Chapter 6
Soil-Plant Relationships of Heavy Metals and Metalloids

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Abstract Nutrient uptake by plants is essential for their development and for the passage of minerals into the food chain, but it also faces several limitations. Whereas soil physicochemical characteristics impose limiting factors on element availability for plants, excess of non-essential metals and metalloids pose a threat for plant health and the environment. To improve nutrient uptake, the plant possesses several mechanisms to explore the soil for minerals such as root development, but the symbiosis with microorganisms clearly improves the ability of plants to overcome these limitations. After metal uptake by the plants, plants make use of different strategies to maintain the metal homeostasis and to limit the metal-induced cellular damage. Also in the research on metal phytotoxicity, microorganisms are shown to be important players in the protection of the plant to excess metal exposure.

Keywords Metal uptake • Metal homeostasis • Toxicity • Deficiency • Metal tolerance • Plant-associated bacteria • Mycorrhiza • Oxidative stress • Anti-oxidative defence

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6.1 Introduction

As important suppliers of dietary minerals for humans and animals, plants form a bridge between the soil elemental composition and the food chain. Consequently, plant nutrient uptake is essential for its central role in element cycling, but also for the growth and development of plants. Soil physicochemical characteristics often impose limiting factors on the bioavailability of elements and mineral deficiencies are often experienced in crop production. Moreover, many regions in the world are unsuitable for growing crops due to contamination with potentially toxic elements such as metals and metalloids. The uptake and transport mechanisms crucial for essential elements also form an entrance for non-essential elements that pose a threat to the plant's fitness and the food chain [166].

In this chapter the focus is on (1) nutritional exploration of the soil by plants and their associated micro-organisms (2) protection by these micro-organisms against excess amounts of (non-)essential elements and (3) the ability of plant cells to cope with metal stress.

6.2 The Soil-Plant Interface: Microbial and Molecular Interactions Define Plant Elemental Uptake

6.2.1 Molecular Interactions Defining Plant Elemental Uptake

Plants obtain essential metals and metalloids (micronutrients) primarily from the soil, so appropriate systems must be in place to ensure adequate uptake of these elements (B, Cu, Fe, Mn, Mo, Ni, and Zn). These elements are heterogeneously distributed in the soil, so exploration of the soil by the root system is the first important parameter to optimize nutrient uptake. Whereas root growth responses to macronutrient deficiencies, especially phosphorus (P) and nitrogen (N), have been intensively studied, responses to micronutrient deficiencies largely remain to be determined. Responses to nutrient deprivation often result in an increased surface area of the root system in a localized part of the soil. Some reports of root developmental responses to Fe deficiency show a stimulatory effect on root hair production in dicotyledonous and non-grass monocotyledonous species [149, 188]. Some plant species that are adapted to growth on very nutrient-poor soils are able to develop cluster roots, which are bottlebrush-like in shape due to dense formation of short lateral roots on the primary root axis. These structures occur in a subset of species of the *Casuarinaceae*, *Leguminosae* and *Proteaceae* families [195] and have been described under iron (Fe)-deficiency [9, 116, 139, 189].

Another parameter determining plant metal uptake is the bioavailability of the elements, which is defined by the chemical properties of the metal cations and by the physicochemical characteristics of the soil [161]. Metals in soil are often

adsorbed to soil particles or present in an insoluble form (for example Fe hydroxides in alkaline soil types [134, 159]). Plant roots can interact with the rhizosphere to increase the bioavailability of minerals and turn them into a form appropriate for uptake by transporters. Roots extrude protons via plasma membrane H^+-ATPases to acidify the rhizosphere. This creates a large membrane potential (−100 to −250 mV), which is the main driving force for cation uptake [160]. Furthermore, the protons can participate in cation exchange, releasing divalent metal ions that are tightly bound to soil particles, and the resulting acidification of the rhizosphere can release metals from their hydroxides [159, 160].

Regarding Fe-uptake, plants have been divided into two groups according to the strategy they use in the interaction with the rhizosphere to solubilize Fe: (1) reduction based or (2) production of chelating molecules. Non-graminaceous plant transport systems take up Fe in the form of Fe^{2+}, whereas Fe in the soil is mostly present as Fe^{3+}. These plants have a reduction-based system in which Fe^{3+} is reduced to Fe^{2+} by the ferric chelate reductase FRO2 [180]. Ferrous ions (Fe^{2+}) are subsequently translocated into the cytoplasm by the high-affinity transporter IRT1 [172]. Graminaceous plants, on the other hand, use a chelation-based strategy. Roots actively secrete compounds, known as phytosiderophores that can function as metal chelators in the soil. Phytosiderophores belong to the mugineic acid family (MAs) and expression of the genes involved in MA biosynthesis is upregulated under Fe-deficiency [159]. Maize roots take up MA-Fe^{3+} complexes through a specific transporter named YS1 [50]. Other species take up the metal-chelator complexes by Yellow stripe-like (YSL) transporter proteins. In barley, phytosiderophores can also assist in the uptake of zinc (Zn) [217] but a role for phytosiderophores in copper (Cu) uptake has not been revealed. The categorisation in graminaceous and non-graminaceous plants regarding reduction-based or chelation-based uptake strategy is not so clear since rice plants have been shown to take up not only Fe^{3+}-chelates, but also Fe^{2+} via OsIRT1 and OsIRT2 transporters (orthologues to Arabidopsis IRT transporters) [91]. There may be an evolutionary aspect to this as rice has been grown and selected on paddy soils where Fe^{2+} is abundant.

After uptake, minerals can migrate into the root apoplastic space, but the impermeable Casparian strip in the endodermal cell layer ultimately blocks this route. Here metals have to be actively transported across the plasmamembrane into the symplast. Ferrous ions (Fe^{2+}) and Cu^+ (after reduction, both by FRO2) are taken up by their respective transporters IRT1 and COPT1 [172]. However, Cu may also enter the plant as Cu^{2+} via a member of the ZIP (ZRT, IRT-related protein) family, a transporter family known to preferentially transport divalent cations. Zinc (Zn^{2+}) is also believed to be transported by ZIP transporters or by the Fe transporter IRT, which can also transport divalent metals other than Fe^{2+} [159].

Metal uptake systems are highly regulated at transcriptional and posttranscriptional levels. Copper is transported as Cu^+ by the COPT1 transporter and COPT1 expression is upregulated under Cu-deficiency [186]. Also ZIP2 and ZIP4 are upregulated by Cu-deficiency, but their functional role as Cu^{2+} transporters remains to be established [159]. IRT1 and FRO2 are co-ordinately regulated at the

transcriptional and posttranscriptional level. FRO2 and IRT1 are induced together under Fe-deficiency and repressed under sufficient Fe-supply [45]. In transgenic plants that overexpress IRT1, increased mRNA accumulation was only translated to increased IRT1 protein in Fe-deficient plants, suggesting posttranscriptional control [44]. Protein levels of IRT1 are tightly controlled via ubiquitination at lysine residues, which leads to proteasome-mediated degradation [101].

High affinity transport systems are indispensable for plants to acquire essential micronutrients, but unspecific metal uptake from the soil seems unavoidable under metal-excess as transporters for essential nutrients also take up non-essential elements. Examples are the metalloid arsenic (As) and the heavy metal cadmium (Cd), which have no demonstrated biological function in higher plants, and for which plants are not expected to have specific uptake mechanisms. Instead, the uptake of Cd seems to occur primarily via calcium (Ca^{2+}), Fe^{2+}, manganese (Mn^{2+}) and Zn^{2+} uptake systems [37, 167]. For example, the Fe^{2+} transporter IRT1 contributes significantly to the uptake of Cd [244]. Arsenate [As(V)] is taken up by the high affinity phosphate transporter system and rapidly reduced to arsenite [As(III)] [141]. In reducing environments, As(III) can be taken up by aquaporin nodulin 26-like intrinsic proteins [21, 90] for example the rice OsNIP2;1/Lsi1, which also transports silicon (Si), a beneficial element for rice [125].

6.2.2 Microbial Interactions Defining Plant Elemental Uptake

Nutrient uptake in plants is in no way a monopoly of the plant itself. From the early days that plants started to colonise the terrestrial environment, microorganisms turned out to be essential partners for the colonisation of the land [26]. Their major task was/is the scavenging for essential elements that are scarce, poorly soluble or immobile in the solid substrates. Both fungi and prokaryotes provide services to plants in terms of nutrient acquisition and protection against biotic and abiotic stresses. This interdependency of plants and microorganisms was shaped by evolution, new symbioses arose in particular plant families – e.g. N-fixing Rhizobia in legumes –, other symbioses were replaced by new innovations on the same theme, such as the ectomycorrhizal fungi that since the Cretaceous replaced the arbuscular mycorrhizal fungi in some woody plant lineages. The evolutionary persistence and ubiquity of the plant-microbe interactions illustrates the positive cost-benefit balance and the synergistic nature of the interactions.

6.2.2.1 The Mycorrhizal Symbiosis

Amongst the plant-microbe interactions, the mycorrhizal symbiosis is the most widespread intimate interaction between plants and fungi. Between 80% and 90% of all seed plant species harbour fungi in their roots, forming structures known as mycorrhizas. Mycorrhizas are a functional part of plant roots where the fungal

hyphae of the external mycelia might be considered as a very fine extension of the absorption roots that provides a cost-effective increase of the absorptive interface between roots and soil [211]. Most mycorrhizal fungi are strict biotrophs, they are morphologically and metabolically very well equipped for mobilising, assimilating and transporting plant nutrients, including essential metals.

Over evolutionary times, different mycorrhizal types and many different fungal species have evolved, showing broad functional diversity and adaptation towards different soil conditions and (or) host plants. And although the functionality of only a limited number of these mycorrhizal interactions has been studied in detail, there is consensus that host plants in a greater or lesser extent experience positive nutritional effects. In general, the nutritional benefit for a host plant seems to be greatest in nutrient poor soils, a condition which is also characteristic for most metal-contaminated soils [140]. Nevertheless, plants differ greatly in their dependence on mycorrhizal fungi, a major aspect being the size and architecture of their root system. Roots with a thick cortex and exodermis suberisation make a fungal symbiont essential, whereas extensive much-branched root systems with very fine elongated fine roots can make the fungal symbioses more futile [26]. Amongst mycorrhizal fungi the efficiency of nutrient uptake and transfer to a host varies significantly both at the intra- and interspecific level [152]. This means that not all fungi are equally effective in plant growth promotion. Mycorrhizal fungi have high nutritional needs themselves and keep significant amounts of assimilated nutrients for their own metabolism and structures. The nutrition of the fungi themselves may also depend on interactions with microbes associated with the hyphae (the mycorrhizosphere) [73, 157]. Recent studies indicate the presence of specific endocellular bacteria in arbuscular mycorrhizal fungi [7], endosymbionts that may have a role in fungal nutrition.

The key role of mycorrhizal fungi in P and N nutrition in plants has been demonstrated in many investigations. Both arbuscular and ectomycorrhizal fungi possess an elaborate set of transporter genes for uptake of a whole range of nutrient sources present in soil solution [135, 136]. There is increasing evidence that both symbiotic partners affect the specific transporter gene or protein expression of each other [80]. Although most mycorrhiza research focuses on the improved macronutrient acquisition in plants, the contribution of mycorrhizal fungi in mobilisation, uptake and transfer of micronutrients has been recognised as well [28]. Weathering of minerals through ectomycorrhizal fungi has been observed *in situ* [24, 86] and is supposed to improve cation uptake in fungi and host plants. Under experimental conditions deficiencies of essential metals in plants can be overcome through inoculation with specific mycorrhizal fungi [105].

6.2.2.2 Plant-Bacteria Partnerships

Beside mycorrhizal symbiosis, plant-associated bacteria can also enhance biomass production and tolerance of plants to trace elements in environments with increased levels of these elements [57, 95, 126, 154, 199, 245]. Some details of chemical

communication between plant roots and their associated bacteria in the rhizosphere were covered in a recent review by Bardi et al. [12]. Endophytic bacteria and their interaction with host plants have also attracted attention [15, 88, 89, 216, 251, 252].

Plant-bacterium partnerships provide a wide range of benefits to the host plants, such as promoting plant growth and development. Plant-associated bacteria can promote plant growth and development (1) directly, by (1a) fixating nitrogen, (1b) increasing the supply of unavailable nutrients such as P, Fe and other mineral nutrients, (1c) producing plant growth regulators such as auxins, cytokinins and gibberilines; and (2) indirectly, by preventing the growth or activity of pathogenic organisms through (2a) competition for space and nutrients, (2b) antibiosis, (2c) production of hydrolytic enzymes, (2d) inhibition of pathogen-produced enzymes or toxins and through (2f) induction of plant defence mechanisms [13, 46, 72, 94, 97, 103, 137, 142, 218, 226, 243, 251, 252, 255, 259].

A number of mineral nutrients in soils, including N, P and Fe, can frequently be limiting and thus restricting the growth of terrestrial plants. Requirements for adding these nutrients accounts for the major portion of fossil fuels used in agricultural systems and minimal application of fertilisers is therefore desirable in order to make feedstock production economically and energetically viable and sustainable. For this reason, strategies to minimize fertiliser inputs by promoting uptake of nitrate or ammonium of biological nitrogen fixation, as well as acquisition of P, Fe and other essential elements are of great interest.

For plants, N needs to be in the form of either ammonia or nitrate before it can be utilized. Plant-associated diazotrophic bacteria possess the enzyme nitrogenase, an O_2-sensitive enzyme that catalyzes the reduction of atmospheric nitrogen to ammonia. The plant growth promoting activity of diazotrophic endophytes has been demonstrated in several greenhouse and field studies of different plant species; for example, sugarcane [23], soybean [147] and rice [23, 132].

Beside N, P is a common limiting mineral nutrient affecting terrestrial plant growth. Phosphate solubilising and phosphate mineralizing bacteria are present in the rhizosphere and inside the plant [181, 243]. These bacteria can either solubilise inorganic phosphates by releasing organic acids, such as gluconic acid and 2-ketogluconic acid, or mineralize organic phosphates by secreting extracellular phosphatases [102].

Iron in the aerobic environment is often present in the highly insoluble forms of ferric hydroxides and oxyhydroxides, making it largely unavailable to plants and microorganisms. To acquire sufficient Fe, many bacteria developed strategies to solubilize this element for a more efficient uptake. One of the most commonly found strategies evolved by bacteria is the production of siderophores, low-molecular-mass Fe chelators with high association constants for complexing Fe. These siderophores are able to bind Fe^{3+} and render it available for reduction into the Fe^{2+} form, which is preferred by plants. As described above, also so-called graminaceous plants release siderophores (*e.g.* mugineic acid in barley and avenic acid in oat) to enhance their Fe uptake, but these phytosiderophores typically have a lower affinity for Fe than microbial siderophores. Plant–microbe interactions involved in the regulation of siderophore production and their role in mediating

competition for iron in the rhizosphere have been the subject of comprehensive reviews by Crowley et al. [49] and Rajkumar et al. [174]. Furthermore, there is evidence that several plant species can also recognize and take up bacterial Fe^{3+}–siderophore complexes. In this way, bacterial Fe^{3+}–siderophore complexes might facilitate uptake of Fe not only into bacteria, but also into plants and this process is considered as crucial for plant Fe uptake, particularly in calcareous soils [100, 197, 198].

Beside these nutrient mobilizing bacteria, phytohormone (such as auxins, cytokinins and gibberellins) producing bacteria can also be applied to increase nutrient uptake. Phytohormones that are produced by plant-associated bacteria can frequently stimulate growth and indeed have been considered the causal agents for altered plant growth and development [218, 220]. The extended root system that is achieved in this way, can contribute to an increased nutrient uptake.

In addition to the above-mentioned beneficial effects on plant growth, both rhizosphere bacteria and endophytes can also contribute to enhanced trace element availability and uptake [111, 112, 127].

Bacteria possessing metabolic pathways for the synthesis of natural chelators (*e.g.* organic acids and siderophores) can mobilize trace elements. As certain plants make use of microbial chelators to increase their Fe uptake (see above), it has been hypothesized that bacterial Fe chelators can eventually also enhance the uptake of other trace elements by plants [25, 187]. The production of these bacterial chelators is in tight equilibrium with plant activity, meaning that trace element mobilization only takes place when plants are active and by consequence can take up the elements. In this way, the risk for leaching of trace elements to the groundwater is limited.

6.3 Plant-Associated Microorganisms: Protection Against Metal Stress

Apart from the nutritional effect, plant-associated micro-organisms may also protect their host plants against various stress factors, including soil toxic compounds and soil-borne pathogens [111, 112, 127, 191]. Such plant protection might be achieved by acting directly on aggressive factors (mainly pathogens and herbivores) or by enhancing plant responses. Plant protective microbial symbionts determine the ecological success of plants; they modify plant communities and related trophic webs.

6.3.1 Mycorrhizal Fungi

There is no doubt that in many metal-polluted environments, mycorrhizal fungi ameliorate metal stress in their host plants [4, 93, 110, 176]. However, the

mechanisms involved are not always clear. Nutritional and hormonal effects often improve plant fitness and thus indirectly stress tolerance. For example, excess metals become more diluted in plant tissues when plants grow better. However, more direct processes that affect the transfer of a metal from soil into the plant may further strengthen such indirect ways of plant protection. In a number of experiments particular mycorrhizal fungi collected from polluted soils, could reduce the accumulation of metals in the shoots of their host plants [3, 110]. Mycorrhizal fungi possess mechanisms involved in metal homeostasis and detoxification of essential and non-essential metals; mechanisms that are probably not different from those present in other eukaryotes [18, 47]. Under high selection pressure these metabolic networks might become more efficient in coping with metal stress and toxicity.

For a long time researchers suggested that soil microorganisms in general exhibit higher tolerance against metal toxicity than plants [83]. They expected little evolutionary adaptation towards elevated tolerance in mycorrhizal fungal communities, as there are sufficient fungi with a high constitutive tolerance that are selected for and thus become dominant in metal-contaminated environments [22, 140].

However, on severely metal contaminated sites, the development of metal tolerant ecotypes in both plant and fungal species has now been demonstrated. For plants, such an influence of soil metal toxicity can easily be demonstrated and there is a lot of evidence for the evolution of adaptive metal tolerance in higher plants. Such evidence is only recently coming up for mycorrhizal fungi. Metal-tolerant arbuscular and ericoid mycorrhizal fungi have been isolated from naturally and anthropogenically polluted sites [6, 117, 138]. The same is true for some higher fungi that form ectomycorrhizas, including *Pisolithus tinctorius* and *P. albus* [67, 96], *Suillus* species [3, 41, 42, 109] and *Cenococcum geophilum* [78]. These fungal ecotypes are mostly adapted towards those specific metals (Al, Ni, Zn, Cu, Cd, . . .) that are in excess in their soil of origin.

Mechanisms involved in metal tolerance in fungi include extracellular processes such as precipitation (e.g. secretion of oxalic acid), chelation and cell-wall binding. Intracellular mechanisms include chelation with organic acids, phytochelatins and other S-compounds, polyphosphates, peptides and transport into intracellular compartments (vacuole) [18]. Some of these mechanisms are constitutively present, whereas others are more activated when excess metals show up in the cytoplasm. Additional antioxidative detoxification systems, which allow the fungus to counteract the accumulation of reactive oxygen species (ROS), directly or indirectly are part of the detoxification response. It is likely that one or more of these mechanisms are modified in the evolution towards adaptive true tolerance in ECM fungi. Homeostasis of essential transition metals such as Cu and Zn requires balanced activities of transporters that mediate import into the cell, distribution to organelles and export from the cell [169]. Transcriptional control is important for the regulation of this cellular homeostasis. Nevertheless, when metals are present in very high concentrations in the environment, the regulatory mechanisms may fail and selection pressure for a more robust homeostasis will increase. In *Suillus* species (Basidiomycete), a strong differential net uptake of Zn is observed among Zn-tolerant and Zn-sensitive ecotypes when the fungi are exposed to elevated Zn [43].

Zinc-tolerant ecotypes accumulate less metal per unit biomass, indicating a metal exclusion system. In sensitive strains, excess Zn is transferred to vacuoles, with little efflux over the plasma membrane. In tolerant strains Zn efflux is much higher and less Zn accumulates in vacuoles.

Metal exclusion mechanisms in mycorrhizal fungi prevent metal stress in fungal cells, but are also of ecological importance for a host plant on metalliferous soil. Tolerant ecotypes are probably better filters than non-tolerant ecotypes because the former more strongly prevent metal transfer to their host. Ashford and Allaway [10] suggest that motile tubular vacuoles are an important vector in the transport chain of mineral nutrients from the site of uptake at hyphal tips to the exchange region in the mycorrhizal root. The observation that Zn-tolerant *Suillus* ecotypes do not store large amounts of Zn into their vacuoles may thus prevent a massive transport of Zn towards the mycorrhizas. Pine seedlings inoculated with metal-tolerant *Suillus* ecotypes in most cases have lower metal concentrations in their needles than seedlings inoculated with sensitive strains [110] confirming that metal-tolerant isolates restrict metal transfer more effectively and thus lead to a more efficient partnership with host plants thriving on metal-polluted soil.

6.3.2 Plant-Associated Bacteria

Under stress conditions including trace element stress, the synthesis of ethylene is increased which negatively affects plant growth [79, 145, 250]. Many plant-associated bacteria are equipped with the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD). This enzyme has no known function in bacteria, but antagonizes ethylene release in plants by cleaving the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). ACCD-producing bacteria therefore can reduce production of stress ethylene and in this way protect plants against trace element toxicity when growing on contaminated soils [77].

For survival in metal-enriched environments, plant-associated bacteria have developed diverse mechanisms to tolerate the uptake of these ions by which they can immobilize or transform the trace elements rendering them inactive. These mechanisms include physical sequestration, exclusion, and complexation or detoxification, etc. Certain efflux-based systems involved in bacterial trace element resistance include post-efflux sequestration of these elements. Extruded trace element ions are prevented from re-entering the cell by precipitation, chelation or by binding to exopolymers [60, 185]. The above-mentioned mechanisms immobilize the trace elements in the rhizosphere and reduce their uptake into the plant root, resulting in a reduced phytotoxicity. For instance, Madhaiyan et al. [127] found that inoculation with *Magnaporthe oryzae* and *Burkholderia* sp. reduces the Ni and Cd uptake in roots and shoots of tomato and also their availability in soil. This effect was attributed to the increased trace element biosorption and bioaccumulation by bacterial strains. Endophytes living in xylem vessels and possessing such systems may contribute to metal detoxification inside their host plants resulting in lowered

phytotoxicity and an increased trace element translocation to the above-ground plant parts [123, 251].

Bacteria equipped with the above characteristics are frequently naturally occurring and even abundant on metal contaminated sites [194]. Hyperaccumulator plants, such as *Thlaspi goesingense*, *Alyssum bertolonii* and *Thlaspi caerulescens*, are able to accumulate large amounts of metals and metalloids in their shoots and provide a specific niche for resistant endophytes [15, 88, 89, 124, 143, 144].

Lodewyckx et al. [124] characterized the cultivable Zn- and Cd-resistant endophytes in the Zn hyperaccumulator *T. caerulescens* subsp. *calaminaria*. Interestingly, shoot and root possessed different microbial communities and among shoot endophytes, *Methylobacterium* strains showed to be highly resistant to Zn, Cd, Co and Ni. Likewise, Barzanti et al. [15] isolated and characterized 83 endophytic strains from the Ni hyperaccumulator *A. bertolonii* endemic to the serpentine outcrops of Central Italy. Most of the isolates showed coresistance to more than one trace element and coresistance to Ni, Cr, Zn and Cu was the most frequent, whereas coresistance to Ni and Co was found less frequently. Idris et al. (2004) identified a wide range of bacteria showing high Ni resistance in the rhizosphere and shoots of the Ni-hyperaccumulator *Thlaspi goesingense*. Among the different bacterial isolates 36% of the endophytes showed ACC deaminase activity.

Recently, Kuffner et al. [112] isolated different rhizospheric and endophytic strains associated with Zn/Cd-accumulating *Salix caprea* ecotypes and investigated their potential to enhance phytoextraction of trace elements. Five of the endophytic strains were further tested for their production of trace element-mobilizing metabolites. Four of the *Actinobacteria* were shown able to mobilize Zn and/or Cd.

To improve phytoextraction efficiency, these naturally abundant strains equipped with the appropriate characteristics can be enriched by means of inoculation. In case these bacteria are not naturally colonizing the plant after isolation, bacteria can also be equipped with metabolic pathways for the synthesis of natural chelators and with trace element sequestration systems [222]. Proof of this concept was provided by Lodewyckx et al. [123] who inoculated Ni-exposed yellow lupine plants with a constructed Ni-resistant endophyte. They introduced the *ncc-nre* nickel-resistance system of *Ralstonia metallidurans* 31 A in the lupin endophytes *Burkholderia cepacia* L.S.2.4 and *Herbaspirillum seropdicae* LMG2284. Inoculation of lupin plants grown on a Ni-enriched substrate with the engineered endophytes, resulted in a 30% increased Ni concentration in the root tissue, whereas, the Ni concentration in the shoots remained comparable with that of the control plants.

Sun et al. [216] isolated and characterized 32 endophytic strains with respect to trace element resistance and production of plant growth promoting factors. In experiments using rape (*Brassica napus*) grown in vermiculite containing 4 mg kg^{-1} of Cu, inoculation with endophytic isolates was found to increase dry weights of roots and aerial tissues when compared to the non-inoculated control. Furthermore, increase in the Cu-content of aerial tissue varied from 63% to 125% in inoculated rape cultivated in the Cu-enriched substrate compared to the non-inoculated control. In another study, trace element resistant endophytic bacteria (*P. fluorescens* G10 and *Microbacterium sp.* G16) colonizing rape roots were investigated for their potential to increase Pb uptake and accumulation [200].

6.4 Plant Metal Stress Responses

6.4.1 Metal Homeostasis: Chelation and Sequestration

After plants take up metals, it is important to keep the free metal concentration under tight control in order to prevent metal-induced cellular damage. Passage across the plasma membrane by metals is enhanced by intracellular binding and sequestration. Once across the plasma membrane, metal ions are either bound to chelators or chaperones. Chelators contribute to metal detoxification by buffering cytosolic free metal concentrations. Chaperones specifically deliver metal ions to organelles and metal-requiring proteins. It is well described that Cu is a cofactor for plastocyanin, Cu/Zn-superoxide dismutase (CuZnSOD), etc. and it is required in different subcellular locations [171, 256]. Several chaperones are identified that deliver Cu to a specific protein in their specific location, such as CCS (Copper Chaperone for SOD) [17, 27, 168]. Phytochelatins, metallothioneins, organic acids and amino acids are well-described metal ion ligands with metal-specific properties [241]. Mugineic acid, nicotianamine, organic acids, histidine and phytate and their role as metal ion ligands for Fe, Zn, Cu, Mn and Ni for metal homeostasis in plants were reviewed by Haydon and Cobbett [84]. Whereas phytochelatins are clear chelators for Cd and As [38, 257], recently Tensteddt et al. [221] observed that phytochelatins function as important chelators of excess Zn^{2+}.

The activities of metal-sequestering pathways in root cells are crucial in determining the rate of metal translocation to the aerial parts. Beside Fe-nicotianamine transport for Fe translocation, this could also be an important mechanism for Mn translocation [92]. The role and characterization of membrane transporters in xylem loading of metal ions/ligands is currently under intense study. Ferric reductase defective 3 (FRD3), a member of the multidrug and toxin extrusion (MATE) family of transporters, is shown to be a citrate transporter involved in the loading of iron into the xylem [66]. A role is attributed to the membrane transporter HMA4 (Heavy metal ATPase 4) in Cd/Zn tolerance more specific in the root-to-shoot translocation of Cd and Zn [48, 82, 253].

6.4.2 Physiological Responses

Generally, plants can withstand metal accumulation until the metal reaches the toxic threshold level in the tissue, leading to growth retardation and toxicity at higher levels. As usual, metal-exposed plants develop reduced, compact roots system and smaller leaf area [193]. Furthermore, weaker growth is often accompanied by different toxicity symptoms such as root browning [170, 239], foliar chlorosis [151, 240], necrotic spots [215, 234], etc. The symptoms may appear in single or complex manner depending on numerous factors including species tolerance, external metal concentration, duration of exposure, etc. As a rule, chlorosis is

stronger in the younger leaves [29], while necrotic spots appear mostly on older leaves, where metal concentrations are higher [232]. The observed root browning most probably is due to enhanced lignifications processes [5]. At low levels of metal contamination, visual phytotoxicity symptoms may be less pronounced or even absent, but an enhanced activity of enzymes involved in plant cellular defense against metal induced oxidative stress, e. g. peroxidases, catalases, superoxide dismutases, as well as $NAD(P)^+$ reducing enzymes may be detected (cfr. Infra) [227, 239].

Due to similar phytotoxicity symptoms, there is an assumption that different metals have similar modes of toxic action. However, Cd, Pb and several other problematic metals have no biological function, while Zn, Cu and Mn are essential micronutrients, which become phytotoxic at supra optimal concentrations [39, 59]. Presumably, as the role of these metals in plant cell metabolism is completely different, their impact on plant performance can be also expected to differ. Some proofs in this aspect have been recently obtained when Cd and Zn were applied in concentrations producing similar (near 50%) inhibition of relative growth rate of durum wheat plants [104]. Briefly, Cd induced classic xeromorphic changes in leaf structure, which were not presented in Zn-exposed plants. In addition, Cd exposure strongly modulated enzyme activities, e.g. ascorbate peroxidase activity, while no significant changes in its activity were observed in Zn-exposed plants

The biochemical bases of metal phytotoxicity is well described and mostly due to three negative effects [61]: (1) metal-induced oxidative stress and damages (cfr. infra), (2) direct effects of metal ions with sulfydryl groups in membrane proteins leading to their dysfunction and (3) inactivation of important enzymes by replacing activation cations with other metal ions [228]. The multiplication of the primary metal toxicity effects leads to functional disorders in the cardinal physiological processes and anatomic-morphological changes and damages.

Metals contact first the root system that shows reduced elongation upon exposure. This often resulted in appearance of “stubby” roots having higher specific dry mass content. Instead of a well-structured root system, brown short laterals are developed. In addition, cell division is lowered due to different damages in the nucleus structures and mitosis [65, 225].

After root metal uptake, deficiencies and imbalances of mineral nutrients can be induced. They can reduce nutrient uptake and translocation through competition, affect root cells membranes, ATP-ases and other carriers resulting in decreased unsuberized root tips and damage of the permeability of root cells [37, 183]. For example, Cd decreased root concentrations of Zn, Cu and Mn in barley plants and *Salix viminalis* cuttings [234, 239]. The decreased essential nutrient content may be also caused by ion leakage from damaged roots and immobilization of elements in roots, resulting in their strong deficiency in the shoots [203]. The most pronounced such effect is K^+ leakage as consequence to Cd-provoked disturbed membrane permeability [146, 229].

Excess metals induce disorders in plant water relations, such as reduced water uptake, translocation and transpiration [11]. The reduced water uptake in metal-exposed plants can be partly explained by root growth inhibition, but binding of

metals, for example Zn, to water-channel proteins in the membrane also occurs [115]. Furthermore, the water movement into xylem vessels is also affected by metals [133]. The reason for the reduced water movement is both a decreased vessel radius and number of vessels due to Cd, Zn and Cr induced inhibition of division, elongation and differentiation of cambium cells [11]. In addition, accumulation of lignin-like insoluble phenols and depositions of Ca oxalate can cause structural disorders in the vessels and hence decrease the water movement. The disturbed plant water relations resulted in decreased relative water content (RWC), water potential (Ψ) of leaves of metal-exposed plants, which may have far going consequences for many physiological processes [233]. Diminished leaf water content decreased transpiration intensity, which in turn enlarged stomatal limitation of photosynthesis [133, 237].

Most of the observed physiological disturbances in metal-exposed plants may finally be focused on photosynthetic performance. In addition to limited access of CO_2 through stomata, metals may affect photosynthesis at other levels, e.g. pigments, thylakoid ultrastructure and electron transport, activities of Calvin cycle enzymes, etc. [107, 230].

The reduced chlorophyll content in metal-exposed plants may be due to inhibition of its biosynthesis [213], metal-induced Fe and Mg deficiency [233], Mg-substitution in the chlorophyll molecule [114], chlorophyll degradation resulting from oxidative damage or enzymatic degradation [129, 249]. Baryla et al. [14] have reported that leaf chlorosis in Cd-exposed oilseed rape was due to neither of the mentioned reasons, but it was attributable to a marked decrease of chloroplast density caused by a reduction in the number of chloroplasts per cell.

Metal-induced disorders in chloroplast and thylakoid ultrastructure – swelling of thylakoids, disruption of envelope, etc, are well documented [8, 231] as well as their negative effects on PSII and PSI activities, analysed *in vitro* [16, 235] and *in vivo* [39, 108]. These negative effects may be partly explained by the enhanced lipid peroxidation at chloroplast level. Evidence for this was obtained by increased ethylene production together with diminished total fatty acids content in isolated thylakoids from Cd- and Cu-exposed barley plants [236, 238]. Furthermore, the electron transport might be limited by changes in the concentrations of the electron carriers [119] due to a decrease in essential cations as well as metal-induced alterations in chlorophyll molecule integration into pigment-protein complexes [87].

A lower photosynthetic performance in metal-exposed plants was observed by several authors [120, 239] and supports the opinion of Krupa et al. [108] that metal-induced alterations in primary C metabolism may lead to a down-regulation of PSII activity due to a reduced demand for ATP and NADPH. That also agrees with a lower capacity for ^{14}C photoassimilation and partitioning of labeled photoproducts [230] as well as increased pools of ATP and ADP in leaves of Cd-exposed plants [204]. Furthermore, metals may indirectly decrease photosynthetic rate by changing the sink-source relationship, with a consequent diminished requirement for photosynthetic products [36]. The metal-induced growth inhibition may cause phloem overloading leading to decreases in enzyme activities and energy consumption by Calvin's cycle reactions, and finally reflecting in downregulation in PSII.

6.4.3 Metal Stress: An Oxidative Challenge

Elevated metal concentrations in the environment cause great losses in plant biomass production worldwide. A better understanding of the underlying molecular mechanisms of metal phytotoxicity is most useful to develop or adjust strategies for growing non-food crops on metal-contaminated agricultural soils. The cellular oxidative stress signature leading to oxidative signalling or damage is an important determinant in metal phytotoxicity [196].

Multiple studies have shown that different metal stresses lead to elevated amounts of reactive oxygen species (ROS) and changes in the antioxidative defence systems in plants (Table 6.1). Depending on the chemical behaviour of metal ions, the metal-induced oxidative stress differs [51, 56, 210]. Metal ions, able to perform monovalent oxidoreduction reactions, easily convert molecular oxygen, O_2, to ROS, e.g. superoxide ($O_2^{\circ -}$) and hydrogen peroxide (H_2O_2) by electron transfer. Furthermore redox-active metals like Cu, Fe... promote hydroxyl radical ($^{\circ}OH$) formation through the Fenton reaction [81; Fig. 6.1], leading to oxidative damage of macromolecules and cellular malfunctioning. Unlike harmful effects, such as DNA-oxidation, lipid peroxidation... that can be a direct consequence of enhanced ROS production, these molecules also exert an important role as signalling molecules. The balance between damage versus signalling is an oxidative challenge imposed by metal ions to the cells and is characterized by the duration, intensity and frequency of metal-induced ROS production [55]. In the next paragraphs emphasis will be on the cellular ROS balance, i.e. ROS production and antioxidative defence, and consecutively the oxidative damage and signalling under metal stress.

In addition to direct metal-induced ROS production through Fenton and Haber-Weiss reactions [190], also other indirect pathways come into play when investigating oxidative stress responses from bivalent cations such as Cd, Zn, Pb not able to perform redox-reactions. Under natural conditions, ROS are produced in organelles with a highly oxidizing metabolic rate or having electron transport chains (e.g. peroxisomes, chloroplasts, mitochondria) [81]. In multiple studies it has been described that metal stress enhances the ROS production in these organelles, by disturbing photosynthesis and respiration (cfr. supra). A quick burst in ROS was detected in *Arabidopsis* cells exposed to Cd as a consequence of morphological and functional changes in mitochondria and chloroplasts [20]. Superoxide levels were elevated under Cr stress by inactivation of the electron transport chain in pea root mitochondria [64]. Elevated H_2O_2 and $O_2^{\circ -}$ contents were observed in the peroxisomes after exposure to Cd [182 and references therein].

Whereas the enzymatic oxidative burst is well studied under pathogenic attack [223], NADPH-oxidases are clearly involved in ROS production after metal application. Remans et al. [178] demonstrated a metal-specific upregulation of NADPH oxidase gene expression in the roots for Cd, whereas Cu caused a metal-specific downregulation of NADPH oxidase genes. Nevertheless ROS production by NADPH-oxidases under Cu stress cannot be ruled out as Navari-Izzo et al. [155] observed an early, but transient induction of NADPH-oxidase activities in Cu-exposed wheat roots.

Table 6.1 Responses of the antioxidant defence system in plants exposed to elevated metal concentrations

Metal	Concentration	Exposure time	Plant	Antioxidant defence mechanisms	References
Cd	3–10–30 μM	Minutes to 24 h	*Medicago sativa*	Altered AsA, GSH	[158]
	5–10–20 μM	24 h	*Arabidopsis thaliana*	Altered transcription, Px↑, GR↓	[209]
	5–10 μM	24 h	*Arabidopsis thaliana*	Altered transcription, CAT↑, APx↑, altered AsA, GSH	[56]
	5 μM	96 h	*Phaseolus vulgaris*	Altered APx, CAT, GR	[31]
	1–10 μM	7 days	*Arabidopsis thaliana*	AsA↓, GSH↓, *GR*↓, APx↑, CAT↑, SOD↑, GR↑, GPOD↑	[192]
	6–30 μM	7 days	*Zea mays*	Altered GSH, APX↑	[177]
	3–10–30 μM	7 days	*Medicago sativa*	AsA↑, biothiols↑, APx↑, GR↑	[212]
	75 μM	7 days	*Arabidopsis thaliana*	α-tocopherol↑	[40]
	10–25–50 μM	15 days	*Brassica juncea, B. napus*	Non-protein SH↑, SOD↓, CAT↓, GR↓, APx↓	[156]
	300–500 μM	21 days	*Arabidopsis thaliana*	SOD↑, GPx↑, APx↑, GR↑	[33]
Pb	1–10–100–200–500 μM 1–10 mM	3, 24 h	*Arabidopsis thaliana*	Altered transcription, SOD↑, Px↑	[122]
	100–1,000 μM	12, 24 h	*Taxithelium nepalense*	AsA↑, GSH↑, Px↓ GR↓	[35]
	10–100–1,000 μM	24 h	*Taxithelium nepalense*	AsA↑, GSH↑, SOD↑, CAT↓, Px↓, GR↓	[34]
	200–500–800 ppm	12 days	*Macrotyloma uniflorum, Cicer arietinum*	SOD↑, CAT↑, Px↑, GR↑, GST↑	[175]
	10–50–100–200 μM	14 days	*Phaseolus vulgaris*	SPx↑, GPx↑, APx↑, GSH-Px↑, DHAR↑, GR↑	[76]
	500–1,000 μM	5, 10, 15, 20 days	*Oryza sativa*	Altered CAT, SOD↑, GPx↑, APx↑	[242]
Cu	100 μM	1 min to 6 h	*Brassica napus*	GSH↓, GR↓ in roots, GSH↑, GR↑ in leaves	[184]
	2–5 μM	24 h	*Arabidopsis thaliana*	Altered AsA, GSH, CAT↓, APx↓, GR↓ in roots CAT↑, APx↑, GR↑ in leaves	[56]
	25 μM	96 h	*Solanum lycopersicon*	GPx↑, SOD↑, CAT↑, APx↑, GR↑	[30]
	15–150–1,500 μM	5 days	*Hordeum vulgare*	Altered non-protein SH, CAT↑, GSH-Px↑, SOD↓	[58]
	50 μM	7 days	*Phaseolus vulgaris*	AsA↑, GSH↑, MDHAR↑, DHAR↑, GR↑, APx↑	[52]
	75 μM	7 days	*Arabidopsis thaliana*	α-tocopherol↑, AsA↑	[40]

(continued)

Table 6.1 (continued)

Metal	Concentration	Exposure time	Plant	Antioxidant defence mechanisms	References
	10–50–75–100–150–200–300 μM	21 days	*Brassica juncea*	SOD↑, APx↑, GPx↑, CAT↑	[207]
Zn	0.1–1–10–100 mM	2, 4, 6, 8 days	*Triticum aestivum*	AsA↑, GSH↓, CAT↓, GPx↓,SOD↓	[163]
	100 μM	96 h	*Phaseolus vulgaris*	Altered Px, MDHAR activities	[31]
	50 μM	7 days	*Phaseolus vulgaris*	APx↓, GR↓ in roots, AsA↑, AsA-GSH pathway↑ in leaves	[53]
	1–5 mM	11 days	*Verbascum thapsus*	APx↑, SOD↑, Px↑, MDHAR↑, GR↓, DHAR↓	[150]
	0.15–0.3 mM	15 days	*Lemna minor*	AsA↑, SOD↑, Px↑, CAT↓	[173]
Al	25 μM	2–48 h	*Arabidopsis thaliana*	Transcription of antioxidant enzymes↑	[179]
	30–50–100 μM	24 h	*Triticosecale*	SOD↑, Px↑	[121]
	10–50 μM	24, 48 h	*Pisum sativum*	Altered transcription, activities, AsA, GSH	[162]
	2–4–6–8 mM	48, 72 h	*Hordeum vulgare*	Altered SOD, Px, APx activities	[205]
	0.001–0.01–0.1–1.0 mM	9 days	*Vigna radiata*	AsA↓, GSH↓, CAT↓, SOD↑, Px↑, GR↑	[164]
	0.15–0.3 mM	15 days	*Lemna minor*	SOD↑, Px↑	[173]
As	10–100–1,000 μM	24 h	*Taxithelium nepalense*	AsA↑, GSH↑, SOD↑, CAT↓, Px↓, GR↓	[34]
	100 μM	3, 10 days	*Arabidopsis thaliana*	*Cu/ZnSOD*↑, Cu/ZnSOD↑, *FeSOD*↓, FeSOD↓	[2]
	2–5 mg/L	5 days	*Phaseolus vulgaris*	Px↑	[214]
	10–50 μM	7 days	*Phaseolus aureus*	SOD↑, GPx↑, GR↑, CAT↓	[206]
	50–100 μM As(III) 100–500 μM As(V)	10 days	*Oryza sativa*	SOD↑, APx↑, Px↑, GR↑	[202]
Cr	100–1,000 μM	12,24 h	*Taxithelium nepalense*	AsA↑, GSH↑, Px↓, GR↓	[35]
	50–100–200 μM	1, 3, 5, 7 days	*Brassica juncea*, *Vigna radiata*	SOD↑, APx↑, CAT↑, GR↑	[63]
	0.1–1–10–100 mM	2, 4, 6, 8 days	*Triticum aestivum*	AsA↑, GSH↓, CAT↓, GPx↓ SOD↓	[163]
	0.2–2–20 μM	1, 2, 3, 4, 5, 15 days	*Brassica juncea*	SOD↑, APx↑, CAT↑, GR↑, GST↑	[165]

Hg	3–10–30 μM	Minutes to 24 h	*Medicago sativa*	Altered AsA, GSH, APx↑	[158]
	6–30 μM	7 days	*Zea mays*	Altered GSH, APx↑	[177]
	3–10–30 μM	7 days	*Medicago sativa*	AsA↑, biothiols↑, GR↓ in roots, GR↑ in leaves	[212]
	10–50 μM	10, 20 days	*Lycopersicon esculentum*	SOD↑, CAT↑, Px↑	[32]
	20–30–40 μM	21 days	*Arabidopsis thaliana*	Transcription of antioxidant enzymes↑	[85]
Mn	183–1,830–18,300 μM	5 days	*Hordeum vulgare*	Altered AsA, CAT↑, GSH-Px↑, SOD↓, APx↓	[58]
	50–100 μM	6 days	*Vigna unguiculata*	Altered AsA, Px	[68]
	600 μM	11 days	*Cucumis sativus*	AsA↓, GSH↓, SOD↑, Px↑, APx↑, DHAR↑, GR↑	[201]
	50–100–250 μM	11 days	*Pisum sativum*	AsA↓, GSH↓, SOD↑, APx↑, DHAR↑, GR↑, CAT↓	[75]
Ni	100 μM	1, 3, 7, 14 days	*Zea mays*	Px↑, APx↑, SOD↑, CAT↓	[113]
	10–200 μM	3, 6, 9 days	*Triticum aestivum*	Px↑, GST↑, SOD↓, CAT↓	[74]
	50–100–200–400–800 μM	7 days	*Luffa cylindrica*	SOD↑, GPx↑, CAT↑	[246]
	200–400 μM	5–20 days	*Oryza sativa*	AsA↑, GSH↓, SOD↑, GPx↑, APx↑, AsA-GSH pathway↑	[128]

For each metal, the effects of acute and prolonged exposure to low or high concentrations are summarized for different plant species based on available literature

Underlined enzymes indicate affected transcript levels

APx ascorbate peroxidase, *AsA* ascorbate, *CAT* catalase, *DHAR* dehydroascorbate reductase, *GPOD* guajacol peroxidase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GSH* glutathione, *GST* glutathione-S-transferase, *MDHAR* monodehydroascorbate reductase, *Px* peroxidase, *SOD* superoxide dismutase, *SPx* syringaldazine peroxidase

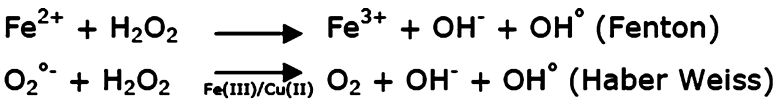

Fig. 6.1 Fenton and Haber Weiss reaction

Besides electron transfer, energy transfer to O_2 results in the conversion to singlet oxygen ($^1O_2$) with a highly oxidizing capacity [70]. Under metal stress, this process can happen in the chloroplast by inefficient transfer to the complexes of the electron transport chain, but $^1O_2$ molecules are also formed as a by-product of lipoxygenase activities [98]. Lipoxygenase gene expression [56, 178, 209, 210] and enzyme activity [99, 219, 258] are elevated under Cd, Hg and Cu stress, possibly leading to a higher production of $^1O_2$. In *Arabidopsis* cell cultures exposed to Cd, an immediate burst of $^1O_2$ was noticed that was not high light dependent (Van Belleghem, personal communication, 2007). Lipoxygenases catalyze the addition of molecular oxygen to polyunsaturated fatty acids that lead to lipid peroxidation but it can also be subsequently modified to bioactive compounds such as oxylipins [69]. Jasmonates are lipid-derived signalling compounds with a role in normal plant growth and development, as well as in the response of plants to (a)biotic stress factors [56, 118, 208, 248].

As sessile organisms, plants cannot escape from toxic surroundings, so in order to counterbalance the stress-induced ROS production plant cells contain a lot of antioxidants, *i.e.* enzymes and metabolites. It is essential to prevent the production of °OH-radicals, having a very short half-life and attacking everything around it [81]. Superoxide radicals are the primary ROS formed after electron transfer and superoxide dismutase (SOD) converts these molecules to H_2O_2 and O_2. In plants 3 different groups of isoforms exist, each containing a redox-active metal in the active site to perform the reaction: CuZnSOD, FeSOD, MnSOD located in different subcellular locations (for a review see 148). Although changes in the SOD activities under metal stress have been described (Table 6.1), currently more information becomes available on the transcript level as well as on the transcriptional and posttranscriptional regulation. In conditions of Cu-deficiency [106, 254] as well as during Cu-excess [1, 153] the presence of GTAC motifs in the promotors of genes are essential in Cu-sensing and homeostasis [62 and references therein]. The presence of Cu induces the *CSD* (CuZnSOD) gene expression and simultaneously inhibits *FSD* (FeSOD) gene expression through the presence of Cu negative *cis*-acting elements in the promotors of miRNA398 (negative posttranscriptional regulator of *CSD* gene transcripts) or the *FSD* gene itself. Recently, the opposite (a downregulation) was observed for the *CSD* gene transcripts under Cd-stress together with un upregulation of *miR398* [56]. Future experiments are needed to reveal the processes by which metal stress affects gene regulation at different biological organisation levels, *i.e.* epigenetics, transcriptional and posttranscriptional regulation.

Hydrogen peroxide is the subsequent ROS in the electron transfer that needs to be scavenged in order to prevent °OH formation. Catalases and peroxidases are

very important scavengers of H_2O_2 and are stimulated under metal stress (Table 6.1). Furthermore peroxiredoxins and associated redoxins come into play to detoxify H_2O_2 by the use of their thiol groups. Although investigations are ongoing for these components in H_2O_2 detoxification [224], the information under metal stress is rather scarce. Conversion of H_2O_2 to H_2O and O_2 links enzymatic systems directly to the antioxidant metabolites, more specifically ascorbate (AsA) and glutathione (GSH). They are electron donors for peroxidases and GSH is also involved in the regeneration of oxidized AsA as well as glutaredoxins. Both AsA and GSH are highly abundant, soluble metabolites present in different cellular compartments [71] and form important constituents of the redox balance in plant cells that can be affected by metal exposure (Table 6.1). During metal stress, special attention should be given to GSH, since its functional group is susceptible for different metals such as Hg, Cd ... showing high affinities to thiols. In this regard it plays a central role in metal chelation (as a precursor for phytochelatins) as well as through its antioxidant capacities.

The steady state level of ROS in the different cellular compartments is determined by the interplay between multiple ROS-producing pathways and ROS-scavenging mechanisms. As mentioned before, ROS are capable of modulating signalling networks that control physiological processes and stress responses [71, 148]. ROS, such as H_2O_2, are ideal signalling molecules as they are small and able to diffuse over short distances. Because H_2O_2 production is an immediate response to increased metal stress [20, 56, 150, 247], it is probably a key molecule that can trigger signal transduction events after plant metal exposure, mediating the acquisition of tolerance [19, 130, 131]. Whereas oxidative signaling is very important under metal stress, the complex interaction with other signaling pathways [54] needs further attention.

In conclusion, the term 'metal-induced oxidative stress' can be more specified in either oxidative damage and oxidative signalling that together forms an oxidative challenge for the cells to cope with metal stress.

6.5 Conclusions

Nutrient uptake by plants is essential for their development and for the passage of minerals into the food chain, but faces several limitations. The plant possesses several mechanisms to explore the soil for minerals such as root development, releasing siderophores, organic acids, etc... but the symbiosis with microorganisms clearly improves the ability of plants to overcome these limitations and needs further attention in the study of plant-soil interactions. Also in the research on plant metal stress and its cellular responses, microorganisms are shown to be important players in the plant protection to excess metal exposure.

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