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Nutrient metal elements in plants

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Plants need many different metal elements for growth, development and reproduction, which must be mobilized from the soil matrix and absorbed by the roots as metal ions. Once taken up by the roots, metal ions are allocated to different parts of the plant by the vascular tissues. Metals are naturally present in the soil, but human activities, ranging from mining and agriculture to sewage processing and heavy industry, have increased the amount of metal pollution in the environment. Plants are challenged by environmental metal ion concentrations that fluctuate from low to high toxic levels, and have therefore evolved mechanisms to cope with such phenomena. In this review, we focus on recent data that provide insight into the molecular mechanisms of metal absorption and transport by plants, also considering the effect of metal deficiency and toxicity. We also highlight the positive effects of some non-essential metals on plant fitness.

1. Introduction

Plants require a complex balance of mineral nutrients to grow and reproduce successfully. In addition to water, oxygen and carbon dioxide, 14 mineral elements are essential to all plants.[1] Among them, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S) and magnesium (Mg) are required in relatively large amounts (>1000 mg kg^{-1} dry weight) and are therefore defined as *macroelements*. In contrast, chlorine (Cl), iron (Fe), boron (B), nickel (Ni), copper (Cu), manganese (Mn), zinc (Zn) and molybdenum (Mo) are needed in smaller amounts (<100 mg kg^{-1} dry weight) and are thus called *micronutrients* or *trace elements*.

The availability and mobility of mineral elements in the soil can fluctuate significantly in time and space reflecting soil properties, seasonal and climatic factors, and the presence of root exudates such as siderophores and organic acids, as well as rhizosphere microorganisms.[2] Plants, as sessile organisms, have evolved adaptive strategies not only to uptake sufficient quantities of essential macronutrients and micronutrients but also to avoid excess accumulation, which would be toxic. Indeed, excess levels of essential free metal ions are toxic to cells because they generate reactive oxygen species (ROS) or substitute other metal ions within metalloproteins, rendering

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them inactive.[3] Furthermore, plants must deal with the non-essential elements such as arsenic (As), mercury (Hg), silver (Ag), antimony (Sb), cadmium (Cd), lead (Pb) and uranium (U), which may potentially be harmful. Plants therefore activate homeostatic mechanisms allowing the uptake and distribution of metals within tissues to respond according to the need for essential mineral nutrients in sufficient amounts for normal growth and development, but to avoid the accumulation of non-essential elements and toxic levels of essential elements.[4]

In order to accumulate in plant cells, metals must be mobilized and absorbed from the soil, sequestered in the root and then loaded into the xylem and transported to the aerial parts of the plant, and finally must be distributed among the leaf cells. Each step requires a complex interaction of chelating compounds and metal transporters that affect the rate of metal accumulation.[5] Over the last few decades, many of the mechanisms that regulate the transport of metal ions have been determined. Several chelating molecules and cation transporters have been identified and characterized. The main players in the micronutrient transport network are members of the ZIP (ZRT zinc regulated transporter, IRT-like protein, iron regulated transporter) family[6,7] and of the NRAMP (natural resistance-associated macrophage protein) family.[8–10] Different metal chelators are also involved in various stages of micronutrient uptake, internal transport, and sequestration in the cytosol or subcellular compartments.[3]

Micronutrients are involved in diverse cellular functions, including energy metabolism, primary and secondary metabolism, defense, gene regulation, hormone perception, signal transduction, and reproduction.[11] Several micronutrients also have redox properties and act as cofactors in metallo-enzymes. Because their availability in the soil can fluctuate substantially, plants may experience both excess accumulation and deficiency at different times. High concentrations of redox-active micronutrients are harmful because they generate ROS which are potentially toxic due to their higher reactivity compared to O_2. ROS may cause nonspecific oxidation of proteins, membrane lipids and nucleic acids.[12] Conversely, inadequate micronutrient supplies cause deficiency symptoms reflecting their essential physiological roles, often manifesting as poor growth and abnormal morphology.[13] Furthermore, certain micronutrients are defined as being beneficial because they stimulate growth under particular environmental conditions or may be essential only for some taxa but not required by all plants.[14] High tissue concentrations of the beneficial element indicate a structural or an osmotic role, whereas low tissue concentrations suggest that the element functions as a cofactor for specific enzymes, often involved in abiotic or biotic stress resistance.[15]

This review focuses on recent advances that have improved our understanding of the molecular mechanisms and genetic factors influencing the absorption, transport and distribution of five essential micronutrients: Zn, Cu, Mo, Mn and Ni. For each of them, we describe how these findings provide insight into their role, the impact of excess accumulation and deficiency. Fe is excluded from this article deliberately because its role has been discussed extensively in recent comprehensive reviews[16,17] whereas Cl and B are not covered because they are not metals. Finally, we discuss the positive effects on plant fitness of five non-essential but in several circumstances beneficial elements: sodium (Na), silicon (Si), selenium (Se), aluminum (Al) and cobalt (Co).

2. Metal absorption and transport

Studies of root uptake, root-to-shoot translocation and maintenance of elemental homeostasis have focused on the characterization of metal transporters.[18] Significant advances have also been made in the study of chelate-based transport, and metal chelators are known to play a role in long-distance transport and detoxification, avoiding nutritional imbalances caused by an excess of or a deficiency of a certain element.[5] The presence of several transporter families in plant genomes suggests the presence of a sophisticated mechanism of metal homeostasis to cope with dynamic changes in nutrient availability.[19] Furthermore, transporters from the same family may

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compete for the transport of multiple cations due to their broad substrate affinity,[20] and different transporter proteins may show different tissue-specific expression profiles and subcellular localizations.[21] Most plant metal transporter families can be assigned to one of the two functional categories: (i) families required for metal sequestration into the cytosol (influx), and (ii) families required for metal remobilization from the cytosol (efflux).[22]

The ZIP, NRAMP, yellow stripe (YS) and copper transporter (COPT) families play a primary role in metal uptake and remobilization from intracellular compartments into the cytosol. In *A. thaliana*, the ZIP family comprises 15 genes involved in the transport of cations (including Zn, Mn, Fe, Ni and Cd) across cellular membranes into the cytoplasm, thus contributing to metal homeostasis.[7] There is partial functional redundancy among the ZIP proteins in terms of substrate affinity for Zn, Fe and Mn.[23] The NRAMP family, mostly characterized in *A. thaliana*, includes at least six members that transport a range of divalent metal cations such as Fe, Mn, Zn and Cd, whereas the YS proteins mediate the absorption of transition elements that are complexed with phytosiderophores (PS) or nicotianamine (NA), and are homologous to proteins in *A. thaliana* named Yellow Stripe-Like (YSL).[24] Members of this family, such as YSL4 and YSL6, are found in vegetative and reproductive tissues, suggesting a role in nutrient translocation within the plant.[25] The COPT proteins, belonging to the CTR Cu transport family, are ubiquitous high-affinity Cu transporters including six COPT proteins in *A. thaliana*.[26]

The efflux of metals from the cytoplasm is carried on by several other families, including the heavy metal-transporting ATPases (HMAs), the cation diffusion facilitator (CDF) family, the cation exchanger (CAX) family, and the multi-drug and toxic compound extrusion (MATE) family, and the plant cadmium resistance (PCR) family and ferroportin (FPN) families also play a role in metal efflux but their precise functions are poorly understood.[22,27–29] The HMAs and CDFs are the largest families. The *A. thaliana* genome encodes eight HMA members belonging to the ubiquitous P-type ATPase superfamily. These pumps use energy from ATP hydrolysis to move Zn, Cu and Cd into several organelles.[30] There are also 12 CDFs in *A. thaliana*, also known as metal tolerance proteins (MTPs). These are implicated in the scavenging of various cations from the cytosol, including Zn, Mn, Cd, Co and Ni, thus contributing to increase metal tolerance.[31]

2.1 Root uptake

Although some nutrients such as SO_2, NH_3 and NO_2 may be absorbed in the form of gases *via* stomata and metabolized directly by leaves,[32,33] most minerals are absorbed *via* the roots. Plants have evolved a number of strategies to increase metal absorption, including acidification, the secretion of organic chelators, and the expression of high-affinity metal transporters.[34] The first of these mechanisms involves the release of protons into the rhizosphere, to increase the solubilization of cations such as Fe, Cu and Zn.[21] This is mediated by ATP-dependent proton pumps (H^+-ATPases) in the plasma membrane of root cells. In *A. thaliana*, some members of this family such as AtAHA1 are expressed constitutively, whereas others such as AtAHA2 are induced under Fe deficiency conditions.[35]

Once solubilized, metals can be adsorbed to the cell wall or move passively through the root apoplast. Redox-active metals, such as Fe, Cu and Mn, are chemically reduced by membrane proteins of the ferric oxidase–reductase (FRO) family.[36] The active uptake of the nutrients into the symplast is then required due to the presence of the Casparian strip in the root stele.[37] This involves Fe^{2+} transport by the main high-affinity iron transporter IRT1,[38] Cu^+ transport by COPT1,[26] and Zn transport predominantly by members of the ZIP family.[6] IRT1 is the best characterized member of the ZIP family and can transfer other cations in plants (Zn, Cd and Ni) and in yeast (Zn, Mn, Co, Ni and Cd), confirming its broad substrate affinity.[39] In *A. thaliana*, Mn is transported by NRAMP1, which is present in the plasma-membrane of root hair cells and upregulated by low Mn availability and also mediates the uptake of Fe and Co.[9,40] It is unclear how Mo is taken up by roots and partitioned among plant tissues. The functional characterization of the *A. thaliana* Mo high-affinity transporter MOT1.1 indicated that this protein is regulated by the external Mo concentration, and expressed in the root differentiation zone and in the mature vascular tissue of both roots and shoots. The subcellular localization of the protein is still not clear, even though a plasma-membrane localization and vesicle localization were suggested,[41] as well as mitochondrial expression.[42] A different mechanism is required for the acquisition of transition metals, based on the release of strong chelating agents into the soil, such as PS compounds, non-proteinogenic amino acids, specifically synthesized from the ubiquitous precursor NA. Once released into the rhizosphere, PS plays a major role in the chelation of Fe^{3+} but also captures other cations such as Zn, Cu, and Ni.[43]

2.2 Root-to-shoot transport

Once absorbed in the root apoplast, cations can be sequestered by root cells or translocated radially into the root stele and subsequently loaded into the xylem. The transpiration stream therefore drives the xylem sap to the shoot, where metals can be allocated to the aerial tissues (Fig. 1).[20] Because transition metals are highly reactive, chelation seems to be required to avoid oxidative stress and to facilitate ion translocation through the vasculature.[44] Metal chelation is achieved by association with amino acids, organic acids, mugineic acids (including PS and NA), and metallothioneins (MTs).

Citrate, in association with other organic acids, has a critical role in metal tolerance and detoxification. It can form stable complexes with Fe and Zn, especially in the xylem sap where the pH is $\sim$5.5–6.0.[45] Ni–citrate complexes were found in the aerial tissues of *Nocceae goesingense* and *Thlaspi arvense*.[34,46] The amino acid histidine is required for Ni translocation and detoxification in some Ni hyperaccumulators from the genus *Alyssum*, confirming its essential role in metal tolerance.[47] Ni can also be bound to NA, found in the roots of *N. cerulescens*,[48] *A. lesbiacum* and *A. montanum*.[47,49] NA forms strong complexes with Mn, Fe, Co, Zn, Ni and Cu, and together with the PSs promote long-distance metal transport.[50]

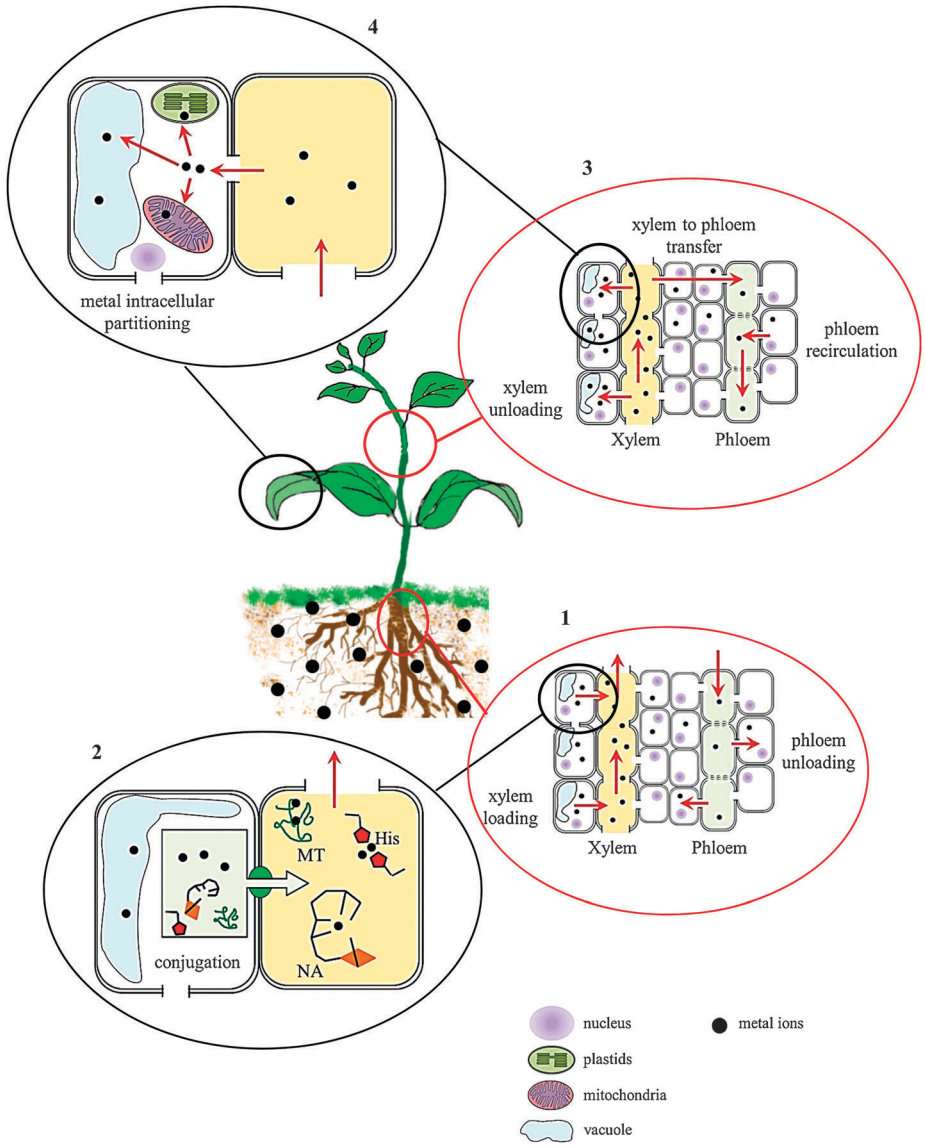

Fig. 1 Main route followed by metal elements in plants. Upon root absorption metal ions are loaded into the xylem (1) as free ions or conjugated forms (2). Following the water stream, ions are delivered to the shoot, exiting the xylem (3). In shoot tissues, metal ions are delivered to cells and subcellularly partitioned (for nutrients) or detoxified (in case of toxic elements) (4). A small portion of ions can be transferred to the phloem and cycle back to the root tissue (3). See the text for a detailed description.

The influx of free or chelated ions into the vascular tissues is regulated by metal-specific transporters. AtHMA5 is thought to regulate the xylem loading of Cu from root cells, whereas AtHMA2/AtHMA4 plays the same role for Zn/Cd. The *hma5* and *hma2*/*hma4* mutants are characterized by much higher metal accumulation in roots compared to wild-type plants, consistent with their long-distance transport activity.[51,52] The upload of Zn and Cd into the xylem by HMA4 is particularly important in the Zn/Cd hyperaccumulator *Arabidopsis halleri*, in which its increased expression, together with gene triplication, lead to hyperaccumulation and hypertolerance of these metals.[53] PCR proteins are also important for the translocation of Zn into the xylem. In *A. thaliana*, PCR2 acts as a membrane Zn-efflux transporter which takes Zn from the roots, ensuring detoxification under excess metal loading.[28] FPN1 is responsible for the long-distance transport of Fe although its presence is also required for the mobilization of Co.[29]

The unloading of the metals from the xylem is not well characterized. Several FRO and ZIP proteins are expressed in the shoot, suggesting a possible role in cation mobilization towards aerial tissues, but additional mechanisms for the long-distance delivery of metals are likely to be required for metal homeostasis.[18] Little is known about the overall processes of chelation and ligand exchange during xylem unloading.[27]

Plants are characterized by another vascular tissue, the phloem, which runs parallel to the xylem and translocates and redistributes the products of photosynthesis and other signal molecules (hormones) and nutrients throughout the entire plant body,

between sources and sinks. Some nutrient ions undergo the so-called phloem recirculation, *i.e.* ions are subjected to a rapid xylem-to-phloem transfer in leaves and stems, and are re-translocated back to the roots (Fig. 1). Such cycling is important in nutrient re-distribution, as for nitrogen, potassium, phosphorous and magnesium.[54] Experiments conducted with labelled metal ions and the analysis composition of the plant fluids showed that some heavy metals may undergo xylem-to-phloem transfer and also phloem cycling, as in the case of the nutrients Fe, Cu, Co, Ni, Zn and Mn and the toxic metal Cd.[55,56] As in the xylem, metal ions stored into the phloem are also conjugated to a variety of compounds, including low and high molecular weight molecules, such as NA, His and phytochelatins (for a recent review, refer to ref. 56). The phloem transport involves (i) apoplastic loading into both companion cells and sieve elements and (ii) unloading at the target sink tissues. Members of the oligopeptide transporter family (OPT) are involved in this process, being able to transport metal-bound amino acids.[57] In *A. thaliana*, the phloem-specific transporter OPT3 mediates transition metal transport rather than small peptides. This protein is involved in Fe loading into the phloem of leaves and in Fe accumulation in developing tissues, such as seeds. AtOPT3 is therefore involved in Fe xylem-to-phloem redistribution and it also participates in Cd partitioning in this tissue.[58]

2.3 Cellular distribution

At the cellular level, metals are partitioned into almost all subcellular compartments. The cytosolic concentration of redox-active metals must be strictly controlled to avoid the generation of ROS. Chelation plays an important role in protection. For example, Cu^{2+} is predominantly bound to histidine, whereas Cu^{+} interacts with MT.[59] In *A. thaliana*, metallo-chaperone proteins have been identified that target Cu to specific metalloproteins, thus modulating the Cu level. Similarly, Zn^{2+} is a strong Lewis acid which is promptly chelated by PSs, glutathione, NA, histidine and MTs, both in the cytoplasm and in the subcellular compartments.[60] The partitioning of metals in the vacuole, chloroplasts and mitochondria are understood in most detail and are discussed below (Fig. 1). The mechanisms that regulate the intracellular homeostasis in other organelles are not well characterized although ZIP, NRAMP and YSL protein families are likely to be involved.[22]

2.3.1 Vacuole. The central vacuole of plant cells has a low metabolic activity compared to other organelles, and is therefore suitable as a storage compartment for the accumulation of metabolites and nutrients. Moreover, the vacuole acts as a buffering pool for non-essential and essential elements, especially Zn and Mn.[61] Only a few proteins are known to be required for the import of Zn into the vacuole, including AtMTP1 (also known as ZAT1) and AtMTP3. AtMTP1 is expressed in the root tips, in leaves and in the vascular tissues of young seedlings, but it is not modulated by external metal concentrations. Therefore, AtMTP1 increases the vacuolar Zn concentration in shoot tissues. Conversely, AtMTP3 is upregulated by exposure to excess Zn, specifically in epidermal and cortex cells of the root hair zone. It therefore reduces the amount of Zn translocated to the leaves when Zn levels become toxic.[62,63] Other transporters are involved in the translocation of Zn across the tonoplast. The *A. thaliana* zinc-induced facilitator 1 (ZIF1) belongs to the major facilitator superfamily (MFS) and may also import free NA into the vacuole. It therefore contributes indirectly to the accumulation of vacuolar Zn, and the over-expression of AtZIF1 causes the accumulation of vacuolar NA.[64] The *A. thaliana* vacuolar pump HMA3 mediates responses to Zn and heavy metal stress, indicating that the vacuolar sequestration of metals is necessary to prevent damage to the cell.[30] The *A. thaliana* vacuolar iron transporter 1 (AtVIT1) mediates the translocation of Fe into the vacuole[65] and the homologous yeast protein CCC1 is a Mn transporter, but this is yet to be demonstrated in plants.[66] Other transporters with a higher affinity for Mn are known to sequester Mn within the vacuole, such as AtCAX2 from the Ca^{2+} cation antiporter family.[67] Metal sequestration inside the vacuole is therefore achieved by several vacuolar transporters, some (*e.g.* MTP1) contributing to basal metal tolerance whereas others (including MTP3, ZIF1 and CAX2) are upregulated under metal stress conditions and act as metal scavengers.[68]

The corresponding proteins that mediate the export of metals from organelles into the cytosol are almost completely unknown. Under severe Cu deficiency conditions, the *A. thaliana* COPT5 vacuolar efflux protein is induced in root vascular tissues, allowing Cu remobilization from the vacuole to the cytosol.[69] AtCOPT6 acts in a similar manner, but it is localized in green tissues and reproductive organs.[70] The remobilization of Mo may be mediated by the vacuolar transporter AtMOT2, which is homologous to AtMOT1.[71] The direct involvement of this transporter has not been confirmed, but the gene is induced in leaves undergoing senescence suggesting an active role in Mo remobilization.[71]

2.3.2 Chloroplasts. Plastid electron transport is a core component of photosynthesis, and this requires large amounts of Fe and Cu. The uptake of Fe into the chloroplast is thought to be mediated by PIC1 (permease in chloroplasts 1), which translocates this ion across the envelope,[72] whereas Cu delivery into the chloroplast is mediated by AtPAA1/HMA6 localized in the envelope and AtPAA2/HMA8 in the thylakoid membrane.[73] AtHMA1 is localized in the envelope and is thought to act as a Cu influx transporter, but it may also detoxify the chloroplast by transferring Zn from the plastid stroma into the cytoplasm.[74]

2.3.3 Mitochondria. The transition metals such as Fe, Zn, Cu are Mn are abundant in mitochondria, whereas Co and Mo are present in trace amounts.[75] Transition metals are required as components of the respiratory electron transport chain and as cofactors in hundreds of enzymes.[76] Fe and Cu deficiencies therefore strongly affect the respiratory electron transport chain and the activity of cytochrome *c* oxidase.[77] Although mitochondria require transition metals in large amounts, the mechanisms that regulate the transport of metals remain mostly unknown.

Mitochondrial iron transporters (MITs) known as mitoferrins have recently been shown to import Fe into the mitochondria in rice, *e.g.* the *mit* knockout mutant is lethal and the lines show a reduction in growth despite abundant Fe accumulation.[78] In *A. thaliana*, FRO8 was identified in the mitochondrial proteome and was tentatively assigned a role in mitochondrial Fe import.[79]

There is evidence that the ATP binding cassette (ABC) protein ATM3 (ABC transporter of the mitochondrion) is a mitochondrial metal efflux transporter, but its specific role is still unknown.[80] In the *A. thaliana atm3* mutant, the maturation of cytosolic Fe–S cluster proteins and the biosynthesis of the molybdenum cofactor (Moco) are impaired. This inhibits the activities of several enzymes dependent on the Fe–S cluster (*e.g.* nitrate reductase and sulfite oxidase) and completely abolishes the activities of others (*e.g.* xanthine dehydrogenase and aldehyde oxidase). In *A. thaliana*, the homologous transporters ATM1 and ATM2 are localized in the mitochondria but their function remains unclear.[81]

The import of Mo into the mitochondria should be mediated by MOT1.1, but as stated above the localization of the transporter remains ambiguous. MOT1.1 may be required to transport the molybdate oxyanion (MoO_4^{-2}) from the mitochondrial intermembrane space to either the cytoplasm or the matrix.[42] Recently, the mitochondrial carrier Pic2 has been identified as the first metal influxer in eukaryotes. This protein imports Cu into the mitochondrial matrix along with the copper ligand (CuL).[82]

3. Micronutrient imbalance: metal stress and plant responses

Micronutrients, including Zn, Ni, Cu, Mo and Mn, are essential for plant growth and development because they are involved in diverse cellular functions such as energy metabolism, the regulation of gene expression, hormone synthesis and perception. These micronutrients contribute as cofactors to the structure and/or catalytic activity of enzymes. Therefore, plants must acquire appropriate quantities of Zn, Ni, Cu, Mo and Mn, and suffer nutrient deficiency symptoms if the supply of any of the metals is insufficient. However, plants require only small amounts of these essential elements so overloading can also cause stress and ultimately toxicity (Table 1), reflecting the ability of excess metals to inhibit enzyme activity, to induce the formation of ROS and to disrupt the ion balance within the plant cell.

3.1 Zinc

Zinc is an essential element and an important component of more than 200 plant enzymes in which it plays both structural and functional roles.[11] It is naturally abundant in the Earth's crust as sulfide, sulfate, oxide, phosphate, silicate and carbonate minerals that can accumulate to produce Zn-rich 'calamine' soils.[14] Zinc availability in the soil depends on several parameters including the mineral and moisture content, pH, weathering rates, organic matter content, plant uptake rate and bacterial population. Under physiological soil conditions, the redox state Zn^{2+} is prevalent and relatively stable. At low pH, Zn is soluble and therefore toxicity may become challenging. At high pH, Zn is more readily adsorbed onto cation exchange sites and its availability is reduced.[14]

Zn-containing enzymes are required for electron transport, energy production, antioxidant activity, chlorophyll biosynthesis and the maintenance of membrane integrity. Such enzymes include oxidoreductases, isomerases, lyases, transferases, ligases and hydrolytic enzymes.[83] Zn may directly contribute to the catalytic mechanism, *e.g.* in carbonic anydrase and Cu/Zn superoxide dismutase (Cu/ZnSOD), or it may play a structural role, *e.g.* in alcohol dehydrogenase, many transcription factors, DNA and RNA polymerases, histone deacetylases, splicing factors and RNA editing enzymes in the mitochondria and chloroplasts.[84] In chloroplasts and mitochondria, Zn-containing enzymes include peptidases[85,86] and metallo-proteases[87] involved in the removal of signal peptides. Moreover, Zn-dependent hydrolytic enzymes are present in the cytoplasm, lysosomes and apoplastic space. These enzymes include nucleases and aminopeptidases, α-mannosidase,[88] the 26S proteasome[89] and matrix metallo proteinases.[90] Zn is also required for the activation and modulation of many enzymes, *e.g.* mitogen-activated protein kinases.[91] Zn plays an essential structural role in ribosomes and is therefore required for protein synthesis.[92] Zn-dependent enzymes involved in carbohydrate metabolism are also influenced by Zn, especially in leaves.

The fundamental role of this element suggests that stress can arise from either deficiency or excess of Zn. Under Zn deficiency conditions, there is a rapid reduction in carbonic anhydrase and fructose-1,6-bisphosphatase activity[93] causing plants to accumulate sugars and starch. The levels of gibberellins and auxins, such as indole-3-acetic acid (IAA), decline, resulting in stunted growth and formation of unusually small leaves.[94] Tomato plants grown under Zn deficiency conditions have shorter stems correlating with the lower concentration of IAA,[95] which in turn probably reflects the inhibition of IAA synthesis or enhanced oxidative degradation.[96] As a component of Cu/ZnSOD, CAT and APX, Zn is required for scavenging H_2O_2 and the superoxide anion $O_2^{\bullet -}$, and its deficiency causes oxidative stress.[97] The increased oxidative stress causes leaf chlorosis and necrosis, reduced shoot elongation and increased membrane permeability.[98,99]

Zn deficiency is widespread in plants growing in acidic and calcareous soils, and in the latter case is often associated with Fe deficiency. The most characteristic symptom of Zn deficiency in dicotyledonous plants is the reduced internodal growth that gives rise to short stems and a rosette-like *habitus*. The leaves are smaller and distorted with puckered margins. In older leaves, Zn deficiency inhibits chlorophyll biosynthesis and thus results in interveinal chlorosis, especially between the margin and midrib, and may eventually cause the formation of necrotic spots.[100] Extreme Zn deficiency causes the shoot apices to "die back".[101]

In cereals, a lack of Zn inhibits shoot elongation and causes the formation of gray-brown necrotic spots on middle-aged leaves, whereas younger leaves turn yellow-green without necrotic patches.[102] In addition, Zn deficiency reduces seed yield, probably by affecting pollen fertility, and causes abnormal grain formation.[103] At the root level, Zn deficiency induces the release of low-molecular-weight exudates, such as amino acids, sugars, phenolics and potassium in dicotyledonous species, and phytosiderophores in graminaceous plants.[104]

Table 1 Summary of the effects due to both deficiency and the toxic level of the nutrient elements discussed in the text

Metal	Deficiency effects	Excess effects
Zn	• Sugar and starch accumulation.[93] • Stunted growth and formation of small leaves.[94] • Increased oxidative stress: leaf chlorosis and necrosis, reduced shoot elongation and increased membrane permeability.[98,99] • Reduction of the seed yield in cereals.[103] • Release of low-molecular-weight exudates from roots.[104]	• Visible leaf chlorosis.[105] • Anthocyanin synthesis and leaf reddening at high concentration.[106] • Necrotic brown spots, growth and yield inhibition.[14] • Photosynthesis inhibition.[107–109] • ROS generation and induction of antioxidant enzymes.[110] • Programmed cell death.[111]
Cu	• Photosynthesis inhibition.[112] • Reduction of carbohydrate synthesis and grain production.[113] • Inhibition of pollen formation and fertilization.[115] • Reduction of legume nodulation and N_2 fixation.[116] • Reduction of lignin biosynthesis: malformation, twisting and weakness of young leaves.[117] • Appearance of chlorosis and formation of necrotic spots.[119] • Stunted growth, delayed maturation and the enhanced formation of tillers in cereals and auxiliary shoots in dicotyledonous plants.[119,120] • Reduction of grain yield and quality.[120] • Increased susceptibility to fungal diseases.[120]	• ROS generation.[122,123] • Stunted growth and inhibition of lateral root initiation and development.[124] • Photosynthesis inhibition.[125,126]
Mo	• Reduction in the efficiency of nitrogen fixing.[13] • Stunted growth, interveinal chlorosis in young leaves and necrosis in older leaves.[133] • Prevention of flower formation and premature abscission.[133]	• Leaf malformation.[134] • Production of molybdocatechol complexes and golden yellow coloration of shoot.[134] • Production of molybdenum–anthocyanin complexes and dark blue coloration of stem.[135]
Mn	• Reduction of thylakoid glycolipids and polyunsaturated fatty acids.[140] • Inhibition of photosynthesis, plant growth and development.[137,138] • Interveinal chlorosis in young leaves, reduced pollen fertility, carbohydrate production and grain yields.[141,142] • Reduction of root length.[143] • Reduced production of phenolic compounds and lignin.[144] • Increased susceptibility to root-infecting pathogens.[145] • Development of dark-brown lesions,[146] discoloration, splitting and deformation of seeds.[147]	• Loss of apical dominance and formation of auxiliary shoots, interveinal chlorosis, deformation of younger leaves (crinkled leaves), and appearance of brown necrotic speckles.[139] • Reduction of shoots and roots length.[148] • Reduction of chlorophyll and carotenoid levels.[148] • Inhibition of photosynthesis rate.[149] • Production of ROS.[150] • Deficiencies of other nutrient, such as Ca, Mg, Fe[151] and Zn.[152]
Ni	• Loss of urease activity in leaves: accumulation of toxic concentrations of urea and disrupting nitrogen metabolism.[154] • Inhibition of root and shoot growth, and unfolding of the terminal leaves.[157] • Premature senescence: appearance of interveinal chlorosis and necrotic spots in younger leaves.[157]	• Inhibition of root growth.[159,160] • Disruption of the water balance: reduction of the transpiration rate[165] and stomatal closure.[166] • Reduction of chlorophyll synthesis, disruption of the thylakoid membranes and inhibition of photosynthesis.[154,167] • Formation of ROS.[168–170] • Deficiency of other nutrients, such as Ca, Mg, Mn, Fe, Cu and Zn.[165] • Reduced accumulation of N[171] and P.[172]

Higher Zn levels are found in soils contaminated by anthropogenic activities such as mining, burning fossil fuels, smelting and the use of phosphate-based fertilizers.[14] The initial symptoms of excess of Zn include visible leaf chlorosis induced by Mg or Fe deficiency, resulting from Zn displacement.[105] At the highest concentrations, Zn also induces anthocyanin synthesis and thus leaf reddening.[106] Necrotic brown spots become visible on the leaves of some species and both growth and yield are inhibited.[14] Zn toxicity also inhibits photosynthesis at different steps through distinct mechanisms. In *Phaseolus vulgaris*, high Zn levels displace Mg from the RuBP carboxylase and the OEC water splitting site of PSII.[107] In *Spinacea oleracea*, excess Zn inhibits plastidial ATP synthesis.[108] In *Beta vulgaris*, excess Zn impairs photosynthesis and thus depletes CO_2 at the RuBisCO carboxylation site as a consequence of reduced stomatal and mesophyll conductance.[109] Although Zn is a non-redox metal, it can generate ROS indirectly and induce antioxidant enzymes such as SOD, CAT and GPX.[110] Moreover, there is a correlation between Zn toxicity and programmed cell death *e.g.* in rice root cells.[111]

3.2 Copper

Copper is an essential nutrient that plays key roles in photosynthesis, respiration, carbon and nitrogen metabolism and protection against oxidative stress. In plants, there are more than 100 different Cu-containing proteins,[112] and about 50% of Cu in plants is localized in the chloroplasts.[11] Like Zn, Cu either acts as an enzyme cofactor or has a structural role, forming stable complexes in proteins. Cu also participates in hormone signaling, cell wall metabolism and stress responses based on its presence in Cu/ZnSOD, chaperones, cytochrome *c* oxidase, ascorbate oxidase, quinol oxidase and laccase (all of these enzymes are inactive in the absence of Cu). In mitochondria and chloroplasts, Cu is involved in redox reactions at

the electron transport chain level, *e.g.* in chloroplasts it is associated with plastocyanin.

Cu has also a key role in PSI activity and its deficiency reduces the rates of photosynthesis and carbohydrate synthesis. Cu-deficient wheat accumulates less carbohydrate than normal and produces few grains.[113] Moreover, severe Cu deficiency also affects PSII, inhibiting CO_2 fixation by $\sim$50%[112] and promoting changes in lipid composition by reducing the synthesis of unsaturated fatty acids.[114] The low level of carbohydrate synthesis inhibits pollen formation and fertilization,[115] and reduces legume nodulation and N_2 fixation.[116]

Two Cu-containing enzymes (polyphenol oxidase and diamine oxidase) are also involved in lignin biosynthesis, so Cu deficiency strongly influences the formation and chemical composition of the cell wall, increasing the abundance of α-cellulose at the expense of lignin.[117] In young leaves this causes malformation, twisting and weakness due to insufficient lignification of xylem vessels, and reduced water transport.[118]

Cu is also required for chlorophyll production, so one of the first Cu deficiency symptoms is chlorosis followed by the appearance of necrotic spots starting at the tip of young leaves and extending down to the leaf margins. These symptoms reflect the impairment of photosynthetic electron transfer, the loss of essential pigments and thylakoid degeneration.[119] The necrosis of apical meristems results in a stunted growth, delayed maturation and the enhanced formation of tillers in cereals and of auxiliary shoots in dicotyledonous plants. Cu deficiency also reduces grain yield and quality, and plants are more susceptible to fungal diseases such as ergot.[120] Finally, under extreme Cu deficiency conditions, leaves abscise before completing their development. The impact of Cu deficiency is species-dependent, *e.g.* oat, wheat and spinach are more sensitive than rye, pea and apple, and the severity of the symptoms also depends on the plant organs, developmental stage and nitrogen supply.[121]

Toxic Cu levels are naturally present in some soils or may be derived from anthropogenic activities, such as the use of Cu-containing fungicides, urban waste management and industrial activity. Cu toxicity primarily reflects the generation of ROS, *e.g.* Cu can generate $OH^{\bullet}$ by redox cycling between the two oxidation states, Cu^{2+} and Cu^{+}.[122] Cu is also involved in the production of ROS directly *via* the Fenton or Haber–Weiss reactions, catalyzing the formation of $OH^{\bullet}$ and $O_2^{\bullet -}$.[123] Plants therefore induce antioxidant responses, such as the activation of APX, MDHAR, DHAR, GR and SODs, in response to excess Cu.[112] Cu toxicity symptoms in plants include stunted growth and the inhibition of lateral root initiation and development. For example, excess Cu disrupts nitrogen metabolism and fixation in soybean (*Glycine max*) and depletes nitrate and free amino acid levels in grapevine (*Vitis vinifera*).[124] Cu toxicity also inhibits photosynthesis by reducing the abundance of chlorophyll, thus increasing susceptibility to photo-inhibition.[125] The most evident effect of Cu toxicity is the inhibition of oxygen evolution, resulting from an interaction between Cu ions and the Tyr_Z and Tyr_D residues on the D2 protein of PSII.[126] Excess Cu also affects the Mn cluster and the extrinsic proteins of the OEC (PsbO, PsbP and PsbQ),[112] and it interacts with non-hemic Fe^{2+} and compromises the redox state of cytochrome b_{559}.[127] Furthermore, photosynthesis may be inhibited indirectly by the effect of excess Cu on enzymes such as RuBisCO and PEPC.[128]

3.3 Molybdenum

Molybdenum is a transition metal present in small amounts in the lithosphere and in soils.[129] Soil pH is one of the most important factors affecting Mo availability. In aqueous solutions with a pH > 4.3, Mo is prevalent as the molybdate oxyanion MoO_4^{2-} (the most highly oxidized form, Mo(VI)), whereas at pH < 4.3 it is primarily found as the protonated species $HMoO_4^{-}$ and $Mo_3(H_2O)$.[130] Although plants require less Mo than any other nutrients, it is an essential component in the active site of several enzymes, such as nitrate reductase, which reduces nitrate to nitrite; xanthine dehydrogenase required for purine degradation; sulfite oxidase, which catalyzes the oxidation of sulfite (SO_3^{2-}) to sulfate (SO_4^{2}); and aldehyde oxidase, which is required for the synthesis of abscisic acid (ABA).[131] Xanthine dehydrogenase, aldehyde oxidase and sulfite oxidase play important roles in stress response and tolerance, *e.g.* plant–pathogen interactions, cold tolerance and protection against damage caused by sulfur dioxide.

Mo is also necessary for nitrogen assimilation in legumes and its deficiency reduces the efficiency of nitrogen fixing. Relatively large amounts of Mo are therefore required in the root nodules of plants that depend on symbiotic nitrogen fixation or if nitrate is the main nitrogen source.[13] If nodulated legumes are starved of Mo, any available Mo accumulates preferentially in the root nodules thus reducing the levels in the shoots and seeds.[132] The principal symptoms of Mo deficiency are therefore similar to those of nitrogen deficiency, *i.e.* stunted growth, interveinal chlorosis in young leaves and necrosis in older leaves. Dicotyledonous species such as cauliflower develop small leaves with atypical blades (whiptail disease) in the absence of Mo, caused by the abnormal early differentiation of vascular tissues.[133] Other symptoms of Mo deficiency include the prevention of flower formation and their premature abscission.[133]

Plants are generally tolerant of excess Mo, but at extreme levels Mo toxicity causes leaf malformation and the production of molybdocatechol complexes in the vacuole that induce the golden yellow coloration of the shoot.[134] In oilseed rape and tomato plants, high Mo concentrations induce dark blue coloration in the stem reflecting the formation of molybdenum–anthocyanin complexes.[135]

3.4 Manganese

Manganese plays an important role in redox reactions as a component of the MnSOD enzyme that protects plants from the damaging effects of ROS.[136] Four Mn atoms are also required as part of the OEC, making Mn essential for the oxygen evolution on the lumenal side of PSII. Therefore, Mn starvation reduces oxygen evolution in wheat[137] and maize,[138] but upon restoration of Mn supply the oxygen evolution rate returns to normal. Mn is a cofactor in more than 30 different enzymes, including catalase, pyruvate carboxylase, malic enzyme, phosphoenol

pyruvate carboxykinase and isocitrate lyase, thus playing a role in oxidation–reduction, decarboxylation and hydrolytic reactions.[11] Mn is also required for the synthesis of proteins, lipids, carotenoids and chlorophyll.

The bioavailability and oxidation state of Mn depend strongly on the soil pH. In acidic soils (pH < 5.5) the more-soluble Mn(II) form is more abundant, increasing the risk of Mn toxicity. However, in neutral to basic soils (pH > 6.5) the less-soluble manganic forms Mn(III), Mn(IV) and Mn(VII) become more abundant, and plants may suffer Mn deficiency. The application of ammonia-based fertilizers causes soil acidification, thus increasing the bioavailability and potential toxicity of Mn.[139]

Mn deficiency causes a reduction in the abundance of thylakoid glycolipids and polyunsaturated fatty acids,[140] and also inhibits photosynthesis, and hence plant growth and development.[138] Chloroplasts are therefore more sensitive to Mn deficiency than other organelles and the principal symptom of Mn deficiency is interveinal chlorosis in young leaves. Other symptoms of Mn-deficient plants include reduced pollen fertility and carbohydrate production, resulting in lower grain yields[141,142] and shorter roots due to the lack of carbohydrates required for cell elongation.[143] Mn deficiency also reduces the production of phenolic compounds and lignin, especially in the roots, because Mn is required as a cofactor in the enzymes phenylalanine ammonia-lyase, which mediates the production of phenolic compounds, and peroxidase involved in the polymerization of cinnamyl alcohols.[144] Because lignin provides an important barrier against fungal infection, Mn-deficient plants are more sensible to root-infecting pathogens.[145] In legumes, the symptoms of Mn deficiency include the development of dark-brown lesions ("marsh spot") in pea[146] and discoloration, splitting and deformation of seeds ("split seed") in lupins.[147]

Mn toxicity causes the loss of apical dominance and promotes the formation of auxiliary shoots (witches' broom), interveinal chlorosis, the deformation of younger leaves (crinkled leaves), and brown necrotic speckles that can be fatal in severe cases.[139] For example, pea plants exposed to toxic Mn concentrations have shorter shoots and roots, lower chlorophyll and carotenoid levels and the activities of glutamine synthetase and glutamate synthase are inhibited.[148] In *Vigna radiata* plants exposed to high levels of Mn, the Hill activity of isolated chloroplasts is inhibited, reducing the rates of photosynthesis and CO_2 uptake and thus a decreased accumulation of carotenoids and chlorophyll.[149] Excess Mn also induces the production of ROS such as H_2O_2 and $O_2^{\bullet -}$,[150] causing oxidative damage to proteins, lipid peroxidation and a compensatory increase in the activities of SOD, PRX, APX, DHAR and GR. Extreme Mn levels can also induce deficiencies of other nutrients, *e.g.* Ca, Mg, Fe[151] and Zn.[152]

3.5 Nickel

Nickel is abundant in the soil as a free ion and in complexes with other metal ions (such as Fe). As is the case for other micronutrients, many human activities contribute to Ni levels in the environment.[153] In soils, Ni exists in different oxidation states but Ni^{2+} is the prevalent and more stable form over a wide range of conditions, such as pH and redox potentials.[154] Ni is a cofactor for urease, which catalyzes the conversion of urea into ammonium, and it is therefore essential for efficient nitrogen metabolism. Ni is not required for the synthesis of the urease protein[155] but is essential for its structure and catalytic activity.[156]

Ni deficiency causes a loss of urease activity in leaves, inducing the accumulation of toxic concentrations of urea and disrupting nitrogen metabolism thus leading to chlorosis and necrosis.[154] In Ni deficient plants, root and shoot growth is inhibited significantly and the terminal leaves fail to unfold.[157] Ni is also essential for normal seed development and grain yields, *e.g.* the viability, germination rate and development of barley seeds are all affected by the presence of Ni.[157] In graminaceous species, Ni deficiency induces premature senescence and causes interveinal chlorosis and necrotic spots in younger leaves similar to those induced by Fe deficiency, partly due to the concomitant reduction in Fe levels.[157] Ni is essential for nitrogen metabolism in legumes and other plants that metabolize ureides.[158] Therefore, the symptoms of Ni deficiency are more severe in plant species with symbiotic relationships involving nitrogen-fixing bacteria. For example, Ni deprivation in soybean plants induces necrotic lesions containing toxic levels of urea, delays nodulation and inhibits early growth.[158]

The typical symptoms of Ni toxicity involve the inhibition of root growth, as observed in *Brassica juncea* plants and wheat seedlings.[159,160] However, in both these species and in others such as maize and pigeon pea, the effects of Ni toxicity are already visible during germination.[161–164] High Ni levels also disrupt the water balance thus reducing the transpiration rate, reflecting the inhibition of leaf growth[165] and the higher levels of endogenous ABA that promote stomatal closure.[166] Ni toxicity also reduces chlorophyll synthesis and disrupts the thylakoid membranes, thus inhibiting photosynthesis. For example, Ni can interact with the extrinsic 16 and 24 kDa polypeptides associated with the OEC of PSII, causing a conformational change that induces their release and the subsequent inhibition of oxygen evolution and electron transport activity.[167] Ni can also compete with Mg and displace it from chlorophyll and enzymes such as RuBisCO.[154] Unlike Fe and Cu, Ni is not a redox-active metal and it does not direct participate in reactions that produce ROS. Nevertheless, exposure to excess Ni levels induces the formation of $O_2^{\bullet -}$, $OH^{\bullet}$ and H_2O_2 in many plants indirectly.[168] For example, levels of H_2O_2 and $O_2^{\bullet -}$ increased in wheat seedlings exposed to excess Ni, resulting in higher rates of lipid peroxidation.[169] Similarly, Ni treatment induced the formation of H_2O_2 in *Alyssum bertoloni* and *Nicotiana tabacum*, whereas in *A. bertolonii* roots the higher endogenous activities of CAT and SOD helped to reduce the resulting oxidative stress.[168] In wheat, the concomitant activation of APX and GPX counteracted the ROS generated by excess Ni and lipid peroxidation did not increase significantly.[170]

Excess Ni can induce deficiency of other nutrients, such as Ca, Mg, Mn, Fe, Cu and Zn, by influencing their uptake from soil and subsequent utilization.[165] High Ni concentrations reduce the accumulation of nitrogen in the leaves and roots of *Cicer arietinum* and *Vigna radiata* plants[171] and the accumulation of phosphorus in *Helianthus annuus* and *Hyptis suaveolens* plants.[172]

4. Non-nutrient elements: beneficial effects on plant fitness

Some elements can stimulate plant growth, especially by playing a role in abiotic–biotic stress resistance and symbiosis, even though they are not essential nutrients, or are essential only for particular plant species. These elements, which include Na, Si, Se, Al and Co, are collectively known as *beneficial elements*, and the functional concentration varies for each element and plant species.[15] Two different mechanisms of action have been proposed to explain the growth-promoting effects of such elements: (i) a structural or an osmotic role, when high concentrations are required for the beneficial effect, and (ii) a role as an enzyme cofactor, when only low concentrations are required for the beneficial effect.[15] Interestingly, the major effects of some beneficial elements on higher plants are particularly noticeable under stress. As a corollary, these elements behave as essential nutrients for a small number of plant species characterized by optimal growth conditions that are prohibitive (*i.e.* stressful) for most taxa. For example, Na is essential for the halophyte *Atriplex vesicaria*, which suffers chlorosis and necrotic lesions when grown at low Na concentrations, and Si is required by silicophilic species such as *Equisetum arvense*, which suffer necrosis and wilting in the absence of Si.[173] Finally, it is worth highlighting the mechanism of hyperaccumulation, *i.e.* the capacity to accumulate high concentrations of metal ions, primarily in shoots, while maintaining a low concentration in roots.[174] Among the hypotheses proposed to explain the ecological role of metal hyperaccumulation, the *elemental defense* hypothesis suggests that increased concentrations of metals and metalloids, especially Zn, Se and Ni, may protect plants through their toxic effects on pathogens and herbivores, ranging from adult and larval insects to small mammals.[175]

4.1 Sodium

Na represents ~3% of the Earth's crust by weight, and it is most abundant in semiarid regions where it is mostly present as NaCl. Na is chemically similar to potassium (K), and can therefore non-selectively enter the cells through K channels, even though several Na-specific transporters have also been discovered.[176] Vascular plants are usually described as natrophilic and natrophobic according to their Na tolerance, the first tolerating (or even requiring) Na and the second showing sensitivity to even low Na concentrations.[177] Some plants (including some halophytic species, such as members of the C4 genus *Atriplex*) require a certain amount of Na to grow normally, and Na deficiency results in stunted growth and chlorosis. Na deficiency in these species inhibits the conversion of pyruvate to phosphoenolpyruvate in mesophyll cells, and reduces the Na^+/H^+ symport of pyruvate across membranes.[178] In *Amaranthus tricolor*, Na enhances nitrate uptake and assimilation in both roots and shoots.[179] Na has a particularly important positive impact in K-depleted soils, again reflecting the chemical similarities between Na and K. In some C3 plants, particularly the Chenopodiaceae family, Na can substitute for K in the vacuole, contributing to the maintenance of osmotic equilibrium in the vacuole and cytoplasm. *Beta vulgaris* growth is enhanced by Na under conditions of K deficiency.[180] A similar mechanism is exploited by parasitic plants (*e.g. Cuscuta attenuata*), which utilize high internal Na concentrations as an osmoticum to drive the extraction of water and possibly other nutrients as the parasite grows within its host.[181] Other mechanisms have been proposed to explain the stimulation of growth by Na, including (i) the higher solute potential in the vacuole, which promotes cellular turgor and cell expansion, and (ii) the better water balance in plants exposed to water deficit, brought about by the faster stomatal closure in plants supplied with Na compared to those supplied with K. This is driven by a mechanism that matches the Na concentration in the apoplast surrounding the guard cells with the transpiration rate, thus controlling the amount of salt delivered to the shoot.[182]

4.2 Silicon

Silicon is abundant in the Earth's crust, comprising more than 50% of the soil mass.[183] Monosilicic acid ($Si(OH)_4$) is the most prevalent soluble form of Si in case of pH < 9.0 and is the typical form found in acidic and neutral soils with low levels of anion adsorption.[184] Silicon is assimilated by passive uptake into the roots, and also through specific transporters discovered in rice, barley and maize, the best characterized being rice Lsi1 and Lsi2.[185] Most of the absorbed Si is then translocated *via* the xylem to the shoot, where silicic acid polymerizes into a silica gel ($SiO_2(H_2O)_n$) which is deposited in the space beneath the cuticle layer of the leaf blade. The epidermis and stem vascular tissue also undergoes silicification, which confers rigidity and prevents compression when the transpiration pressure is high. Different plant species show great variations in their capacity of accumulating Si, and some Graminaceae and Cyperaceae species are known for accumulating particularly high levels of this mineral.[186]

Si accumulation can stimulate plant growth and reduce biotic stress symptoms, *e.g.* conferring resistance to bacteria, fungi and also small arthropods, such as hoppers, leaf spiders and mites. Two mechanisms are thought to be involved: (i) the silica gel deposited in the apoplast beneath the cuticle acts as a physical barrier, mechanically impeding penetration and infection by fungi and pests; and (ii) symplastic Si may act as a modulator of the host resistance, inducing the production of antimicrobial compounds and stress signals, such as phenolic compounds and phytoalexins, and enhancing the activity of defense enzymes such as chitinases, peroxidases and polyphenoloxidases.[15,184]

Si also protects wheat, barley, tomato, rice, maize and cucumber plants from a variety of abiotic stresses, such as UV irradiation, drought, freezing and chemical stress, including osmotic stress, nutrient imbalance and heavy metal toxicity. The deposited layers of silica gel reflect UV radiation and reduce transpiration through the leaf cuticle, thus limiting the impact of drought stress and the resulting osmotic stress caused by water loss.[187] It can also trap toxic metal ions to reduce their concentration in solution, *e.g.* Mn in cowpea and Na in rice.[184,187] In some plant species, such as barley and tomato, Si also promotes the activity of endogenous cellular

ROS-scavenging enzymes, including SOD, PRX and GR, which may reduce lipid peroxidation and the toxicity caused by high levels of salts and heavy metals.[187]

4.3 Selenium

The soil chemistry of Se resembles that of sulfur, with Se present in a variety of oxidation states. The most common soluble (and toxic) forms in aerobic soils are selenite [SeO_3^{2-}, Se(IV)] and selenate [SeO_4^{2-}, Se(VI)], whereas Se^0 and selenide (Se^{2-}) are more prevalent under anaerobic and reducing conditions. Selenium is an essential trace nutrient in bacteria, animals and algae, because it is a component of tRNA, and seleno-enzymes such as glutathione peroxidase, which contain the modified amino acid selenocysteine. Seleno-proteins similar to those found in microbes, animals and algae have not yet been identified in plants and therefore the status of Se as a micronutrient in higher plants remains controversial.[188] The chemical similarity of Se and sulfur means that the two elements share the same root uptake systems and metabolic pathways, so their accumulation is strongly linked.[189] Plants that tolerate and accumulate high levels of Se (hyperaccumulators) are characterized by enhanced selenocysteine methyltransferase activity, which detoxifies the selenocysteine and selenomethionine formed when Se replaces sulfur during the synthesis of amino acids. Se hyperaccumulators (*e.g.* members of the genera *Astragalus*, *Xylorhiza*, *Stanleya* and the family Brassicaceae) also show higher selenate/sulfate discrimination indices, suggesting that the transporters expressed in these species are more selective for selenate.[190]

Despite the enhanced growth of Se hyperaccumulators in seleniferous soils, low doses of Se generally improve plant growth and reproduction. The addition of Se reduced the accumulation of heavy metals and enhanced resistance to UV irradiation in several species, mediated by the Se-enhanced activity of glutathione peroxidase and reduced lipid peroxidation.[191] There is also evidence that Se induces the synthesis of jasmonic acid and ethylene, as well as defense-related proteins, thus protecting plants from biotic stress.[189] In Se hyperaccumulators, the high concentrations of Se appear to protect the plants both from small arthropods and fungal pathogens, *e.g. B. juncea* is protected from its pathogen *Alternaria brassiciola*, and also deter mammalian herbivory *e.g.* the consumption of *Stanleya pinnata* by the black-tailed prairie dog.[192,193] This beneficial element may also protect plants against abiotic stress, such as drought, salt, water deficit, high temperatures and heavy metals.[194] For example, the addition of selenite to *Lactuca sativa* plants significantly reduced the accumulation of toxic heavy metals such as Pb and Cd. Similarly, prior exposure to Se protected *Helianthus annuus* plants from the negative effect of Cd, probably by enhancing the activities of ROS-scavenging enzymes such as CAT, APX and GPX.[194]

4.4 Aluminum

Aluminum is one of the most abundant elements in the Earth's crust, forming alumino silicate clays and aluminum hydroxide minerals, and its dynamic bioavailability in the soil is high, particularly at pH < 5.5. Al toxicity is common in acidic soils because Al is released in soluble forms, the most phytotoxic being Al^{3+}, such as $Al(OH)^{2+}$ and $Al(OH)_2^{+}$.[195] Aluminum is toxic to most plants and its phytotoxicity depends on the speciation, concentration and ionic strength of the solution.[196] Aluminum toxicity reflects several mechanisms that inhibit root elongation and nutrient and water uptake, such as the inhibition of cell division and elongation, the formation of micronuclei and chromosome breaks,[197,198] and interference with cytoskeletal organization and stability.[199] Oxidative stress, resulting from the production of ROS and lipid peroxidation, is also induced by excess Al.[200]

Despite the above, low levels of Al are beneficial for plants, particularly those adapted to acidic soils, where the levels of acidity and the bioavailability of nutrient metal ions (*e.g.* Fe, Zn and Cu) and phosphorous are strictly interconnected.[1] For example, the application of Al to *Camellia sinensis* and *Melastoma malabathricum* enhances their growth, nutrient accumulation (especially phosphorus) and fitness, reflecting several distinct phenomena: (i) Al-induced root activity, reflecting the stimulation of root cell elongation and the development of numerous secondary roots, at least in *M. malabathricum*;[201] (ii) a counteraction effect against Fe toxicity, reducing Fe assimilation and transport, otherwise enhanced to phytotoxic levels by the low soil pH;[202,203] and (iii) the activation of SOD, CAT and APX, which scavenge the ROS produced by normal plant metabolism, eventually enhancing plant fitness.[204] Aluminum is also thought to enhance resistance against biotic stress in *Solanum tuberosum*, where Al treatment induces biochemical changes that improve the response to challenges with *Phytophthora infestans*.[205]

4.5 Cobalt

Co is found in many different chemical forms in minerals such cobaltite, smaltite and erythrite,[206] and is widespread in trace amounts (15–25 ppm) in most soils.[15] Cobalt is an essential nutrient in bacteria and animals, but not in plants.[207] As for other heavy metals, excess Co causes toxicity symptoms in plants, ranging from diffuse leaf chlorosis and necrosis in tomato,[208] to reduced biomass accumulation and nutrient uptake in cauliflower.[209] However, small amounts of Co have beneficial effects on the growth of leguminous plants, such as *Pisum sativum* and *Lupinus angustifolius*, resulting from enhanced nodule activity.[210,211] This is because Co is required as a cofactor for the coenzyme cobalamin in *Rhizobium* spp. during nodulation, nitrogenfixation and leghemoglobin synthesis.[212]

5. Conclusion

The bioavailability of metals in the soil is highly dynamic, reflecting a variety of physical, chemical and biological factors. Even if the metabolism of a particular metal is usually treated as a singular process, the different ions often share transport proteins, therefore competing for transport across membranes. In terms of plant nutrition, the abundance of one nutrient

greatly affects the absorption, distribution and even the function of other nutrients, which means that interactions between nutrients can induce specific deficiency or toxicity symptoms in plants even if the nutrient availability is plenty in the environment. Similarly, nutrient interactions may counteract stress caused by the imbalance (excess or deficiency) of potentially toxic metals. Examples of this intricate network include the beneficial effects of Si which are particularly evident during phosphorus deficiency due to the reduced uptake of Fe and Mn;[185] Si also alleviates toxicity of both Cd and Al.[213] Zn fertilizers may increase Cd uptake by displacing Cd ions from their binding sites in the soil and increasing their availability. Such considerations should be borne in mind in soil management programs, which also contribute to change the physical, chemical and biological characteristics of the soil, resulting in a variety of conditions that allow metals to cause either beneficial or harmful effects. For example, liming may reduce Cd uptake by increasing the pH and hence the competition between Ca and Cd ions, but this strategy can also increase Cd uptake by reducing the concentration of Zn.[214] Finally, the activity of microorganisms populating the rhizosphere, may influence metal bioavailability, thus also contributing to plant growth and fitness.

Abbreviations

ABA	Abscisic acid
ABC	ATP binding cassettes
APX	Ascorbate peroxidase
ATM3	ABC transporter of the mitochondrion
CAT	Catalase
CAX	Cation exchanger
CDF	Cation diffusion facilitator
COPT	Copper transporter
CuL	Copper ligand
DHA	Dehydroascorbate
DHAR	DHA reductase
FPN	Ferroportin
FRO	Ferric oxidase–reductase
GPX	Glutathione peroxidase
GR	Glutathione reductase
H^+-ATPase	ATP-dependent proton pump
H_2O_2	Hydrogen peroxide
HMA	Heavy metal-transporting ATPase
IRT	Iron-regulated transporter
MATE	Multi-drug and toxic compound extrusion
MDHA	Monodehydroascorbate
MDHAR	MDHA reductase
MFS	Major facilitator superfamily
MIT	Mitochondrial iron transporter
Moco	Molybdenum cofactor
MoO_4^{-2}	Molybdate oxyanion
MOT	Molybdenum transporter
MT	Metallothionein
MTPs	Metal tolerance proteins
NA	Nicotianamine
NRAMP	Natural resistance-associated macrophage protein
$O_2^{\bullet -}$	Superoxide anion
OEC	Oxygen evolving complex
$OH^{\bullet}$	Hydroxyl radical
PCR	Plant cadmium resistance
PEPC	Phosphoenolpyruvate carboxylase
PIC	Mitochondrial carrier family
PRX	Peroxidase
PS	Phytosiderophore
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate carboxylase oxygenase
SOD	Superoxide dismutase
VIT	Vacuolar iron transporter 1
YS	Yellowstripe
YSL	Yellow-stripe like
ZIF	Zinc-induced facilitator
ZIP	ZRT/IRT-like protein
ZRT	Zinc-regulated transporter

References

1 K. Mengel, E. A. Kirkby, H. Kosegarten and T. Appel, *Principles of plant nutrition*, Kluwer Academic Publishers, Doredrecht, 2001.

2 W. W. Wenzel, M. Bunkowski, M. Puschenreiter and O. Horak, Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil, *Environ. Pollut.*, 2003, **123**, 131–138.

3 M. J. Haydon and C. S. Cobbett, Transporters of ligands for essential metal ions in plants, *New Phytol.*, 2007, **174**, 499–506.

4 L. Williams and D. E. Salt, The plant ionome coming into focus, *Curr. Opin. Plant Biol.*, 2009, **12**, 247–249.

5 S. Clemens, Molecular mechanisms of plant metal tolerance and homeostasis, *Planta*, 2001, **212**, 475–486.

6 M. L. Guerinot, The ZIP family of metal transporters, *Biochim. Biophys. Acta*, 2000, **1465**, 190–198.

7 D. J. Eide, Zinc transporters and the cellular trafficking of zinc, *BBA, Biochim. Biophys. Acta, Mol. Cell Res. Mol. Cell Res.*, 2006, **1763**, 711–722.

8 L. E. Williams, J. K. Pittman and J. L. Hall, Emerging mechanisms for heavy metal transport in plants, *Biochim. Biophys. Acta, Biomembr.*, 2000, **1465**, 104–126.

9 C. Curie, J. M. Alonso, M. L. E. Jean, J. R. Ecker and J. F. Briat, Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport, *Biochem. J.*, 2000, **347**, 749–755.

10 S. Thomine, F. Lelievre, E. Debrabieux, J. I. Schroeder and H. Barlier-Brygoo, AtNRAMP3, a multi-specific vacuolar metal transporter involved in plant responses to iron deficiency, *Plant J.*, 2003, **34**, 685–695.

11 R. Hänsch and R. R. Mendel, Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B Cl), *Curr. Opin. Plant Biol.*, 2009, **12**, 259–266.

12 A. Schützendübel and A. Polle, Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization, *J. Exp. Bot.*, 2002, **53**, 1351–1365.

13 L. Taiz and E. Zeigler, *Plant Physiology*, Sinauer Associates, Sunderland, Massachusetts, 4th edn, 2006.

14 M. R. Broadley, P. J. White, J. P. Hammond, I. Zelko and A. Lux, Zinc in plants, *New Phytol.*, 2007, **173**, 677–702.

15 E. A. Pilon-Smits, C. F. Quinn, W. Tapken, M. Malagoli and M. Schiavon, Physiological functions of beneficial elements, *Curr. Opin. Plant Biol.*, 2009, **12**, 267–274.

16 T. Kobayashi and N. K. Nishizawa, Iron uptake, translocation, and regulation in higher plants, *Annu. Rev. Plant Biol.*, 2012, **63**, 131–152.

17 S. Thomine and G. Vert, Iron transport in plants: better be safe than sorry, *Curr. Opin. Plant Biol.*, 2013, **6**, 322–327.

18 N. Grotz and M. L. Guerinot, Molecular aspects of Cu, Fe and Zn homeostasis in plants, *Biochim. Biophys. Acta*, 2006, **1763**, 595–608.

19 A. Migeon, D. Blaudez, O. Wilkins, B. Montanini, M. M. Campbell, P. Richaud, S. Thomine and M. Chalot, Genome-wide analysis of plant metal transporters, with an emphasis on poplar, *Cell. Mol. Life Sci.*, 2010, **67**, 3763–3784.

20 S. Clemens, M. G. Palmgren and U. Krämer, A long way ahead: understanding and engineering plant metal accumulation, *Trends Plant Sci.*, 2002, **7**, 309–315.

21 C. Palmer and M. L. Guerinot, Facing the challenges of Cu, Fe and Zn homeostasis in plants, *Nat. Chem. Biol.*, 2009, **5**, 333–340.

22 E. P. Colangelo and M. L. Guerinot, Put the metal to the petal: metal uptake and transport throughout plants, *Curr. Opin. Plant Biol.*, 2006, **9**, 322–330.

23 M. J. Milner, J. Seamon, E. Craft and L. V. Kochian, Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis, *J. Exp. Bot.*, 2013, **64**, 369–381.

24 C. Curie, Z. Panaviene, C. Loulergue, S. L. Dellaporta, J. F. Briat and E. L. Walker, Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake, *Nature*, 2001, **409**, 346–349.

25 S. S. Conte, H. H. Chu, D. Chan-Rodriguez, T. Punshon, K. A. Vasques, D. E. Salt and E. L. Walker, *Arabidopsis thaliana* Yellow Stripe1-Like4 and Yellow Stripe1-Like6 localize to internal cellular membranes and are involved in metal ion homeostasis, *Front. Plant Sci.*, 2013, **4**, 1–15.

26 V. Sancenon, S. Puig, I. Mateau-Andrés, E. Dorcey, D. J. Thiele and L. Penarrubia, The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development, *J. Biol. Chem.*, 2004, **279**, 15348–15355.

27 U. Krämer, I. N. Talke and M. Hannikenne, Transition metal transport, *FEBS Lett.*, 2007, **581**, 2263–2272.

28 W. Y. Song, K. SamChoi, D. Y. Kim, M. Geisler, J. Park, V. Vincenzetti, M. Schellenberg, S. H. Kim, Y. P. Lim, E. W. Noh, Y. Lee and E. Martinoia, *Arabidopsis* PCR2 is a zinc exporter involved in both zinc extrusion and long-distance zinc transport, *Plant Cell*, 2010, **22**, 2237–2252.

29 J. Morrissey, I. R. Baxter, J. Lee, L. Li, B. Lahner, N. Grotz, J. Kaplan, D. E. Salt and M. L. Guerinot, The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*, *Plant Cell*, 2009, **21**, 3326–3338.

30 M. Morel, J. Crouzet, A. Gravot, P. Auroy, N. Leonhardt, A. Vavasseur and P. Richaud, AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*, *Plant Physiol.*, 2009, **149**, 894–904.

31 B. Montanini, D. Blaudez, S. Jeandroz, D. Sanders and M. Chalot, Phylogenetic and functional analysis of the Cation Diffusion Facilitator (CDF) family: improved signature and prediction of substrate specificity, *BMC Genomics*, 2007, **23**, 107.

32 L. Yang, I. Stulen and L. J. De Kok, Impact of sulfate nutrition on the utilization of atmospheric SO_2 as sulfur source for Chinese cabbage, *J. Plant Nutr. Soil Sci.*, 2006, **169**, 529–534.

33 D. M. I. Vallano and J. P. Sparks, Quantifying foliar uptake of gaseous nitrogen dioxide using enriched foliar $\delta^{15}N$ values, *New Phytol.*, 2008, **177**, 946–955.

34 W. E. Rauser, Structure and function of metal chelators produced by plants, *Cell Biochem. Biophys.*, 1999, **31**, 19–48.

35 S. Santi and W. Schmidt, Dissecting iron deficiency-induced proton extrusion in *Arabidopsis* roots, *New Phytol.*, 2009, **183**, 1072–1084.

36 N. J. Robinson, C. M. Procter, E. L. Connolly and M. L. Guerinot, A ferric-chelate reductase for iron uptake from soils, *Nature*, 1999, **397**, 694–697.

37 T. Chen, X. Cai, X. Wu, I. Karahara, L. Schreiber and J. Lin, Casparian strip development and its potential function in salt tolerance, *Plant Signaling Behav.*, 2011, **6**, 1499–1502.

38 G. Vert, N. Grotz, F. Dedaldechamp, F. Gaymard, M. L. Guerinot, J. F. Briat and C. Curie, IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth, *Plant Cell*, 2002, **14**, 1223–1233.

39 S. Nishida, C. Tsuzuki, A. Kato, A. Aisu, J. Yoshida and T. Mizuno, AtIRT1, the primary iron uptake transporter in the root, mediates excess nickel accumulation in *Arabidopsis thaliana*, *Plant Cell Physiol.*, 2011, **52**, 1433–1442.

40 R. Cailliatte, A. Schikora, J. F. Briat, S. Mari and C. Curie, High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions, *Plant Cell*, 2010, **22**, 904–917.

41 H. Tomatsu, J. Takano, H. Takahashi, A. Watanabe-Takahashi, N. Shibagaki and T. Fujiwara, An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 18807–18812.

42 I. Baxter, B. Muthukumar, H. C. Park, P. Buchner, B. Lahner, J. Danku, K. Zhao, J. Lee, M. J. Hawkesford, M. L. Guerinot and D. E. Salt, Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1), *PLoS Genet.*, 2008, **4**, e1000004.

43 S. Takagi, K. Nomoto and S. Takemoto, Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants, *J. Plant Nutr.*, 1984, **7**, 469–477.

44 A. Pich, R. Manteuffel, S. Hillmer, G. Scholz and W. Schmidt, Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration?, *Planta*, 2001, **213**, 967–976.

45 D. A. Cataldo, K. M. McFadden, T. R. Garland and R. E. Wildung, Organic constituents and complexation of nickel (ii), iron (iii), cadmium (ii) and plutonium (iv) in soybean xylem exudates, *Plant Physiol.*, 1988, **86**, 734–739.

46 U. Krämer, R. D. Smith, W. W. Wenzel and I. Raskin, Salt DE. The role of metal transport and tolerance in Nickel hyperaccumulation by *Thlaspi goesingense* Halacsy, *Plant Physiol.*, 1997, **115**, 1641–1650.

47 U. Krämer, D. C. H. Janet, J. M. Charnock, A. J. M. Baker and J. C. Smith, Free histidine as a metal chelator in plants that accumulate nickel, *Nature*, 1996, **379**, 635–638.

48 V. Vacchina, S. Mari, P. Czernic, L. Marques, K. Pianelli, D. Schaumloffel, M. Lebrun and R. Lobinski, Speciation nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation, *Anal. Chem.*, 2003, **75**, 2740–2745.

49 L. Kerkeb and U. Krämer, The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*, *Plant Physiol.*, 2003, **131**, 716–724.

50 N. Von Wirén, S. Klair, S. Bansal, J. F. Briat, H. Khodr, T. Shioiri, R. A. Leigh and R. C. Hider, Nicotianamine chelates both Fe(III) and Fe(II). Implications for metal transport in plants, *Plant Physiol.*, 1999, **119**, 1107–1114.

51 N. Andres-Colas, V. Sancenon, S. Rodriguez-Navarro, S. Mayo, D. J. Thiele, J. R. Ecker, S. Puig and L. Penarrubia, The Arabidopsis heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots, *Plant J.*, 2006, **45**, 225–236.

52 D. Hussain, M. J. Haydon, Y. Wang, E. Wong, S. M. Sherson, J. Young, J. Camakaris, J. F. Harper and C. S. Cobbett, P-Type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis, *Plant Cell*, 2004, **16**, 1327–1339.

53 M. Hanikenne, I. N. Talke, M. J. Haydon, C. Lanz, A. Nolte, P. Motte, J. Kroymann, D. Weigeland and U. Krämer, Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of HMA4, *Nature*, 2008, **453**, 391–395.

54 S. Hall and D. A. Baker, The chemical composition of *Ricinus* phloem exudate, *Planta*, 1972, **106**, 131–140.

55 O. Riesen and U. Feller, Redistribution of Nickel, Cobalt, Manganese, Zinc, and Cadmium *via* the phloem in young and maturing wheat, *J. Plant Nutr.*, 2005, **28**, 421–430.

56 A. Álvarez-Fernández, P. Díaz-Benito, A. Abadía, A. F. López-Millán and J. Abadía, Metal species involved in long distance metal transport in plants, *Front. Plant Sci.*, 2014, **5**, 105.

57 C. Curie, Z. Panaviene, C. Loulergue, S. L. Dellaporta, J.-F. Briat and E. L. Walker, Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake, *Nature*, 2001, **409**, 346–349.

58 Z. Zhai, S. R. Gayomba, H. Jung, N. K. Vimalakumari, M. Piñeros, E. Craft, M. A. R. J. Danku, B. Lahner, T. Punshon, M. L. Guerinot, D. E. Salt, L. V. Kochian and O. K. Vatamaniuk, OPT3 is a phloem-specific iron transporter that is essential for systemic iron signaling and redistribution of iron and cadmium in Arabidopsis, *Plant Cell*, 2014, **26**, 2249–2264.

59 J. L. Burkhead, A. Kathryn, K. G. Reynolds, S. E. Abdel-Ghany, C. M. Cohu and M. Pilon, Copper homeostasis, *New Phytol.*, 2009, **182**, 799–816.

60 S. Sharma and K. J. Dietz, The relationship between metal toxicity and cellular redox imbalance, *Trends Plant Sci.*, 2009, **14**, 43–50.

61 H. Kupper, F. J. Zhao and S. P. McGrath, Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*, *Plant Physiol.*, 1999, **119**, 305–311.

62 A. G. Desbrosses-Fonrouge, K. Voigt, A. Schröder, S. Arrivault, S. Thomine and U. Krämer, *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation, *FEBS Lett.*, 2005, **579**, 4165–4174.

63 S. Arrivault, T. Senger and U. Krämer, The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply, *Plant J.*, 2006, **46**, 861–879.

64 M. J. Haydon, M. Kawachi, M. Wirtz, S. Hillmer, R. Hell and U. Krämer, Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in Arabidopsis, *Plant Cell*, 2012, **24**, 724–737.

65 S. A. Kim, T. Punshon, A. Lanzirotti, L. Li, J. M. Alonso, J. R. Ecker, J. Kaplan and M. L. Guerinot, Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1, *Science*, 2006, **314**, 1295–1298.

66 P. J. Lapinskas, S. J. Lin and V. C. Culotta, The role of the *Saccharomyces cerevisiae* CCC1 gene in the homeostasis of manganese ions, *Mol. Microbiol.*, 1996, **21**, 519–528.

67 J. K. Pittman, T. Shigaki, J. L. Marshall, J. L. Morris, N. H. Cheng and K. D. Hirschi, Functional and regulatory analysis of the *Arabidopsis thaliana* CAX2 cation transporter, *Plant Mol. Biol.*, 2004, **56**, 959–971.

68 J. S. Peng and J. M. Gong, Vacuolar sequestration capacity and long-distance metal transport in plants, *Front. Plant Sci.*, 2014, **5**, 1–5.

69 S. Klaumann, S. D. Nickolaus, S. H. Furst, S. Starck, S. Schneider, H. Ekkehard Neuhaus and O. Trentmann, The tonoplast copper transporter COPT5 acts as an exporter and is required for interorgan allocation of copper in *Arabidopsis thaliana*, *New Phytol.*, 2011, **192**, 393–404.

70 A. Garcia-Molina, N. Andrés-Colás, A. Perea-García, U. Neumann, S. C. Dodani, P. Huijser, L. Pen Arrubia and S. Puig, The *Arabidopsis*COPT6 transport protein functions in copper distribution under copper-deficient conditions, *Plant Cell Physiol.*, 2013, **54**, 1378–1390.

71 A. Gasber, S. Klaumann, O. Trentmann, A. Trampczynska, S. Clemens, S. Schneider, N. Sauer, I. Feifer, F. Bittner, R. R. Mendel and H. E. Neuhaus, Identification of an

Arabidopsis solute carrier critical for intracellular transport and inter-organ allocation of molybdate, *Plant Biol.*, 2011, **13**, 710–718.

72 D. Duy, R. Stube, G. Wanner and K. Philippar, The chloroplast permease PIC1 regulates plant growth and development by directing homeostasis and transport of iron, *Plant Physiol.*, 2011, **155**, 1709–1722.

73 S. E. Abdel-Ghany, P. Muller-Moulè, K. K. Niyogi, M. Pilon and T. Shikanai, Two P-type ATPase are required for copper delivery in *Arabidopsis thaliana* chloroplasts, *Plant Cell*, 2005, **17**, 1233–1251.

74 S. Boutigny, E. Sautron, G. Finazzi, C. Rivasseau, A. Frelet-Barrand, M. Pilon, N. Rolland and D. Seigneurin-Berny, HMA1 and PAA1, two chloroplast-envelope P IB-ATPases, play distinct roles in chloroplast copper homeostasis, *J. Exp. Bot.*, 2014, **65**, 1529–1540.

75 Y. F. Tan, N. O'Toole, N. L. Taylor and A. H. Millar, Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function, *Plant Physiol.*, 2010, **152**, 747–761.

76 G. Vigani, D. Maffi and G. Zocchi, Iron availability affects the function of mitochondria in cucumber roots, *New Phytol.*, 2009, **182**, 127–136.

77 C. Nouet, P. Motte and M. Hanikenne, Chloroplastic and mitochondrial metal homeostasis, *Trends Plant Sci.*, 2011, **16**, 395–404.

78 K. Bashir, Y. Ishimaru, H. Shimo, S. Nagasaka, M. Fujimoto, H. Takanashi, G. An, H. Nakanishi and N. K. Nishizawa, The rice mitochondrial iron transporter is essential for plant growth, *Nat. Commun.*, 2011, **322**, 2, DOI: 10.1038/ncomms1326.

79 J. L. Heazlewood, J. S. Tonti-Filippini, A. M. Gout, D. A. Day, J. Whelan and A. H. Millar, Experimental analysis of the *Arabidopsis* mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins, *Plant Cell*, 2004, **16**, 241–256.

80 J. Teschner, N. Lachmann, J. Schulze, M. Geisler, K. Selbach, J. Santamaria-Araujo, J. Balk, R. R. Mendel and F. Bittner, a novel role for *Arabidopsis* mitochondrial ABC transporter ATM3 in molybdenum cofactor biosynthesis, *Plant Cell*, 2010, **22**, 468–480.

81 S. Chen, R. Sanchez-Fernandez, E. Lyver, A. Dancis and P. Rea, Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of *Arabidopsis* half-molecule ATP-binding cassette transporters implicated in iron homeostasis, *J. Biol. Chem.*, 2007, **282**, 21561–21571.

82 K. E. Vest, S. C. Leary, D. R. Wige and P. A. Cobine, Copper import into the mitochondrial matrix in *Saccharomyces cerevisiae* is mediated by Pic2, a mitochondrial carrier family protein, *J. Biol. Chem.*, 2013, **288**, 23884–23892.

83 S. F. Sousa, A. B. Lopes, P. A. Fernandes and M. J. Ramos, The Zinc proteome: a tale of stability and functionality, *Dalton Trans.*, 2009, 7946–7956.

84 U. Krämer and S. Clemens, Function and homeostasis of zinc, copper, and nickel in plants, *Top. Curr. Genet.*, 2005, **14**, 215–271.

85 S. Richter and G. K. Lamppa, Structural properties of the chloroplast stromal processing peptidase required for its function in transit peptide removal, *J. Biol. Chem.*, 2003, **278**, 39497–39502.

86 P. Luciano, K. Tokatlidis, I. Chambre, J. C. Germanique and V. Geli, The mitochondrial processing peptidase behaves as a zinc-metallopeptidase, *J. Mol. Biol.*, 1998, **280**, 193–199.

87 A. Ståhl, P. Moberg, J. Ytterberg, O. Panfilov, H. Brockenhuus Von Lowenhielm, F. Nilsson and E. Glaser, Isolation and identification of a novel mitochondrial metalloprotease (PreP) that degrades targeting presequences in plants, *J. Biol. Chem.*, 2002, **277**, 41931–41939.

88 S. M. Snaith and G. A. Levvy, Alpha-mannosidase as a zinc-dependent enzyme, *Nature*, 1968, **218**, 91–92.

89 J. A. Sullivan, K. Shirasu and X. W. Deng, The diverse roles of ubiquitin and the 26S proteasome in the life of plants, *Nat. Rev. Genet.*, 2003, **4**, 948–958.

90 J. M. Maidment, D. Moore, G. P. Murphy, G. Murphy and I. M. Clark, Matrix metalloproteinase homologues from *Arabidopsis thaliana*. Expression and activity, *J. Biol. Chem.*, 1999, **274**, 34706–34710.

91 C. W. Lin, H. B. Chang and H. J. Huang, Zinc induces mitogen-activated protein kinase activation mediated by reactive oxygen species in rice roots, *Plant Physiol. Biochem.*, 2005, **43**, 963–968.

92 H. Obata and M. Umebayashi, Effect of zinc deficiency on protein synthesis in cultured tobacco plant cells, *Soil Sci. Plant Nutr.*, 1988, **34**, 351–357.

93 C. K. Shrotri, P. Mohanty, V. C. Rathore and M. N. Tewari, Zinc deficiency limits the photosynthetic enzymes activation in *Zea mays* L, *Biochem. Physiol. Pflanz.*, 1983, **178**, 213–217.

94 H. Sekimoto, M. Hoshi, T. Nomura and T. Yokota, Zinc deficiency affects the levels of endogenous gibberellins in *Zea mays* L., *Plant Cell Physiol.*, 1997, **38**, 1087–1090.

95 C. Tsui, The role of zinc in auxin synthesis in the tomato plant, *Am. J. Bot.*, 1948, **35**, 172–179.

96 I. Cakmak, H. Marschner and F. Bangerth, Effect of zinc nutritional status on growth, protein metabolism and levels of indole-3-acetic acid and other phytohormones in bean (*Phaseolus vulgaris* L.), *J. Exp. Bot.*, 1989, **40**, 405–412.

97 I. Cakmak and H. Marschner, Enhanced superoxide radical production in roots of zinc-deficient plants, *J. Exp. Bot.*, 1988, **39**, 1449–1460.

98 I. Cakmak and H. Marschner, Increase in membrane permeability and exudation in roots of zinc deficient plants, *J. Plant Physiol.*, 1988, **132**, 356–361.

99 I. Cakmak, Tansley Review No. 111. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species, *New Phytol.*, 2000, **146**, 185–205.

100 C. P. Sharma, *Plant micronutrients*, Science Publishers, Enfield, NH, USA, 2006.

101 R. Boardman and D. O. McGuire, The role of zinc in forestry. I. Zinc in forest environments, ecosystems and tree nutrition forest ecology, *For. Ecol. Manage.*, 1990, **37**, 167–205.

102 I. Cakmak, A. Yilmaz, H. Ekiz, B. Torun, B. Erenoglu and H. J. Braun, Zinc deficiency a critical nutritional problem in wheat production in Central Anatolia, *Plant Soil*, 1996, **180**, 165–172.

103 M. V. Wiese, Wheat and other small grains, in *Nutrient Deficiencies and Toxicities in Crop Plants*, ed. W. F. Bennett, APS Press, St. Paul, MN, 1993.

104 F. Zhang, V. Romheld and H. Marschner, Release of zinc mobilizing root exudates in different plant species as affected by zinc nutritional status, *J. Plant Nutr.*, 1991, **14**, 675–686.

105 R. Sagardoy, F. Morales, A. F. López-Millán, A. Abadía and J. Abadía, Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics, *Plant Biol.*, 2009, **11**, 339–350.

106 R. L. F. Fontes and F. R. Cox, Effects of sulfur supply on soybean plants exposed to zinc toxicity, *J. Plant Nutr.*, 1995, **18**, 1893–1906.

107 F. van Assche and H. Clijsters, Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentrations of zinc: effects on electron transport and photo-phosphorylation, *Physiol. Plant.*, 1986, **66**, 717–721.

108 M. Teige, B. Huchzermeyer and G. Schultz, Inhibition of chloroplast ATPsynthase/ATPase is a primary effect of heavy metal toxicity in spinach plants, *Biochem. Physiol. Pflanz.*, 1990, **186**, 165–168.

109 R. Sagardoy, S. Vázquez, D. Florez-Sarasa, A. Albacete, M. Ribas-Carbó, J. Flexas, J. Abadía and F. Morales, Stomatal and mesophyll conductance to CO_2 are the main limitations to photosynthesis in sugar beet (*Beta vulgaris*) plants grown with excess zinc, *New Phytol.*, 2010, **187**, 145–158.

110 K. Prasad, P. P. Saradhi and P. Sharmila, Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*, *Environ. Exp. Bot.*, 1999, **42**, 1–10.

111 H. B. Chang, C. W. Lin and H. J. Huang, Zinc-induced cell death in rice (*Oryza sativa* L.) roots, *Plant Growth Regul.*, 2005, **46**, 261–266.

112 I. Yruela, Copper in plants: acquisition, transport and interactions, *Funct. Plant Biol.*, 2009, **36**, 409–430.

113 R. D. Graham, The distribution of copper and soluble carbohydrates in wheat plants grown at high and low levels of copper supply, *Z. Pflanzenernaehr. Bodenkd.*, 1980, **143**, 161–169.

114 M. B. Ayala, J. L. Gorgé, M. Lachica and G. Sandmann, Changes in carotenoids and fatty acids in photosystem II of Cu deficient pea plants, *Physiol. Plant.*, 1992, **84**, 1–5.

115 R. D. Graham, Male sterility in wheat plants deficient in copper, *Nature*, 1975, **254**, 514–515.

116 B. Cartwright and E. G. Hallsworth, Effects of copper deficiency on root nodules of subterranean clover, *Plant Soil*, 1970, **33**, 685–698.

117 A. D. Robson, R. D. Hartley and S. C. Jarvis, Effect of copper deficiency on phenolic and other constituents of wheat cell walls, *New Phytol.*, 1981, **89**, 361–371.

118 R. D. Graham, Anomalous water relations in copper-deficient wheat plants, *Aust. J. Plant Physiol.*, 1976, **3**, 229–236.

119 M. Droppa and G. Horváth, The role of copper in photosynthesis, *Crit. Rev. Plant Sci.*, 1990, **9**, 111–123.

120 E. Solberg, I. Evans and D. Penny, *Copper Deficiency: Diagnosis and Correction*, Agdex 532–3, Agriculture, Food, and Rural Development, Government of Alberta, Edmonton, AB (1999).

121 B. J. Alloway and A. R. Tills, Copper deficiency in world crops, *Outlook Agric.*, 1984, **13**, 32–42.

122 Y. Li, A. Seacat, P. Kuppusamy, J. L. Zweier, J. D. Yager and M. A. Trush, Copper redox-dependent activation of 2-tert-butyl(1,4)hydroquinone: formation of reactive oxygen species and induction of oxidative DNA damage in isolated DNA and cultured rat hepatocytes, *Mutat. Res.*, 2002, **518**, 123–133.

123 M. E. Letelier, A. M. Lepe, M. Faúndez, J. Salazar, R. Marín, P. Aracena and H. Speisky, Possible mechanisms underlying copper-induced damage in biological membranes leading to cellular toxicity, *Chem.–Biol. Interact.*, 2005, **151**, 71–82.

124 N. Llorens, L. Arola, C. Bladé and A. Mas, Effects of copper exposure upon nitrogen metabolism in tissue cultured *Vitis vinifera*, *Plant Sci.*, 2000, **160**, 159–163.

125 E. Pätsikkä, M. Kairavuo, F. Sersen, E. M. Aro and E. Tyystjärvi, Excess copper predisposes photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll, *Plant Physiol.*, 2002, **129**, 1359–1367.

126 W. Maksymiec and T. Baszynski, The role of Ca^{2+} ions in modulating changes induced in bean plants by an excess of Cu^{2+} ions. Chlorophyll fluorescence measurements, *Physiol. Plant.*, 1999, **105**, 562–568.

127 M. Roncel, J. M. Ortega and M. Losada, Factors determining the special redox properties of photosynthetic cytochrome *b*559, *Eur. J. Biochem.*, 2001, **268**, 4961–4968.

128 A. M. B. Pahlsson, Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants, *Water, Air, Soil Pollut.*, 1989, **47**, 287–319.

129 S. A. Barber, *Soil nutrient bioavailability*, John Wiley and Sons, New York, 1984.

130 K. S. Smith, L. S. Balistrieri, S. M. Smith and R. C. Severson, Distribution and mobility of molybdenum in the terrestrial environment, in *Molybdenum in agriculture*, ed. U. C. Gupta, Cambridge University Press, New York, 1997, pp. 23–46.

131 L. Xiong, M. Ishitani, H. Lee and J. K. Zhu, The *Arabidopsis* LOS5/ABA3 locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression, *Plant Cell*, 2001, **13**, 2063–2083.

132 J. Ishizuka, Characteristics of molybdenum absorption and translocation in soybean plants, *Soil Sci. Plant Nutr.*, 1982, **28**, 63–77.

133 W. Bussler, Molybdenum deficiency symptoms and their development, *Z. Pflanzenernaehr., Dueng., Bodenkd.*, 1970, **125**, 50–64.
134 C. Hecht-Buchholz, Molybdänverteilung und-verträglichkeit bei Tomate, Sonnenblume und Bohne, *Z. Pflanzenernaehr. Bodenkd.*, 1973, **136**, 110–119.
135 K. L. Hale, S. P. McGrath, E. Lombi, S. M. Stack, N. Terry, I. J. Pickering, G. N. George and E. A. Pilon-Smits, Molybdenum sequestration in *Brassica* species. A role for anthocyanins?, *Plant Physiol.*, 2001, **126**, 1391–1402.
136 D. J. Kliebenstein, R.-A. Monde and R. L. Last, Superoxide dismutase in Arabidopsis: an eclectic enzyme family with disparate regulation and protein localization, *Plant Physiol.*, 1998, **118**, 637–650.
137 P. E. Kriedemann, R. D. Graham and J. T. Wiskich, Photosynthetic dysfunction and *in vivo* changes in chlorophyll a fluorescence from manganese-deficient wheat leaves, *Aust. J. Agric. Res.*, 1985, **36**, 157–169.
138 X. Gong, Y. Wang, C. Liu, S. Wang, X. Zhao, M. Zhou, N. Li, Y. Lu and F. Hong, Effects of manganese deficiency on spectral characteristics and oxygen evolution in maize chloroplasts, *Biol. Trace Elem. Res.*, 2010, **136**, 372–382.
139 T. Dučic and A. Polle, Transport and detoxification of manganese and copper in plants, *Braz. J. Plant Physiol.*, 2005, **17**, 103–112.
140 G. Constantopoulos, Lipid metabolism of manganese-deficient algae. I. Effect of manganese deficiency on the greening and the lipid composition of *Euglena gracilis* Z., *Plant Physiol.*, 1970, **45**, 76–80.
141 C. P. Sharma, P. N. Sharma, C. Chatterjee and S. C. Agarwala, Manganese deficiency in maize affects pollen viability, *Plant Soil*, 1991, **138**, 139–142.
142 N. E. Longnecker, N. E. Marcar and R. D. Graham, Increased manganese content of barley seeds can increase grain yield in manganese-deficient conditions, *Aust. J. Agric. Res.*, 1991, **42**, 1065–1074.
143 K. H. Neumann and F. C. Steward, Investigations on the growth and metabolism of cultured explants of *Daucus carota*. I. Effects of iron, molybdenum and manganese on growth, *Planta*, 1968, **81**, 333–350.
144 P. H. Brown, R. D. Graham and D. J. D. Nicholas, The effects of manganese and nitrate supply on the levels of phenolics and lignin in young wheat plants, *Plant Soil*, 1984, **81**, 437–440.
145 Z. Rengel, R. D. Graham and J. F. Pedler, Manganese nutrition and accumulation of phenolics and lignin as related to differential resistance of wheat genotypes to the take-all fungus, *Plant Soil*, 1993, **151**, 255–263.
146 J. D. Reynolds, Marsh spot of peas: A review of present knowledge, *J. Sci. Food Agric.*, 1955, **6**, 725–734.
147 G. H. Walton, The effect of manganese on seed yield and the split seed disorder of sweet and bitter phenotypes of *Lupinus angustifolius* and *L. Cosentinii*, *Aust. J. Agric. Res.*, 1978, **29**, 1177–1189.
148 S. Gangwar, V. P. Singh and J. N. Maurya, Responses of *Pisum sativum* L. to exogenous indole acetic acid application under manganese toxicity, *Bull. Environ. Contam. Toxicol.*, 2011, **86**, 605–609.
149 S. Sinha, S. Mukherji and J. Dutta, Effect of manganese toxicity on pigment content, Hill activity and photosynthetic rate of *Vigna radiata* L. Wilczek seedlings, *J. Environ. Biol.*, 2002, **23**, 253–257.
150 R. Millaleo, M. Reyes-Diaz, A. G. Ivanov, M. L. Mora and M. Alberdi, Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms, *Jpn. J. Soil Sci. Plant Nutr.*, 2010, **10**, 470–481.
151 W. J. Horst, The physiology of maganese toxicity, in *Manganese in soils and plants*, ed. R. D. Graham, R. J. Hannan and N. C. Uren, Kluwer Academic Publishers, Doredrecht, 1988, vol. 33, pp. 175–188.
152 A. de Varennesa, J. P. Carneiro and M. J. Gossb, Characterization of manganese toxicity in two species of annual medics, *J. Plant Nutr.*, 2001, **24**, 1947–1955.
153 B. J. Alloway, The origin of heavy metals in soils, in *Heavy Metals in Soils*, ed. B. J. Alloway, Springer Science & Business Media, 1995, pp. 38–56.
154 M. Yusuf, Q. Fariduddin, S. Hayat and A. Ahmad, Nickel: an overview of uptake, essentiality and toxicity in plants, *Bull. Environ. Contam. Toxicol.*, 2011, **86**, 1–17.
155 R. G. Winkler, J. C. Polacco, D. L. Eskew and R. M. Welch, Nickel is not required for apourease synthesis in soybean seeds, *Plant Physiol.*, 1983, **72**, 262–263.
156 R. V. Klucas, F. J. Hanus, S. A. Russell and H. J. Evans, Nickel: A micronutrient element for hydrogen-dependent growth of *Rhizobium japonicum* and for expression of urease activity in soybean leaves, *Proc. Natl. Acad. Sci. U. S. A.*, 1983, **80**, 2253–2257.
157 P. H. Brown, R. W. Welch and E. E. Cary, Nickel: A micronutrient essential for higher plants, *Plant Physiol.*, 1987, **85**, 801–803.
158 D. L. Eskew, R. M. Welch and E. E. Cary, Nickel: an essential micronutrient for legumes and possibly all higher plants, *Science*, 1983, **222**, 691–693.
159 M. M. Alam, S. Hayat, B. Ali and A. Ahmad, Effect of 28-homobrassinolide treatment on nickel toxicity in *Brassica juncea*, *Photosynthetica*, 2007, **45**, 139–142.
160 E. Gajewska, M. Sklodowska, M. Slaba and J. Mazur, Effect of nickel on antioxidative enzyme activities, proline and chlorophyll content in wheat shoots, *Biol. Plant.*, 2006, **50**, 653–659.
161 K. V. M. Rao and T. V. S. Sresty, Antioxidative parameters in the seedlings of pigeonpea *(Cajanus cajan L.)* Millsp auga in response to Zn and Ni stress, *Plant Sci.*, 2000, **157**, 113–128.
162 R. Bhardwaj, N. Arora, P. Sharma and H. K. Arora, Effects of 28-homobrassinolide on seedling growth, lipid peroxidation and antioxidative enzyme activities under nickel stress in seedlings of *Zea mays* (L), *Asian J. Plant Sci.*, 2007, **6**, 765–772.
163 E. Gajewska and M. Sklodowska, Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation, *Plant Growth Regul.*, 2008, **54**, 179–188.

164 C. P. Sharma, R. Bhardwaj, N. Arora, H. K. Arora and A. Kumar, A Effects of 28-homobrassinolide on nickel uptake, protein content and antioxidative defence system in *Brassica juncea*, *Biol. Plant.*, 2008, **52**, 767–770.
165 C. Chen, D. Huang and J. Liu, Functions and toxicity of nickel in plants: recent advances and future prospects, *Clean*, 2009, **37**, 304–313.
166 J. Molas, Changes in morphological and anatomical structure of cabbage (*Brassica oleracea* L.) outer leaves and in ultrastructure of their chloroplasts caused by an *in vitro* excess of nickel, *Photosynthetica*, 1997, **34**, 513–522.
167 S. Boisvert, D. Joly, S. Leclerc, S. Govindachary, J. Harnois and R. Carpentier, Inhibition of the oxygen-evolving complex of photosystem II and depletion of extrinsic polypeptides by nickel, *BioMetals*, 2007, **20**, 879–889.
168 R. Boominathan and P. M. Doran, Ni-induced oxidative stress in roots of the Ni hyperaccumulator, *New Phytol.*, 2002, **156**, 205–215.
169 F. Hao, X. Wang and J. Chen, Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings, *Plant Sci.*, 2006, **170**, 151–158.
170 E. Gajewska and M. Sklodowska, Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves, *BioMetals*, 2007, **20**, 27–36.
171 R. Athar and M. Ahmad, Heavy metal toxicity in legume–microsymbiont system, *J. Plant Nutr.*, 2002, **25**, 369–386.
172 S. V. Pillay, V. S. Rao and K. V. N. Rao, Effect of nickel toxicity in *Hyptis suareeolens* (L.) Poit. and *Helianthus annuus* L. Indian, *J. Plant Physiol.*, 1996, **1**, 153–156.
173 C. H. Chen and J. Lewin, Silicon as a nutrient element for *Equisetum arvense*, *Can. J. Bot.*, 1969, **47**, 125–131.
174 U. Krämer, Metal hyperaccumulation in plants, *Annu. Rev. Plant Biol.*, 2010, **61**, 1–18.
175 A. Kazemi-Dinan, S. Thomaschky, R. J. Stein, U. Krämer and C. Müller, Zinc and cadmium hyperaccumulation act as deterrents towards specialist herbivores and impede the performance of a generalist herbivore, *New Phytol.*, 2014, **202**, 628–639.
176 B. G. Hua, R. W. Mercier, Q. Leng and G. A. Berkowitz, Plants do it differently. A new basis for potassium/sodium selectivity in the pore of an ion channel, *Plant Physiol.*, 2003, **132**, 1353–1361.
177 G. S. Smith, K. R. Middleton and A. S. Edmonds, Sodium nutrition of pasture plants II, Effect of sodium chloride on growth, chemical composition and the reduction of nitrate nitrogen, *New Phytol.*, 1980, **84**, 603–612.
178 J. P. Martínez, J. M. Kinet, M. Bajji and S. Lutts, NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L., *J. Exp. Bot.*, 2005, **56**, 2421–2431.
179 D. Ohta, S. Yasuoka, T. Matoh and E. Takahashi, Sodium stimulates growth of *Amaranthus tricolor* L. plants through enhanced nitrate assimilation, *Plant Physiol.*, 1989, **89**, 1102–1105.
180 A. Wakeel, D. Steffens and S. Schubert, Potassium substitution by sodium in sugar beet (*Beta vulgaris*) nutrition on K-fixing soils, *J. Plant Nutr. Soil Sci.*, 2010, **173**, 127–134.
181 C. K. Kelly and K. Horning, Acquisition order and resource value in *Cuscuta attenuate*, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 13219–13222.
182 G. Kerstiens, W. Tych, M. F. Robinson and T. A. Mansfield, Sodium-related partial stomatal closure and salt tolerance of *Astertripolium*, *New Phytol.*, 2002, **153**, 509–515.
183 E. Epstein, Silicon, *Annu. Rev. Plant Physiol.*, 1999, **50**, 641–664.
184 J. F. Ma and N. Yamaji, Silicon uptake and accumulation in higher plants, *Trends Plant Sci.*, 2006, **11**, 392–397.
185 J. F. Ma and N. Yamaji, Functions and transport of silicon in plants, *Cell. Mol. Life Sci.*, 2008, **65**, 3049–3057.
186 M. J. Hodson, P. J. White, A. Mead and M. R. Broadley, Phylogenetic variation in the silicon composition of plants, *Ann. Bot.*, 2005, **96**, 1027–1046.
187 H. J. Gong, D. P. Randall and T. J. Flowers, Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow, *Plant, Cell Environ.*, 2006, **29**, 1970–1979.
188 L. H. Fu, X. F. Wang, Y. Eyal, Y. M. She, L. J. Donald, K. G. Standing and G. Ben-Hayyim, A selenoprotein in the plant kingdom. Mass spectrometry confirms that an opal codon (UGA) encodes selenocysteine in *Chlamydomonas reinhardtii* glutathione peroxidase, *J. Biol. Chem.*, 2002, **277**, 25983–25991.
189 M. Tamaoki, J. L. Freeman and E. A. Pilon-Smits, Cooperative ethylene and jasmonic acid signaling regulates selenite resistance in Arabidopsis, *Plant Physiol.*, 2008, **146**, 1219–1230.
190 P. J. White, H. C. Bowen, B. Marshall and M. R. Broadley, Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants, *Ann. Bot.*, 2007, **100**, 111–118.
191 R. Feng, C. Wei and S. Tu, The roles of selenium in protecting plants against abiotic stresses, *Environ. Exp. Bot.*, 2013, **87**, 58–68.
192 B. Hanson, G. F. Garifullina, S. D. Lindbloom, A. Wangeline, A. Ackley, K. Krämer, A. P. Norton, C. B. Lawrence and S. E. H. Pilon Smits, Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection, *New Phytol.*, 2003, **159**, 461–469.
193 C. F. Quinn, J. L. Freeman, M. L. Galeas, E. M. Klamper and E. A. H. Pilon Smits, The role of selenium in protecting plants against prairie dog herbivory: implications for the evolution of selenium hyperaccumulation, *Oecologia*, 2008, **155**, 267–275.
194 I. Saidi, Y. Chtourou and W. Djebali, Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings, *J. Plant Physiol.*, 2014, **171**, 85–91.
195 L. Kochian, O. A. Hoekenga and M. A. Piñeros, How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency, *Annu. Rev. Plant Biol.*, 2004, **55**, 459–493.
196 F. P. C. Blamey, D. C. Edmeades, C. J. Asher, D. G. Edwards and D. M. Wheeler, Evaluation of solution culture techniques for studying aluminum toxicity in plants, in *Plant-Soil Interactions at Low pH*, ed. R. J. Wright, V. C. Baligar and

R. P. Murrmann, Kluwer Academic Publisher, Doredrecht, 1991, vol. 45, pp. 905–912.
197 N. V. Bulanova, B. I. Synzynys and G. V. Kozmin, Aluminum induces chromosome aberrations in cells of wheat root meristem, *Russ. J. Genet.*, 2001, **37**, 1455–1458.
198 S. Mohanty, A. B. Das, P. Das and P. Mohanty, Effect of a low dose of aluminum on mitotic and meiotic activity, 4C DNA content, and pollen sterility in rice, *Oryza sativa* L. cv. Lalat, *Ecotoxicol. Environ. Saf.*, 2004, **59**, 70–75.
199 W. J. Horst, N. Schmohl, M. Kollmeier, F. Baluška and M. Sivaguru, Does aluminum affect root growth of maize through interaction with the cell wall-plasma membrane-cytoskeleton continuum?, *Plant Soil*, 1999, **215**, 163–174.
200 Y. Yamamoto, Y. Kobayashi, S. R. Devi, S. Rikiishi and H. Matsumoto, Oxidative stress triggered by aluminum in plant roots, *Plant Soil*, 2003, **255**, 239–243.
201 T. Watanabe, S. Jansen and M. Osaki, The beneficial effect of aluminium and the role of citrate in Al accumulation in *Melastoma malabathricum*, *New Phytol.*, 2005, **165**, 773–780.
202 T. Watanabe, S. Jansen and M. Osaki, Al–Fe interactions and growth enhancement in *Melastoma malabathricum* and *Miscanthus sinensis* dominating acid sulphate soils, *Plant, Cell Environ.*, 2006, **29**, 2124–2132.
203 R. Hajiboland, J. Barceló, C. Poschenrieder and R. Tolrà, Amelioration of iron toxicity: A mechanism for aluminum-induced growth stimulation in tea plants, *J. Inorg. Biochem.*, 2013, **128**, 183–187.
204 F. Ghanati, A. Morita and H. Yokota, Effects of aluminum on the growth of tea plant and activation of antioxidant system, *Plant Soil*, 2005, **276**, 133–141.
205 M. Arasimowicz-Jelonek, J. Floryszak-Wieczorek, K. Drzewiecka, J. Chmielowska-Bąk, D. Abramowski and K. Izbiańska, Aluminum induces cross-resistance of potato to *Phytophthora infestans*, *Planta*, 2014, **239**, 679–694.
206 H. F. Li, C. Gray, C. Mico, F. J. Zhao and S. P. McGrath, Phytotoxicity and bioavailability of cobalt to plants in a range of soils, *Chemosphere*, 2009, **75**, 979–986.
207 E. W. Bolle-Jones and A. Mallikarjuneswara, A beneficial effect of cobalt on the growth of the rubber plant (*Hevea brasiliensis*), *Nature*, 1957, **179**, 738–739.
208 R. Gopal, B. K. Dube, P. Sinha and C. Chatterjee, Cobalt toxicity effects on growth and metabolism of tomato, *Commun. Soil Sci. Plant Anal.*, 2003, **34**, 619–628.
209 J. Chatterjee and C. Chatterjee, Phytotoxicity of cobalt, chromium and copper in cauliflower, *Environ. Pollut.*, 2000, **109**, 69–74.
210 N. Gad, Increasing the efficiency of nitrogen fertilization through cobalt application to pea plant, *Res. J. Agric. Biol. Sci.*, 2006, **2**, 433–442.
211 I. T. Riley and M. J. Dilworth, Recovery of cobalt-deficient root nodules in *Lupinus agustifolius* L., *New Phytol.*, 1985, **100**, 361–365.
212 Y. Zhang and V. N. Gladyshev, Comparative genomics of trace elements: emerging dynamic view of trace element utilization and function, *Chem. Rev.*, 2009, **109**, 4828–4861.
213 Y. Lyang, W. Sun, Y. G. Zhu and P. Christie, Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review, *Environ. Pollut.*, 2007, **147**, 422–428.
214 N. Sarwar, S. Saifullah, S. Malhi, M. H. Zia, A. Naeem, S. Bibi and G. Farid, Role of mineral nutrition in minimizing cadmium accumulation by plants, *J. Sci. Food Agric.*, 2010, **90**, 925–937.

