plants are able to compensate, at least to some extent, for the effects of the stresses and maintain their water status (De Silva et al. 2012).

### Effects of heavy metals on transpiratory water loss

Heavy metals affect a number of morphological and anatomical features that influence water loss via leaves (Barceló and Poschenrieder 1990). It has been found that the leaf area decreased in *P. vulgaris* exposed to Cd (Barceló et al. 1986a, 1988), *C. sativus* treated with Cu (Vinit-Dunand et al. 2002) and *Nicotiana tabacum* subjected to an excess of Zn (Bazihizina et al. 2014). In *P.*

*sativum*, Cd toxicity resulted in a decrease in leaf size and the thickness of the lamina, reduced intercellular spaces in the mesophyll, small pavement cells and a high density of stomata (Tran et al. 2013). Previous studies have indicated that the number of stomata per unit of leaf area increased in *Helianthus annuus* treated with Pb, Cd, Cu and Zn (Kastori et al. 1992), and in *Beta vulgaris* exposed to Cd (Greger and Johansson 1992). It was proposed that an apparent increase in the number of stomata is not due to the enhanced stomata formation, but rather to the reduction of guard cell sizes generated by metal-induced water deficit in leaves (Neelu et al. 2000; Wainwright and Woolhouse 1977). On the other hand, some reports demonstrate that the amount of stomata might decline on account of metal toxicity. Stomatal frequency was reduced significantly by Cd and Cu treatments in *S. bicolor* (Kasim 2006) as well as by Zn in *P. vulgaris* (Kasim 2007) and in *B. vulgaris* (Sagardoy et al. 2010). The number of intact stomata per unit of leaf area decreased in leaves of *B. vulgaris* exposed to Cd; furthermore, metal ions induced closure of intact stomata (Greger and Johansson 1992). Experiments with greening etiolated leaves of *Hordeum vulgare* showed that a Pb-induced decrease in stomata opening was correlated with the presence of Pb deposits in the cuticle covering guard cells and subsidiary cells (Woźny et al. 1995). It has been widely documented that the quantity of anomalous (non-functional) stomata significantly increases in response to various heavy metals. The presence of poorly differentiated elliptical-shaped, sunken stomata was observed in Cr-treated *B. juncea* (Han et al. 2004). Numerous defective and undeveloped stomata were found in Cd-treated *B. vulgaris* (Greger and Johansson 1992), as well as in Cd- and Zn-exposed *Z. mays* (Souza et al. 2005). These stomata had small apertures and probably lacked a functional closing mechanism (Greger and Johansson 1992; Souza et al. 2005). Many stomata were permanently closed as a result of exposing *Vicia faba* (Neelu et al. 2000) and *Vigna radiata* (Tomar et al. 2000) to Cd and Pb, respectively. In Zn-treated *B. vulgaris*, stomata were round in shape and smaller than in control plants, and, in many cases, were covered by a wax-like seal of unknown nature (Sagardoy et al. 2010). These stomata did not respond to either chemical (exogenous abscisic acid—ABA) or hydraulic signals (leaf desiccation, relative humidity treatment) (Sagardoy et al. 2010). In *V. radiata*, As-induced disruption of microtubules caused alteration of the regular pattern of cell division, which resulted in the formation of abnormal stomata and arrested stomatal development at various stages (Gupta and Bhatnagar 2015). It was suggested that the disruption of the stomatal pore microtubule bundle, coupled with disorganization of cytoplasmic organelles, leads to a lack of differential wall thickenings, which was responsible for opening the stomatal pore when the guard

cells become turgid. The defective stomata remained permanently closed on account of exposure to As (Gupta and Bhatnagar 2015).

Nevertheless, it can be assumed that metal-treated plants with a less disorganized structure and function in their epidermal cells might regulate the degree of stomata opening, and by this means be capable of a plastic response or adaptation to water stress caused by metal toxicity. This postulate is confirmed by observations carried out on leaves of *P. vulgaris* (Barceló et al. 1988). Although almost all stomata were closed or had very small apertures in Cd-treated plants, guard cells did not show severe ultrastructural changes. As a result, a significant decline in stomatal conductance and transpiration was observed (Barceló et al. 1988). In Cd-treated *G. max*, stomata closure contributed to an elevated RWC under conditions of lower leaf water availability evoked by an increase in root resistance (Marchiol et al. 1996). Studies on As accumulation and distribution in *Atriplex atacamensis* have revealed that young seedlings close their stomata for osmotic regulation to avoid secondary water stress (Vromman et al. 2011). It was indicated that *P. guajava* responds to low Ni concentration by means of a significant decrease in stomatal conductance and transpiration, and was able to maintain a growth rate similar to that of control plants (Bazihizina et al. 2015). Studies with epidermal peels of *H. annuus* and *Z. mays* floating on solutions containing Pb, Cd, Ni and Tl (Bazzaz et al. 1974; Carlson et al. 1975) showed that heavy metals may directly induce stomata closure. Cd-induced stomatal movement from epidermal peels of *Arabidopsis thaliana*, *Vicia faba* or the monocotyledonous plant, *Commelina communis* revealed a dual effect (Perfus-Barbeoch et al. 2002). Nanomolar to micromolar Cd concentrations induced a strong inhibition in stomata opening, whereas at higher concentrations the inhibitory effect was progressively suppressed. However, analyses on *A. thaliana*, with Cd taken up by the whole plant, indicated that an inhibition of stomatal aperture correlated with metal concentration in a dose-dependent manner (Perfus-Barbeoch et al. 2002).

Increased stomatal resistance (or decreased stomatal conductance) has been described in many experiments as a result of plant exposure to toxic metal concentrations: Cd (Becerril et al. 1989; Costa and Morel 1994; Costa and Spitz 1997; Pandey and Sharma 2002; Perfus-Barbeoch et al. 2002; Poschenrieder et al. 1989), Co (Chatterjee and Chatterjee 2000; Pandey and Sharma 2002; Rauser and Dumbroff 1981), Ni (Pandey and Sharma 2002; Rauser and Dumbroff 1981), Pb (Becerril et al. 1989), and Zn (Rauser and Dumbroff 1981). It has been shown that a high resistance to Cd in *Setaria veridis* results from a decrease in the number and size of stomata and a closing of the stomata followed by a significant decrease in stomatal conductance

(Kaznina et al. 2014). Stomata closure caused by water deficiency stress can be brought about by hydropassive (loss of leaf turgor) or hydroactive (ABA-mediated) mechanisms (Danquah et al. 2014; Gill et al. 2013; Wasilewska et al. 2008). Poschenrieder et al. (1989), based on studies performed on Cd-treated *P. vulgaris*, postulated that the inhibition of the stomata opening may result from three different mechanisms, depending on both the Cd concentration and exposure time, i.e., depending on the degree of toxicity suffered by the plants. Under relatively short exposure to a low Cd concentration, an increase in stomatal resistance occurred without either a decrease in leaf turgor or an increase in bulk leaf ABA. This may be due to small changes in the pool of active ABA, the effects of Cd on root metabolism, or direct interaction between Cd and guard cells. Longer exposure to Cd caused an increase in the bulk leaf ABA level, leading to further stomata closure. Under further extension of treatment to higher Cd concentrations, wilting and hydropassive stomata closure may occur (Poschenrieder et al. 1989).

In plants subjected to water and other abiotic stresses, ABA has several ameliorative functions that involve a cascade of signaling intermediates, causing rapid induction of stomata closure, which reduce water loss from leaves (Chmielowska-Bąk et al. 2014; León et al. 2014; Neill et al. 2008; Wilkinson and Davies 2010; Yao et al. 2013). Whether or not ABA controls metal-dependent stomatal movement and mediates the drop in leaf conductance is still under debate. Increased ABA concentrations in leaves of *P. vulgaris* exposed to Zn and Ni (Rauser and Dumbroff 1981) as well as in leaves of *O. sativa* treated with Cu (Chen et al. 2001) concomitant with increased stomatal resistance (Rauser and Dumbroff 1981) seems to confirm the hypothesis that hydroactive mechanism might be responsible for metal-induced stomata closure. However, a study with *P. vulgaris* exposed to Co (Rauser and Dumbroff 1981) did not show higher leaf ABA levels in spite of enhanced stomatal resistance. It cannot be excluded that an increase in stomatal resistance may be due to a small pool of active ABA not detectable when bulk leaf ABA is analyzed (Poschenrieder et al. 1989). It has been shown that under a water deficit, total ABA concentration in leaves did not always correlate with stomatal conductivity, whereas a more strict correlation was found between stomatal conductivity and ABA concentration in the xylem sap (Li et al. 2011). The role of ABA in stomata regulation under water deficiency is also linked with hormone localization in the leaf (Kudoyarova et al. 2013). With present knowledge, it is not clear to what extent ABA distribution among leaf tissues can be made responsible for metal-induced stomatal resistance.

It has been suggested that roots are able to communicate stress situations to the leaves by chemical signals such as

ABA Kudoyarova et al. (2013, 2015). It was established that in drying roots, ABA biosynthesis was enhanced and then the hormone was transported along the xylem to the shoot (Sharp and Davies 2009). It can be expected that in metal-stressed plants, root-derived ABA or ABA-induced signals may also play a role in leaf responses, such as stomatal movements (Poschenrieder et al. 1989). Since ABA signaling is attributed to desiccation, it seems that this hormone cannot be produced in roots that are well hydrated in spite of increased metal concentration in this organ (Rucińska-Sobkowiak et al. 2013). Moreover, metal amounts in roots and shoots could not exceed some critical concentrations. Studies on *B. vulgaris* exposed to Zn indicated that xylem ABA concentration was reduced (Sagardoy et al. 2010). Anatomical analyses revealed that Zn and Ni at high concentrations caused severe damage in the roots of *N. tabacum* and *P. guajava* (Bazihizina et al. 2014, 2015) and Zn affected the functionality of the root plasma membrane (Bazihizina et al. 2014). It can be supposed that enhanced metal concentrations could have a detrimental effect on ABA synthesis in roots (Sagardoy et al. 2010). Further studies are required to provide more extensive data demonstrating the influence of heavy metals on root ABA biosynthesis and xylem export to the shoots.

In addition to ABA as a positive root signal for stomata closure, cytokinins may play a role as a negative signal in this process (Davies et al. 2005). It has been shown that external application of kinetin caused a significant increase in stoma opening in greening etiolated leaves of *H. vulgare* (Woźny et al. 1995). In the presence of Pb alone or in combination with kinetin, the percentage of open stomata as well as pore size decreased, indicating that the effect of kinetin may be reversed by metal ions (Woźny et al. 1995). Root tips are a major site for cytokinin synthesis, and the effect of heavy metals on root growth is clearly related to a decrease in cytokinin production (De Smet et al. 2015). It has been proven that exposure to Cd significantly inhibits the growth of *G. max* and declined cytokinin fractions: zeatin and zeatin riboside, wherein zeatin was the most severely affected (Hashem 2014). In *S. bicolor*, Cd decreased root length and root/shoot ratio (Haroun et al. 2003). The inhibition of growth in Cd-treated plants was relieved either partially or completely when grains were presoaked in kinetin. Moreover, the alleviating effect of kinetin for Cd toxicity was observed with respect to water relations: total leaf conductivity, transpiration rate and relative water content (Haroun et al. 2003). The addition of kinetin improved water status in *Carthamus tinctorius* exposed to Pb (Sayed 1999) as well as in *Lupinus termis* treated with Cu and Zn (Gadallah and El-Enany 1999), but this effect was attributed to increased leaf membrane stability, and thus to improved water retention properties. There is no direct evidence to prove that metal toxicity may



affect cytokinin export from roots to shoots and by this means influence stomata movement.

It is thought that both  $K^+$  and  $Ca^{2+}$  play an important role in the regulation of stomata opening (Osakabe et al. 2014). Experiments on *Vicia faba* guard cell protoplasts showed that stomata closure induced by Cd is not mediated directly by the regulation of K channels (Perfus-Barbeoch et al. 2002). It was found that Cd may compete with Ca for voltage-dependent channels and by that means cause perturbations in Ca signaling (Perfus-Barbeoch et al. 2002). On the other hand, the reduction in the size of the stomatal aperture observed in *S. veridis* in the presence of Cd was attributed to the leakage of both K and Ca from guard cells due to increased membrane permeability (Kaznina et al. 2014). These results suggest that Cd may affect guard cell regulation in an ABA-independent manner (Perfus-Barbeoch et al. 2002).

Regardless of the mechanism involved in the opening and closing of the stomatal pore, restricted transpiration is to be expected due to high stomatal resistance values. A loss of transpiration was found in shoots of *P. sativum* and *O. sativa* subjected to excess of Cd (Hernández et al. 1997) and Cu (Chen et al. 2004), respectively. An inhibitory effect was also described in leaves of *Brassica oleracea* exposed to Co, Ni and Cd (Chatterjee and Chatterjee 2000; Pandey and Sharma 2002), as well as in leaves of *M. crystallinum* treated with Cu and Zn (Kholodova et al. 2011). In *Lolium Perenne* leaves, Cr-induced water loss was reduced via suppression of transpiration (Vernay et al. 2007). The intensity of transpiration was suppressed by Cd in metal-resistant *S. veridis* (Kaznina et al. 2014), while a high rate of photosynthesis was maintained. In contrast to these data, an excess of Cu and Cr increased the transpiration rate in leaves of *B. oleracea* correlated with decreased diffusive resistance (Chatterjee and Chatterjee 2000). Enhanced transpiration in plants stressed by high metal concentrations has been previously attributed to increased stomatal density resulting from a reduction in leaf area or to cuticle damage that increased cuticular transpiration (Greger and Johansson 1992).

A reduction in the leaf-cell sap osmotic potential following exposure to an excess of metals might be considered as a symptom of water deprivation, as well as one of the most important components of the plant's adaptive strategy to water deficit. Exposure of *P. vulgaris* to Ni resulted in a large drop in water potential in leaves (Rauser and Dumbroff 1981). This effect might be explained by prior observations that sugars tend to accumulate in the leaves of this species in response to Ni (Samarakoon and Rauser 1979). The sucrose concentration in the leaf of *Cucumis dativus* also rose significantly with Cu addition (Vinit-Dunand et al. 2002). The assumption that reduced potential was involved in osmotic adjustment in *P. vulgaris*

was confirmed by the fact that RWC was not affected in Ni-treated plants and water stress did not develop (Rauser and Dumbroff 1981). A similar effect was observed in *B. oleracea*, where decreased water potential was followed by increased RWC (Chatterjee and Chatterjee 2000) or decreased water saturation deficit (WSD) (Pandey and Sharma 2002) in response to Co and Cd, respectively. The substantial difference in osmotic potential between a control and Cr-stressed leaves of *Lolium perenne* might suggest that changes in osmotic potential are not only due to passive water losses, but also due to osmotic adjustment (Vernay et al. 2007).

Some adaptative mechanisms to the negative effects of heavy metals on water status were observed in facultative halophyte *M. crystallinum*, which represent a relatively high potential metal tolerance (Kholodova et al. 2005, 2011). In short-time experiments with both metal ions, a reduction in root sap exudation and water deficits in leaf tissues became evident (Kholodova et al. 2011). Several primary adaptive events, including a rapid decrease in the transpiration rate and progressive declines in the leaf-cell sap osmotic potential were observed. Longer metal treatments resulted in reductions in total and relative water contents, as well as in proline accumulation. Although proline concentrations were higher in metal-treated plants than controls, proline was not attributed to the adjustment of osmotic potential (Kholodova et al. 2011). This amino acid was probably not acting as a compatible solute (Gagneul et al. 2007), but rather as a metal chelator, an antioxidative defense molecule and a signaling molecule (Hayat et al. 2012a; Szabados and Saviouré 2010). Leaf expression of the McTIP2;2 gene, which encodes tonoplast aquaporin, was suppressed, thus representing one of the earliest responses to metal treatment. The expression of three additional aquaporin genes was also reduced, and this effect became more prominent upon longer metal exposure. According to Kholodova et al. (2011), these results indicate that heavy metals induce critical rearrangements in the water relations of *M. crystallinum* plants based on the rapid suppression of the transpiration flow and strong inhibition of root sap exudation. These effects then triggered an adaptive water-conserving strategy involving differential regulation of aquaporin gene expression in leaves and roots, further reductions in transpiration, and an accelerated switch to CAM photosynthesis (Kholodova et al. 2011).

## Conclusions

It seems that in plants responding to heavy metal stress, disturbance of the water balance is an early stress-induced event. A complex set of changes related to water status occurred in the whole plant. In roots, heavy metals induce a

decrease in water uptake and the suppression of short-distance water transfer in the symplast as well as in the apoplast (Fig. 1). Impairment of water transport through the membranes is most likely attributable to the inhibition of aquaporin activity and/or to changes in the expression of these proteins. In the case of vacuolar transport, deposition of callose in cell walls seems to be responsible for the reduction in the rate of water movement through the vacuolar continuum. Moreover, cell wall thickening caused by metal deposits and/or incrusting substances in the cell walls increase apoplast resistance to water flow. These changes affect the movement of water into the vascular system and, in turn, decrease root sap exudation. Inhibition of long-distance water transport leads to a reduction in leaf water content and subsequently to the development of a water deficit in leaves (Fig. 2.). A rapid decrease in osmotic potential, root vacuolisation and rearrangement of stem and leaf tissues might be considered as events that enhance water retention ability in plants. Long before visible symptoms of water status disturbance appear in leaves, information about the immediate changes in roots is rapidly spreading across the plant and initiates a cascade of adaptive processes, including a decrease in the transpiration rate and differential expression of aquaporin genes. It is postulated that alterations in the aquaporin permeability are associated with the modification of these proteins by their phosphorylation/dephosphorylation (the process, which may be controlled by ABA). However, there is a lack of research examining whether metal-dependent changes in the levels of aquaporin phosphorylation are involved in regulation of transmembrane water flow.

Plant exposure to heavy metals may induce an increase in various signaling compounds, including calcium ions, nitric oxide, polyamines and plant hormones such as ethylene, auxins and jasmonic (JA), and salicylic (SA) and abscisic acid (ABA). Under water stress, ABA, among other molecules, is involved in signal pathways, causing the rapid induction of stomata closure, which reduces water loss from leaves. However, the role of root-derived ABA or leaf-accumulated ABA in controlling stomatal function in metal-treated plants remains somewhat unclear. Further investigations are required to understand the cross talk between signaling elements and their role in the transduction of metal-induced signals from roots to leaves. These studies should provide better insight into the mobilization of defense mechanisms and the regulation of the expression of genes involved in protecting against water deficit in metal-exposed plants.

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