Metal toxicity and tolerance in plants

Martin J. Hodson (Oxford Brookes University, UK)

Metal toxicity is an important factor limiting the growth of plants in many environments. Some metals, such as copper and zinc, are micronutrients at low concentrations and become toxic at higher levels, whereas others (e.g. aluminium and lead) are only known for their toxicity. In some cases, soils are naturally very high in metals. An example is the serpentine soils, which have elevated levels of chromium, cobalt, iron, magnesium and nickel, but are very low in calcium. Low soil pH makes most metals more available for plant uptake and can indirectly lead to metal toxicity (e.g. through acid rain). Some acidic soils have high aluminium availability, and this can be an important limiting factor for agriculture. Localized pollution caused by human activity (e.g. mining or smelting) often leads to toxicity problems. For example, the spoil heaps at Old Gang mine in Swaledale in North Yorkshire, UK, are still very high in plant available lead (Figure 1). Sewage sludge that is released on to agricultural land often contains high levels of toxic metals. But what happens to a plant when it is growing in an environment with toxic levels of metals, and how do some plants tolerate these environments? This brief article considers some of the recent trends in the study of metal toxicity and tolerance in plants.

Mechanisms of metal toxicity

Metal toxicity is responsible for many visual symptoms in plants. Root growth is often reduced, and leaves may change colour. Competition with nutrient ions for uptake by roots can cause deficiency symptoms (e.g. aluminium causes calcium or magnesium deficiency). In a similar way, binding of toxic metals with proteins and other compounds because they resemble essential metals can also cause toxicity. The plasma membrane of root cells is often damaged by exposure to toxic metals, resulting in leakage of cellular solutes.

In common with many other plant stresses, the toxicity mechanism that has been a topic of much research in recent years has been the involvement of reactive oxygen species (ROS). This is a complex area of research as was well illustrated by the work of Rodriguez-Serrano et al. on cadmium toxicity in pea plants. They studied the effect of cadmium on ROS, antioxidants and nitric oxide (NO). Cadmium affected the expression of superoxide dismutase (SOD) isoenzymes and SOD activity was reduced. Calcium deficiency caused by the cadmium treatment was probably the cause of SOD down-regulation. Under these conditions, ROS are overproduced, but this effect could be prevented by increasing exogenous calcium. Treatment with calcium also prevented the depression of NO production that was caused by cadmium treatment. Cadmium stress led to the up-regulation of the PrP4A and chitinase proteins and the heat-shock protein 71.2, probably as a protective measure. These proteins appear to be regulated by the plant hormones jasmonic acid and ethylene, which are increased by cadmium treatment. ROS seem to be involved both in toxicity effects and in signalling.

Tolerance to toxic metals

Whereas most plants are badly affected by high concentrations of toxic metals, others are able to tolerate toxic environments, such as those on serpentine soils. Sometimes normally non-tolerant plants have evolved races that are tolerant to the toxic soil, known as edaphic ecotypes, and these are particularly common around old mine workings. Some plants appear to tolerate metals either by excluding them from the shoot or by accumulating metals in older leaves and then dropping them. Others are hyperaccumulators and contain very high concentrations of metals up to four orders of magnitude above the non-tolerant species. Hyperaccumulators have been the subject of much research and many have potential applications for phytoremediation, where they can take up metals from contaminated soils and remove them from the environment.

Key words: hyperaccumulator, metal toxicity, metallothionein, phytochelatin, phytochelatination, reactive oxygen species

Abbreviations: ABC, ATP-binding cassette; ALMT, aluminium activated malate transporter; MATE, multidrug and toxic compound exudation; MT, metallothionein; MTP, metal transporter protein; PC, phytochelatin; ROS, reactive oxygen species; SOD, superoxide dismutase
tolerance in plants

magnitude higher than those found in most plants. Obviously all hyperaccumulators are metal-tolerant, but there are many tolerant plants that do not show this trait. Hyperaccumulators can be found in at least 500 plant species (or about 0.2% of taxa), and accumulate metals that include aluminium, cadmium, copper, lead, nickel and zinc (Table 1). Spring sandwort or leadwort (*Minuartia verna*) is a lead hyperaccumulator plant (Figure 2). The most well-known aluminium hyperaccumulator is tea (*Camellia sinensis*), and leaves accumulate high concentrations of the element.

Although tolerance mechanisms at the whole plant level are important in some cases, those at the molecular and cellular levels will occupy our attention for the rest of this article (Figure 3). Binding of toxic metals to root cell walls can occur, but these soon become saturated with ions, and this mechanism is unlikely to be whole answer to tolerance. Many toxic metals cannot easily pass across the plasma membrane because of their large atomic radius and valence. Active efflux of toxic metals can occur through the plasma membrane of some plants. For example, Kim et al. showed that the ATP-binding cassette (ABC) transporter AtPDR8 is a cadmium-extrusion pump in *Arabidopsis thaliana*, and that an active pump conferred resistance to the metal. It has been known for some time that the membranes of tolerant plants show less metal-induced damage than those of sensitive plants, but the mechanism is still not entirely clear. It is, however, now evident that toxic metal-induced oxidative stress is usually greater in sensitive plants than in tolerant plants, which show reduced lipid peroxidation. Antioxidant defences consist of enzymatic and non-enzymatic components including SOD, ascorbate peroxidase (APX), peroxiredoxins (PRXs) and catalase (CAT). Glutathione (GSH) and ascorbate build up in chloroplasts and mitochondria because of the ascorbate–GSH cycle, which is also present in peroxisomes. Glutathione reductase (GR), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) are responsible for maintaining the redox state of GSH and ascorbate. These have a major role in defence against the oxidative damage caused by ROS.
It appears that plant enzymes are rarely adapted to high concentrations of metal and enzyme activity is usually decreased or lost altogether. So decreasing the concentration of toxic metals at sensitive sites is crucial, and this is our next topic.

The importance of complexation, chelation and compartmentation

Besides ROS and antioxidants, the other major areas of current research into metal toxicity and tolerance concern complexation, chelation and compartmentation. For over 20 years, I have been interested in the interaction between aluminium and silicon in plants. It has been convincingly demonstrated that, under some conditions, aluminium toxicity can be ameliorated by the presence of silicon in culture solutions. When both elements are present, non-toxic hydroxyaluminosilicate complexes can form in both the bulk solution and the cell walls of plant roots. In some plants, enough of both elements are transported to the shoot for co-deposition in leaf cell walls to occur. This phenomenon has been frequently reported in conifer needles where co-deposition may remove toxic aluminium from the apoplast.

Organic acids have been intensively studied as they are well known chelators of some metals and can decrease metal toxicity. In some cases, the acids are secreted into the rhizosphere and decrease metal activity before it reaches the plant. In wheat roots, exudation of malate is implicated in aluminium tolerance, with tolerant cultivars secreting more malate than sensitive cultivars. Ma et al. found that an aluminium-resistant cultivar (cv. Juanxi) of buckwheat (Fagopyrum esculentum Moench) secretes oxalate from its roots into the rhizosphere in response to aluminium stress. Buckwheat also accumulates aluminium in the leaf cells as an aluminium-oxalate complex which is non-toxic.

Once the toxic metal has crossed the plasma membrane into the cytosol, plants have several possible options. Phytochelatins (PCs) are metal-complexing peptides that act as chelators, thereby decreasing metal availability. Work on PCs has mostly concerned cadmium tolerance, with less on arsenic and mercury. PCs have the structure (γ-Glu-Cys) Gly and their production is induced by metal treatment. They are synthesized from glutathione (GSH; γ-Glu-Cys-Gly) and related compounds in a reaction catalysed by the enzyme PC synthase, which is activated in the presence of metal. As might be expected, Arabidopsis mutants with lowered levels of PC synthase are more sensitive to cadmium. Undoubtedly, PCs have a role in cadmium, arsenic and mercury tolerance, all of which are non-essential elements, but there is less evidence for a role in tolerance to toxic levels of essential metals. It might well be asked why plants have developed such elaborate systems for dealing with toxic elements to which they were hardly exposed before the human industrial era? It seems possible that the answer lies in other roles that PCs may perform, such as the storage and homeostasis of essential metals present at lower concentrations in plants.

### Table 1. Hyperaccumulation of some common elements by higher plants. Data mostly extracted from Krämer and concentrating on those elements and plant species mentioned in the text.

<table>
<thead>
<tr>
<th>Element</th>
<th>Critical deficiency level (μg·g⁻¹)</th>
<th>Critical toxicity level (μg·g⁻¹)</th>
<th>Hyperaccumulation concentration criterion (μg·g⁻¹)</th>
<th>Typical plant species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>n.r.</td>
<td>200*</td>
<td>&gt;1000</td>
<td>Tea (Camellia sinensis); Buckwheat (Fagopyrum esculentum)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>n.r.</td>
<td>6–10</td>
<td>&gt;100</td>
<td>Solanum nigrum; Thlaspi caerulescens</td>
</tr>
<tr>
<td>Copper</td>
<td>1–5</td>
<td>20–30</td>
<td>&gt;1000</td>
<td>Haumaniastrum katangense</td>
</tr>
<tr>
<td>Lead</td>
<td>n.r.</td>
<td>0.6–28</td>
<td>&gt;1000</td>
<td>Leadwort (Minuartia verna)</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.002–0.004</td>
<td>10–50</td>
<td>&gt;1000</td>
<td>Thlaspi goesingense</td>
</tr>
<tr>
<td>Zinc</td>
<td>15–20</td>
<td>100–300</td>
<td>&gt;10000</td>
<td>Arabidopsis halleri</td>
</tr>
</tbody>
</table>

*Average concentration found in plant tissues. It is difficult to determine a critical toxicity level for this element as often little is transported to shoots, and most toxicity effects are on roots.

n.r. = no known requirement
Metallothioneins (MTs) are another type of cysteine-rich peptide found in plants and other organisms. There are between 45 and 87 amino acids in plant MTs, and between 10 and 17 cysteine residues per molecule. MTs are involved in tolerance to metals as they are able to bind ions using the thiol groups of their cysteine residues. As with PCs, synthesis of MTs is induced by exposure to metals. Recently, it has also been shown that MTs are involved in scavenging of ROS, but how these two roles interact is not yet clear.

So plant cells can pump toxic metals out of the plasma membrane, or they can chelate metals using PCs and MTs in the cytosol, but ultimately many toxic metals are sequestered in the cell vacuole. In most plant cells, the vacuole occupies 95% or more of the cell volume and, if not devoid of enzymes, they have few that are essential for metabolism. The plant cell vacuole is a dumping site for many waste products and toxic substances, and toxic metals are no exception. It is interesting to compare hyperaccumulator and related non-hyperaccumulator species in this respect. Sequestration in the vacuole seems to rely on constitutive overexpression of genes that encode proteins involved in transferring metal across the vacuolar membrane, the tonoplast. Metal transporter proteins (MTPs) have been implicated in cation efflux from the cytosol, and the MTP1 gene which encodes a protein localized at tonoplast is overexpressed in the leaves of zinc/nickel hyperaccumulators. MTP1 may also have a role in increasing accumulation of zinc at lower micronutrient levels. Vacular storage of nickel in the shoots of Thlaspi goesingense, a nickel hyperaccumulator, also involves MTPs. Moreover, it is also possible that MTP1 is involved in nickel and zinc efflux transport from the cytosol to the cell wall as the protein is localized in both the tonoplast and the plasma membrane. Once in the vacuole, the toxic metals are usually chelated with small ligands, particularly organic acids. In T. goesingense leaves, citrate is the most important ligand involved in chelation of nickel. Cadmium is chelated by citrate and acetate in the leaves of Solanum nigrum. Malate is involved in complexing zinc in Arabidopsis halleri and cadmium in Thlaspi caerulescens. I have already noted that buckwheat uses oxalate to complex aluminium in its leaf cell vacuoles.

The future

In the last few years, we have seen significant advances in our knowledge of metal toxicity and tolerance in plants. What does the future hold? I consider two promising areas of current research.

The first is the idea of using hyperaccumulation of metals to clean up contaminated land, one possible phytoremediation technique. In what is known as phytoaccumulation, plants are grown on contaminated soil, where they take up toxic metals, and then the shoots that have accumulated the metal are harvested. The metal might subsequently be extracted from the harvested shoots, a process known as phytomining. Over time, hyperaccumulators will eventually decontaminate the soil of the metal. However, many hyperaccumulators have low biomass and grow very slowly. For example, T. caerulescens, a zinc/cadmium hyperaccumulator, only produces a maximum of 2 tons-ha⁻¹ of shoot dry matter, although this can be increased to 5 tons-ha⁻¹ by plant breeding. It might be possible to genetically modify faster-growing plants to become hyperaccumulators. Somatic hybrids have been generated between T. caerulescens and Brassica napus and the hybrid had high biomass and zinc tolerance.
Moreover, it accumulated zinc at levels that would be toxic to *B. napus*, suggesting that metal hyperaccumulation can be transferred. At present, this whole area is very much still under development and it is uncertain whether phytoremediation or phytomining are economically viable, although this may well change in the future.

Finally, improving the aluminium tolerance of crop plants so that they can grow better on acid soils has been the focus of much research. As we saw above, organic acid efflux from roots is an important mechanism of aluminium tolerance in plants. This mechanism is controlled by members of the *ALMT* (aluminium-activated malate transporter) and *MATE* (multidrug and toxic compound exudation) gene families and high levels of expression confer tolerance. In rice and Arabidopsis, aluminium induces the expression of *MATE* and *ALMT* genes, whereas in wheat and barley, the genes are constitutively expressed. The use of genetic engineering to improve aluminium tolerance has largely concentrated on increasing organic acid efflux from sensitive plants. *MATE* and *ALMT* genes have been cloned and used to enhance the aluminium tolerance of barley, wheat and sorghum. A number of other genes which encode other mechanisms are now available, providing other possibilities for increasing the aluminium tolerance of crops.

![Diagram of metal transport](image.png)

**Figure 3.** A summary of some of the tolerance mechanisms used by higher plants when exposed to toxic metals. 1. Metals are adsorbed by cation-exchange sites in cell walls. 2. Because of their size and valence metal movement across the plasma membrane is restricted. 3. Metals are pumped out of the cell, possibly by MTPs. 4. In the cytosol, metals are chelated by organic acids, PCs and MTs. 5. Particularly in the case of aluminium, acids are exuded from root cells into the rhizosphere, and complex with metals there, reducing their toxicity. 6. It is now thought that metals are mostly stored in the vacuole as complexes with various acids. 7. Metals are pumped into the vacuole by MTPs, reducing their concentration in the cytosol. This diagram is based on Hall, and has been updated to take into account more recent research findings.

References


Martin Hodson took his degree in Botany at Swansea University, and stayed on for a doctorate, studying salt tolerance in plants. He then had postdoctoral contracts in Bangor, Jerusalem, Toronto and Birmingham, and arrived at Oxford Brookes University in 1989. Martin’s research has mostly been in plant mineral nutrition, and particularly aluminium toxicity and silica deposition. He recently published Functional Biology of Plants (Wiley-Blackwell) an undergraduate text book which he co-authored with John Bryant of Exeter University. Email: mjhodson@brookes.ac.uk