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# Cellular and Ultrastructure Alteration of Plant Roots in Response to Metal Stress

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## Abstract

Metal stress is among the important environmental stresses, which influences the growth and development of plants and crops in many areas in the biosphere. Root is an important gate for the absorption of water and mineral nutrition which in many types of lands is also accompanied by a higher concentration of metal elements, either essential (such as Fe, Mn, and Cu) or non-essential metal elements or heavy metals (such as Al, Pb, Hg, Cd, and Ag). In response to metal stress, plant roots sometimes develop a cellular structure to prevent excessive concentration of metal components to avoid toxic effects and cellular damage. Physiological and biochemical responses at the cellular level, which result in ultrastructure changes may occur due to or to avoid the negative effect of metal toxicity. In many cases it was followed by the reduction of root growth followed by discontinuing entirely plant growth. On the other hand, the structural changes are an important part of root mechanism to sustain the plant from metal toxicity. In this chapter, different changes in the cellular ultrastructure resulting from toxic damage or indicating tolerance response to metal stress will be elucidated.

**Keywords:** metal stress, cellular ultrastructure, root anatomy, heavy metal, metal toxicity

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## 1. Introduction

In nature, plants will face diverse environmental circumstances including unfavorable conditions due to the presence of toxic compounds such as metal elements at toxic concentrations. On the one hand, plants as autotroph organisms require several essential elements from their environment which are mostly metal elements such as Cu, Zn, Mn, Fe, Mo, Co, and Ni but

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in small amounts as microelements (trace elements). These elements are essential for crucial biological processes and developmental pathways [1]. But in excessive amounts they will be toxic [2]. On the other hand, their environment sometimes also contains non-essential metallic elements, such as Al, which are normally abundant in the soils with lower pH or even heavy metals such as Pb, Cd, Hg, and Cr on post-mining lands as well as contaminated lands from industrial waste [3, 4]. The existence of these elements causes plants to experience stress, which consequently inhibits the growth of the roots and canopy and can even cause death.

Metal stress occurs due to the absorption of metal elements that exceeds the required concentration threshold which in turn leads to toxicity. For non-essential metallic elements such as Pb, Cd, Cr, and Hg, even at low concentrations, if they are absorbed by plants, they can be toxic for them. The toxic effects of these elements include decreased photosynthesis rate, cell division inhibition, free radical formation, or the inhibition of water absorption rate, which finally cause root growth and plant canopy to be strongly inhibited [5, 6]. Growth is the most easily recognizable morphological parameter of plants undergoing metal stress, where root growth is commonly the most affected. Furthermore, slow growth will result in low crop production if it occurs in cultivated plants.

Some plant species may become resilient to those conditions which allow them to live in environments with higher levels of metals. Some plant species are even able to absorb large amounts of metals in their body that are known as hyper-accumulators such as in *Alyssum bertolonii* and *Berkheya coddii* [7, 8] and *Camellia sinensis* [9]. There are several mechanisms that allow plants to keep growing well in environments with high metal content, including: (a) plants having the ability to keep metal ions from entering into cells, (b) plants having the ability to absorb metals in high concentrations and allocating them certain tissues/organs, and (c) plants having mechanisms that allow metals to be detoxified so that they do not to disrupt plant growth. There are several evidences to show that metal toxicity have a direct effect on growth inhibition of many species, either in roots or in shoots, but the detailed discussion on this response, especially on the perspective of cellular growth, is still rarely found. This will discuss the general feature of growth inhibition of roots in response to metal toxicity and the tentative mechanisms of tolerant plants which are able to sustain their growth under higher metal concentration. This chapter is prepared to present the simple and holistic concept of plant response to metal stress especially in the context of plant growth extracted from newer references and advance researches. The scope is restricted in growth because the initial stage that can be recognized is the inhibition of growth, especially root growth, followed by other morphological and physiological parameters depending on the tolerance level of the plants.

## 2. Metal source and contaminants in nature

In nature, the abundance of metal elements comes from several sources: (a) from natural parent rocks [10], (b) environmental conditions that influence metal elements to dissolve and cause toxicity to plants such as flooded lands with lower pH [3], and (c) anthropogenic factors, derived from human activities such as mining, industry, and intensive farming activities. Some areas of the Earth have high metal content [11, 12]; one example is the ultramafic bedrock

in Sulawesi, Indonesia, which contains magnesium, iron, and nickel in high quantities [13]. Such soils usually have extreme characteristics because the macronutrient content such as nitrogen, phosphorus, potassium, and calcium is very low while the micronutrient content such as nickel is so high that it is difficult for plants to grow well because of toxicity [14].

Environmental conditions may have set up the abundance of metal elements due to acidified soil. Acid sulfate soil is an example of this which is characterized by an excess of potentially acidic pyritic material over acid-neutralizing free carbonate, adsorbed base, and easily weatherable minerals [15], which cause the accumulation of  $H^+$ ,  $Al^{3+}$ ,  $Fe^{2+}$ , and organic acid that are toxic to plants [16].

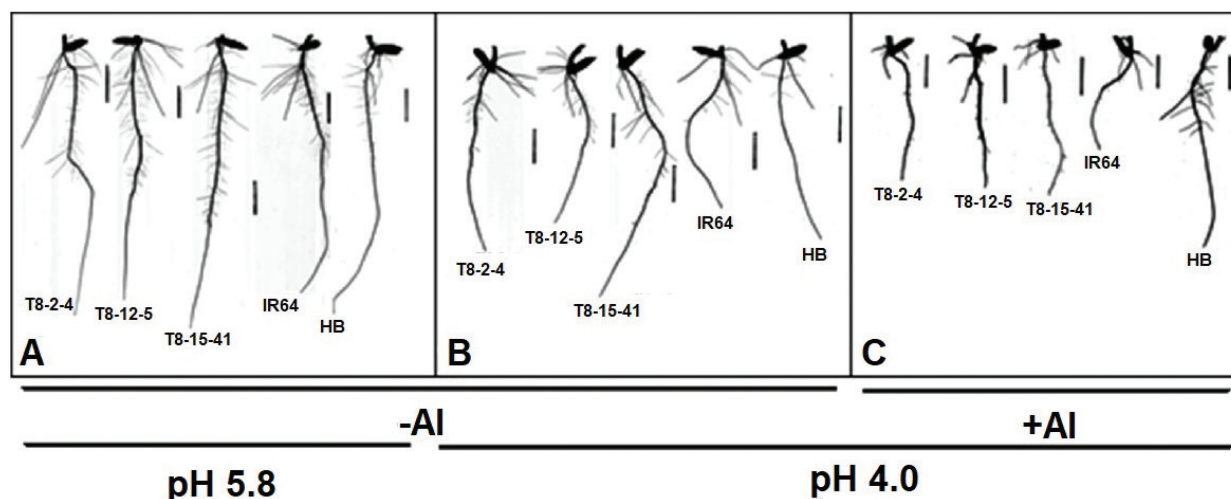
Human activities have influenced the dispersion of metal elements including heavy metals such as Pb, Cd, Ag, Hg, and Cr due to several activities including traditional and mining activities, and intensive agricultural practices such as pesticide and fungicide applications have increased the contamination of metal elements [17–19]. Therefore, heavy metals, especially, have been addressed as critical substances concerning human health and environmental issues due to their high occurrence as contaminants, low solubility in biota, and some heavy metals also have been classified as having carcinogenic and mutagenic effects [20, 21].

Based on plant requirements, metal elements are divided into two groups, essential and non-essential metal elements. Some metal elements such as copper, iron, zinc, manganese, molybdenum, and nickel have important roles in a wide range of physiological processes in plant organs, especially for enzyme activities, which are also known as essential micronutrients or trace element [6]. However, at higher concentrations, they can also be toxic to the plants [22, 23]. Another group of metals such as chromium, arsenic, cadmium, mercury, and lead are non-essential and potentially very toxic to the plants even under lower concentrations [22]. Metal toxicity can inhibit photosynthesis and water absorption, disturb carbohydrate metabolism, and initiate the secondary stresses such as oxidative stress, which influences plant growth and development [24].

### 3. Growth inhibition due to metal stress

Plant growth is among the morphological characteristics, which is normally inhibited by metal stress, and root growth is the most affected, and therefore root growth sometimes becomes an important parameter to analyze plants tolerant to metal stress [25]. The inhibition of roots in several species in response to metal stress has been reported by many authors, species such as rice [26, 27], soybean, sorghum [28], and wheat [29] in higher aluminum concentrations; Brassica species [30] and soybean [31] in Zn toxicity; as well as tea plants [32] and tomato-sensitive as well as tolerant genotypes [33] in cadmium toxicity.

In *Vigna unguiculata*, Al exposure caused great root inhibition even only 5 h after treatment, even though after 18 h the growth recovered with a higher rate for tolerant genotypes while it was lower in sensitive genotypes [34]. **Figure 1** also shows an example of root inhibition in sensitive, transgenic, and tolerant rice in response to aluminum exposure of 15 ppm at lower pH [27]. Lower pH (4.5) decreased the root length of tolerant (HB), transgenic (TS34, TS13-5,



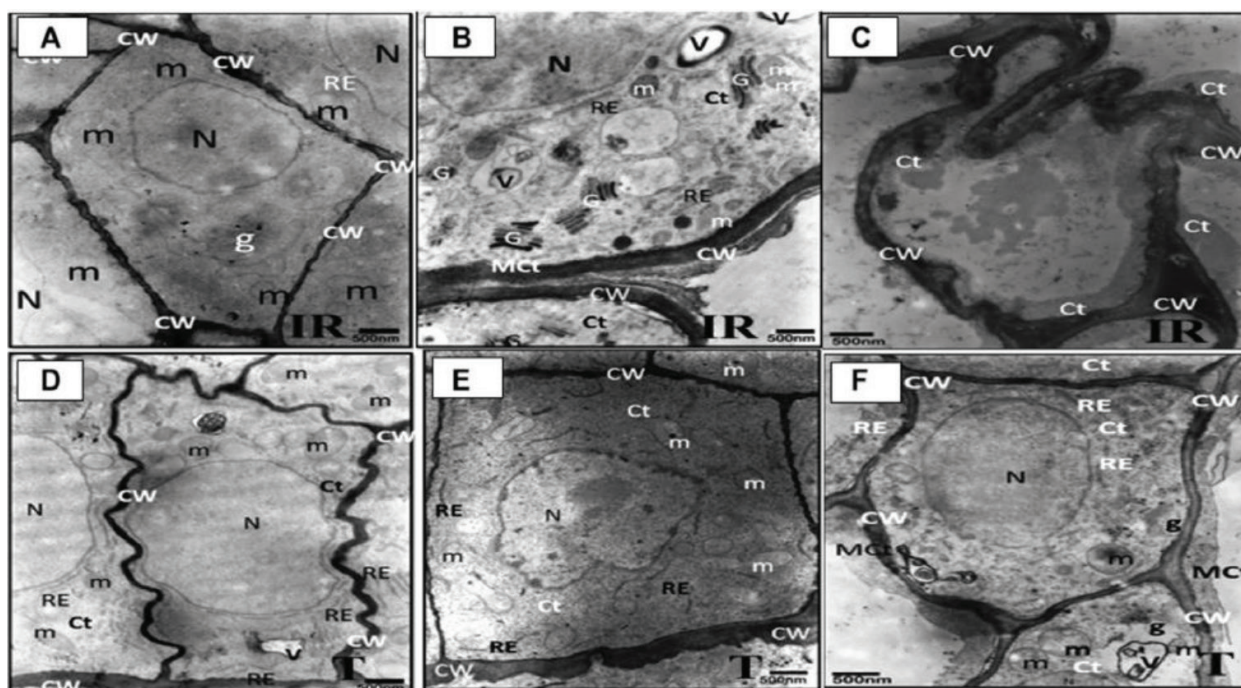
**Figure 1.** Root growth responses of five rice genotypes to low pH and 15 ppm Al stress. Rice seedlings were grown on nutrient solution at (A) pH 5.8, (B) pH 4.0, and (C) pH 4.0 + 15 ppm of Al. +Al = 15 ppm Al; -Al = 0 ppm Al (control); HB = Al-tolerant rice; IR64 = Al-sensitive rice; T8-2-4, T8-12-5, and T8-15-41 = T4 generations of transgenic lines of IR64. Bar = 1000 mm (After [27]).

TS 15-41) and sensitive rice (IR64) altogether, but Al treatment at 15 ppm caused root inhibition more severe with sensitive variety (IR64) had the lowest root length (**Figure 1**).

At the tissue level, metal toxicity may cause damage to certain tissues such as epidermis, cortex, as well as vascular tissues. The damage of epidermis and cortex tissues was observed when rice seedlings were treated with a high concentration of cadmium [35]. A greater number of nucleoli and vacuoles and enlarged vacuoles were observed in transgenic cotton cultivars exposed to cadmium [36].

At cellular level, metal toxicity has a direct as well as indirect effect on plant physiology and biochemical mechanisms which result in growth inhibition. The direct effect of metal toxicity can be categorized as membrane damage, the alteration of enzyme activity, and the inhibition of root growth, while the indirect effect of metal toxicity can be the disturbance of hormone balance, the deficiency of essential nutrients, the inhibition of photosynthesis, changes in photo-assimilate translocation, the alteration of water relations, and so on, which further enhance metal-induced growth reduction [22]. Therefore, root growth inhibition is sometimes followed by damage to root cells that can be observed from cellular ultrastructure as shown in **Figure 2**. Aluminum-sensitive plant roots treated with a concentration of 15 ppm experienced ultrastructural damage and the cells underwent plasmolysis and had irregular shapes, while the transgenic plant cell structure was still intact with a normal tetrahedron shape (**Figure 2**).

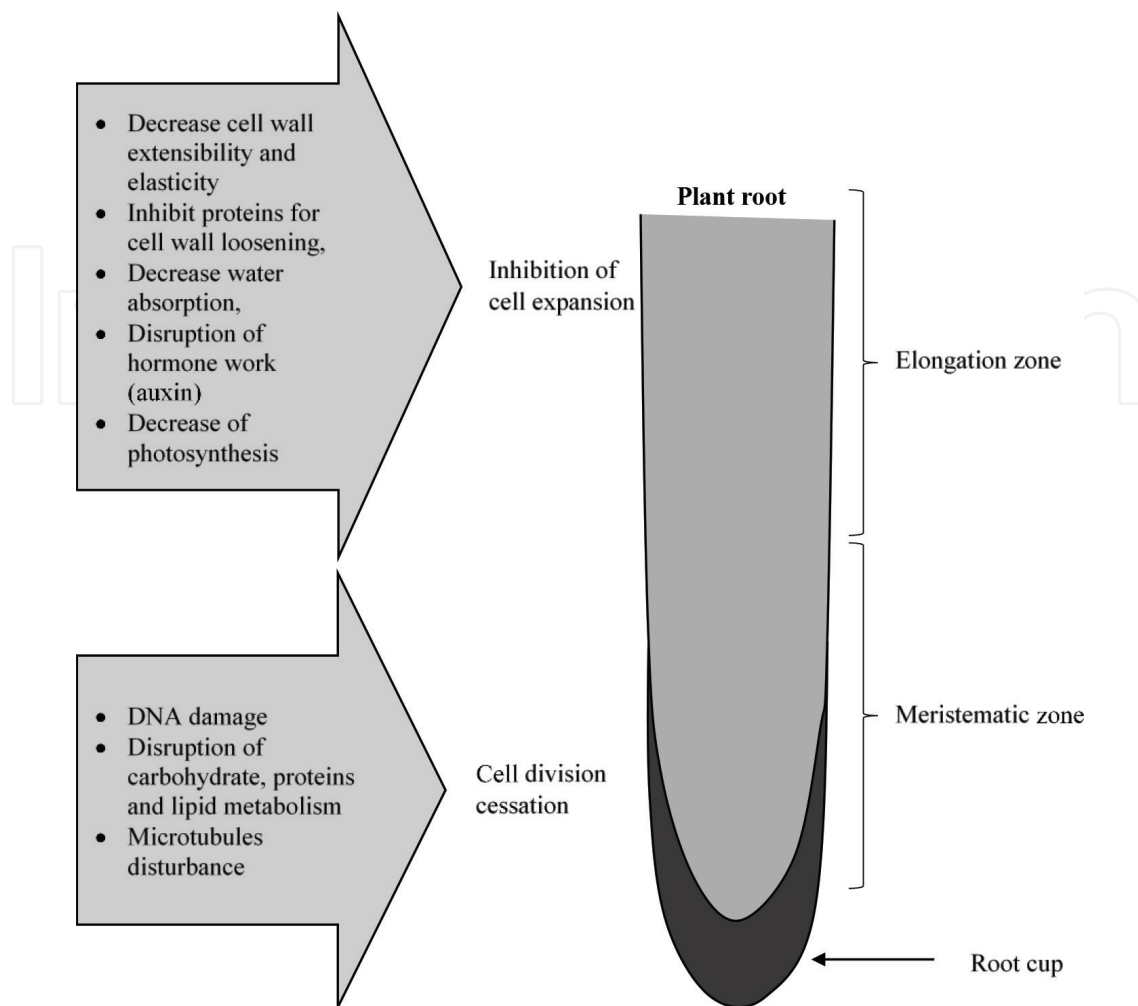
Growth restrictions, especially in roots of plants that undergo heavy metal stress, are caused by two fundamental reasons: (a) inhibition of cell division and (b) decrease of cell expansion (**Figure 3**). During the process of growth, cell division in meristematic tissues is an initial stage that must go, by which if cell division is disturbed, the growth will slow down. Higher cellular activity in the meristematic region of the root tip is a key factor that may be disrupted by abiotic stress including metal stress. The inhibition of cell division or cessation of mitosis due to metal stress has been documented in many species, such as cowpea plant (*Vigna*



**Figure 2.** Root tip cell structure after treated with and without 15 ppm Al treatment for 72 h using TEM. (A and D) control treatment without Al pH 5.8; (B and E) control treatment without Al pH 4.0; and (C and F) treatments with 15 ppm Al pH 4.0. Ct = cytoplasm; Cw = cell wall; G = golgi apparatus; IR = IR64; M = mitochondria; MCt = membrane of cytoplasm; N = nucleus; RE = reticulum of endoplasm; T = transgenic rice; TEM = transmission electron microscope; V = vacuole. Magnification 10,000 $\times$ . Bar = 500 nm (After [27]).

*unguicalata*) exposed to Al stress [34], *Zea mays*, and *Lemna minor* exposed to Pb [37, 38]. The disorder of cell division often occurs when the basic material for the formation of new cells such as carbohydrates, lipids, and nucleic acids (DNA) is disrupted. Damage to proteins and DNA is one of the effects of metal stress that occurs in many plant species such as in *Urtica dioica* [39]. In addition, some heavy metal such as Pb has caused microtubule disruption in *Zea mays* which caused mitosis inhibition [38].

In addition to cell division, the capacity of plant growth is also determined by cell enlargement and expansion. Cell expansion is an important aspect of cellular growth. During cell expansion, cell wall stress relaxation occurs and results in a decrease in cell water potential and turgor pressure, creating the necessary water potential gradient for water uptake and the irreversible process of cell wall expansion [40]. The process of cell expansion involves important aspects including cell wall loosening or wall stress relaxation, followed by the absorption of water by cells which enlarge and stretch the cells [41, 42]. Therefore, the decrease of cell expansion is mostly triggered by several factors: (1) decrease in cell wall extensibility and elasticity, (2) inhibiting proteins that work in cell wall loosening, (3) decreasing water absorption, (4) the disruption of hormone work, especially auxin which plays an important role in the growth processes, and (5) the decrease of photosynthesis. Wolf et al. [43] suggested that environmental stresses such as salt, heavy metals, osmotic stresses, microbial enzymes, or mechanical injury can threaten the integrity of the rearranging carbohydrate and glycoprotein networks. There are a lot of papers that have explained that some metals including Al are



**Figure 3.** Effect of metal toxicity on roots cell growth involving multifaceted physiological inhibition and disruption including inhibition of cell division in meristematic tissues and inhibition of cell expansion. Cell division cessation may be caused by DNA damage, disruption of carbohydrate, protein and lipid metabolism, and microtubule disturbance. Inhibition of cell expansion could be caused by decrease of cell wall extensibility, inhibition of proteins that work in cell loosening, decrease water absorption, disruption of hormone work and decrease of photosynthesis.

bound to the cell wall such as in algal cells like *Chara coralline* [44], okra hypocotyl [45], and tobacco cells [46], which in turn caused decreased cell wall extensibility and consequently root growth inhibition [45, 47].

Cell wall loosening is a direct cause and an initial part of cell wall expansion which subsequently results in cell growth [48]. Cell wall loosening during cell expansion also involves a group of proteins known as expansins which catalyze the pH-dependent extension and stress relaxation of cell wall [6]. Under normal conditions the decrease of pH in the cell wall will initiate cell wall loosening and cell relaxation. Expansins have the ability to non-enzymatically trigger a pH-dependent relaxation of the cell wall, which loosens and softens it, thus enabling cell expansion. This group of proteins is required in almost all plant physiological developmental aspects, from germination to fruiting, by reducing adhesion between adjacent wall polysaccharides [48]. Some experiments indicated that metal stress caused the inhibition of this group of proteins significantly [49]. In broad beans, some expansin family was also inhibited by Cu and Cd toxicity [50].

Decreased water absorption is one of general effects of metal toxicity, especially generated by heavy metal stresses such as Cd and Hg [51]. The interference with water absorption is partly due to the inactivation of water channel proteins by heavy metals [25]. In addition, the decrease in water potential was probably due to decreased cell wall extensibility or elasticity by cross-linking the pectin carboxyl groups in the walls with heavy metals [22]. In addition to the interference with the absorption of water, metal stress is also suspected to cause the hampering of plant hormones, especially auxins [52]. Although indirect, the decline of photosynthesis also affects cell enlargement, considering that this process will produce the needed materials to form new cell walls. In this phase, photosynthesis also has an important role, so the decline of the photosynthetic rate will result directly in the occurrence of cell division barriers. Data suggest that metal stress results in a decrease in photosynthesis rates such as Cd and Cr [51] and excessive Cu [53, 54].

#### **4. Physiological responses and oxidative stress induced by metal toxicity**

In response to metal toxicity, there are several physiological mechanisms exhibited by plants involving biochemical processes as well as cellular and ultrastructural changes (**Table 1**). These mechanisms may be species specific and are associated with its characteristics and tolerance levels to metal toxicity, which comprise two basic mechanisms: (1) retaining metal elements out of cellular cytoplasm through cell wall component binding or active transport excluding the cell and (2) detoxification of metals using chemical compounds such as phytochelatin and metallothioneins and accumulating them in vacuoles (**Figure 4**), which are also known as avoidance and tolerance types [55].

To keep metal elements out of the cytoplasm, cell wall has an important role, because cell wall is a complex structure composed of cellulose microfibrils and non-cellulosic neutral polysaccharides embedded in a physiologically active pectin matrix, cross-linked with structural proteins and sometimes with lignin [56]. The ability of the cell wall to bind divalent metal cations depends on the number of functional groups such as  $-\text{COOH}$ ,  $-\text{OH}$ , and  $-\text{SH}$  occurring in cell wall compounds containing cellulose, hemicellulose, and pectin, which are able to bind metal elements [57, 58]. In higher plants, the most significant role is especially determined by polysaccharides abundant in the carboxyl group homogalacturonans (HGA) [59, 60]. In addition to polysaccharide compounds, other compounds such as proteins, amino acids, and phenolics also take part in metal element binding [55].

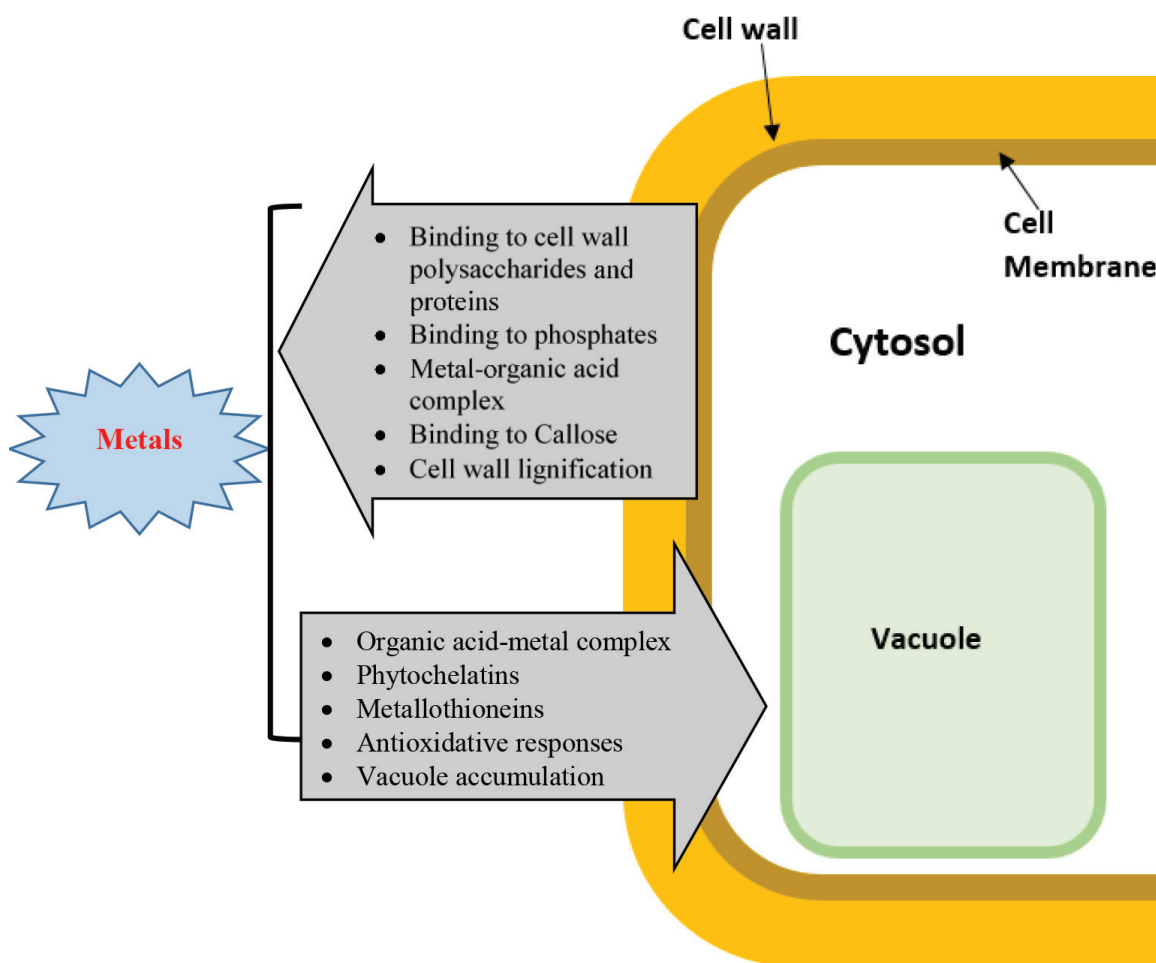
Accumulation and secretion of organic acids was observed in many species exposed to metal stress, especially Al, Cd, and Pb [9, 61–64]. This organic acid accumulation is associated with the inhibition and avoidance of metals from entering the metabolic-active cellular part through forming metal–organic acid complexes in the cytosol or at the root-soil interface [9]. Cell wall thickening and lignification are also important histological responses of the plants to avoid metal toxicity [35, 42, 63, 65].

It has been well known that plants exposed to heavy metal stresses undergo oxidative stress specified by producing higher free radicals [82–84]. At the cellular level, the generation of reactive



Metal elements	Plant species	Physiological responses	References
Al	<i>Camellia sinensis</i>	Malate secretion	[9]
	<i>Triticum aestivum</i>	Citrate secretion	[61]
	<i>Phaseolus vulgaris</i>	Citrate secretion	[66]
	<i>Zea mays</i>	Citrate secretion	[67]
	<i>Glycine max</i>	Oxalate secretion	[68]
	<i>Colocasia esculenta</i>	Oxalate and citrate	[62]
	<i>Brassica napus</i>	Oxalate and citrate	[69]
	<i>Avena sativa</i>	Oxalate and citrate	[70]
	<i>Raphanus sativus</i> Secale cereale	Oxalate and citrate	[71]
	<i>Fagopyrum tataricum</i>	Lower pectin in cell wall	[72]
	Pea ( <i>Pisum sativum</i> )	Lower pectin in cell wall of tolerant cultivar	[73]
	Rice	$\alpha$ -expansins involved in the root cell wall loosening	[49]
	<i>Medicago sativa</i>	exogenous IAA improve tolerance	[2]
	Cd	Rice	Cell wall thickening
Cotton		Greater number of nucleoli and vacuoles and enlarged vacuoles	[36]
Maize		Lignin accumulation and the role of apoplastic collenchyma and phloem lignification for metal new bound site	[64]
<i>Brassica napus</i>		Induced phytochelatin and glutathione	[74]
Tomatoes genotypes		Induced proline and antioxidant enzymes (APX, GR, CAT)	[75]
<i>Avena strigosa</i>		Induced antioxidant enzymes and phytochelatin	[76]
White lupin		Induced Phytochelatin	[77]
<i>Sedum alfredii</i>		Induced Phytochelatin	[78]
Cd and As	Rice	Disturb IAA biosynthesis	[52]
		Alter the lateral root primordia	
Cd and As	<i>Pteris vittata</i> (fern)	Metabolite deposition in intercellular space	[63]
		Induced GSH and phytochelatin (Pcs)	
		Cell wall thickening in epidermis and Increase cuticle	
Cu and Cd	<i>Broad bean</i>	Inhibition of a phytochelatin synthase and/or a member of the $\alpha$ -expansin family	[50]
Fe	Wheat	Regulation of phytosiderophore and induction of antioxidative enzymes (CAT, POD, GR) and elevated glutathione, cysteine, and proline.	[79]
Hg	Maize	Induced lipid peroxidation and proline content	[80]
Pb	<i>Dianthus carthusianorum</i>	The development and role of pericyclic tissues	[81]
Pb	<i>Paraserianthes falcataria</i>	Citrate secretion	[64]

**Table 1.** Physiological and ultrastructural changes in response to metal toxicity.



**Figure 4.** The role of root cells to mitigate metal toxicity involving (1) cell wall barriers such as polysaccharides and proteins binding sites, phosphate binding sites, callose development and cell wall lignification to prevent metals enter to the cells; and (2) cellular resistance mechanism including metal efflux assisted by ATPase-based transporter, phytochelatines, metallothioneins, enzymatic as well as non-enzymatic antioxidant mechanism and accumulation in vacuole.

oxygen species (ROS) which includes superoxide anion ( $O_2^-$ ), hydroxyl radical ( $^*OH$ ), alkoxy ( $RO^*$ ), peroxy ( $ROO^*$ ), hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), and so on due to metal stress results in oxidative damages to lipids, proteins, and fatty acids which disrupt biomembrane, ultrastructural cellular components, DNA, and causes programmed cell death [85, 86].

Oxidative damage is among the cause of growth inhibition of roots as well as shoots. These reactive oxygen species (ROS) react with lipids, proteins, pigments, and nucleic acids which led to the occurrence of lipid peroxidation, membrane damage, and inactivation of enzymes, thus destroying cell viability [32]. Lipid peroxidation is the general indicator of oxidative stress which is recognized by the accumulation of malondialdehyde (MDA) in the cells or tissues when the plants are under stress [87], and MDA content is often used as an indicator for the extent of oxidative stress [88, 89]. Some experiments showed that cadmium exposure caused gradual the increase of MDA and  $H_2O_2$  content in the leaves as well as roots of resistant as well as sensitive tomatoes [33]. In *Camellia sinensis*, the application of cadmium up to 400  $\mu M$  caused a linear increase of MDA content, while it caused a significant decrease of chlorophyll and protein content [32]. The significant increase of MDA content was also observed in sensitive rice IR64 treated with 15 mM of Al, while the increase was moderate in tolerant varieties [27].

The plants have specific mechanisms to overcome oxidative stress which in general involves (a) antioxidant enzyme activities and (b) non-enzymatic antioxidant processes. Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), glutathione peroxidase (GPX), and dehydroascorbate reductase (DHAR) are among the enzymes that have important roles in cellular scavenging from ROS [82, 90–92]. In *Camellia sinensis*, for example, transcription levels of glutathione reductase (GR), an enzyme involved in the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), showed up-regulation on cadmium exposure [32].

In addition to antioxidant enzymes, to deal with the oxidative stress caused by metal toxicity, the plants sometimes accumulate some non-enzymatic antioxidant compounds such as organic acids, glutathione, tocopherol, phytochelatin, metallothionein, and non-protein thiol [9, 51, 63, 77, 78]. These compounds are important in protecting the cells from the damage caused by heavy metal stress so that plants that have the ability to accumulate such compounds are tolerant to heavy metal stresses [82, 93]. The indication of oxidative stress induced by heavy metals was also demonstrated by the application of several agents such as ascorbic acid, oxalic acid, citric acid, and malic acid [9]. Using Al-sensitive wheat (cv. Scout 66), Ma et al. [29] showed that Al exposure at 10  $\mu\text{M}$  caused a substantial decrease of the roots' elongation of wheat. However, the application of malate, oxalate, and citrate gradually recovered the inhibition of Al to the root elongation as compared to the control, without organic acid application, even though the most effective treatment was using citric acid [29]. Data show that organic acid has an important role in metal toxicity especially Al with different specificities among plant species. Organic acid accumulation including oxalic acid, malic acid, citric acid and glycolic acid was also observed in tea plants treated by high concentrations of aluminum until 2 mM, even though they were decreased when the plant was treated with 4 mM of Al [9].

Glutathione (GSH) is also an antioxidant compound that is known to alleviate the plant from environmental stress, including metal toxicity [51, 94]. GSH is very important because it involves cell protection from free radicals generated from heavy metal toxicity including  $\text{H}_2\text{O}_2$ . In many species, the increase of GSH concentration in the cell has been observed in response to heavy metal treatments, since this compound is known as the precursor of phytochelatin (PC), a typical metal chelator found in plants that facilitates metal sequestration into vacuoles [95], and this has been believed to be part of heavy metal tolerance [96]. Interestingly, the exogenous application of glutathione was also able to alleviate the toxic effect of metal stress especially from Hg toxicity [93]. He explained that the exogenous glutathione application effectively prevented mercury absorption by roots and improved plant tolerance to mercury toxicity by significantly decreased  $\text{H}_2\text{O}_2$  and  $\text{O}_2^-$  levels and lipid peroxidation, while it improves the chlorophyll content of *Arabidopsis thaliana*, tobacco, and pepper in the presence of Hg. He also suggested that GSH is a potent molecule capable of conferring Hg tolerance by inhibiting Hg accumulation in plants [93].

Interestingly, the exogenous application of  $\text{H}_2\text{O}_2$  on *Brassica napus* was able to reduce oxidative stress induced by cadmium application indicated by the decrease of MDA and  $\text{H}_2\text{O}_2$  accumulation in the plant and the increase of antioxidant enzyme activities, such as APX, DHAR,

catalase, GR, and GST as well as ascorbate and glutathione content significantly [97]. In this regard, H<sub>2</sub>O<sub>2</sub> may become an important substance required to induce antioxidant enzyme activities in the plants when the plant undergoes stress.

## 5. Accumulator plants are resistant to metal toxicity

Although heavy metals cause plant toxicity, there are some groups of plants that have the ability to accumulate large quantities of metal elements which are known as accumulator plants. These plants are not only able to grow in the area with high metal concentrations but also even able to grow better under high metal contents, even though some plants have slower growth rate. Tea plants (*Camellia sinensis*), for example, have the ability to accumulate aluminum in higher amounts. In his experiments Li et al. [9] showed the growth of *C. sinensis* plant on the medium with Al content ranging from 0, 0.1, 0.4, 2, to 4 mM for 4 weeks, and the best growth was shown by plants treated with Al 0.4 mM. He also showed that even when the plants received Al treatment up to 2 M concentrations they had better growth than control plants [9]. This shows that *C. sinensis* has a high tolerance to Al. Several plant species such as *Alyssum bertolonii*, *Brassica juncea*, *Eichhornia crassipes*, and *Iberis intermedia* have been recognized to accumulate metals in higher concentrations and therefore have been considered to be used in the phytomining of Ni, Co, Tl, Ag, and Au [7, 8, 98, 99]. In an ultramafic area in Tuscany, Italy, *Alyssum bertolonii* was able to extract nickel till 0.7% of its dry weight [7], a very high value of metal component that was there in the plant. Another species *Brassica juncea* was also grown using similar methods that accumulated Au up to 57 mg/kg dry mass [99]. Therefore these plants are categorized as hyper-accumulator plants.

Plants may have ultrastructure modification in shoots as well as root cells in response to metal stress to anticipate the binding or deposition of the metal element when they enter into the cell of accumulator plants. Krzesłowska [55], for example, presented the TEM ultrastructure analysis of poplar root protonema apical cell exposed to lead of 32 μM, and she found that in the cell wall there were extremely large crystalline-like deposits of Pb which thickened the cell wall. She also found internalization of Pb deposits together with pectin in the protonema apical of *Funaria hygrometrica* exposed to 1000 μM of lead.

In maize leaves, the increase of the transversal area occupied by collenchyma in the foliar nervure as well as of the cell wall lignification was pronounced in response to cadmium treatment in combination with lime, even though collenchyma's lignification was not found in the treatment without lime [64]. Another example is cotton, where ultrastructure analysis found cadmium in the form of crystals and electron-dense granules both in the vacuoles and attached to the cell walls, which reveals that the sequestration of cadmium was possibly facilitated by binding with the non-functional parts of the cell, and the increase in number and size of vacuoles and greater number of nucleoli might be important characters of tolerant genotypes to cadmium toxicity in cotton plants [36]. Data show that the accumulation of metals for accumulator or even hyper-accumulator plants may be facilitated by both the capacity of the

cell wall to bind particular metals and the ability to detoxify and have a safer metal-transport mechanism to cell vacuole or other non-active organs. This response may be supported by the dynamic modification of physiological, anatomical, and even ultrastructural changes which allow the plant to sustainably grow under metal stress.

## 6. Conclusions

Metal toxicity is one of the conditions plants face in the growing environment. Essential trace elements such as Cu, Zn, Fe, and Mn are important to support metabolic processes in the plant, but under high concentrations, they can result in metal toxicity. The presence of non-essential metals such as Al, Pb, Cd, Cr, and Hg in plant media is very toxic to plants even at lower concentrations. Common responses of plants to metal poisoning are the inhibition of growth, chlorosis and necrosis at the leaves, decreased photosynthesis, and even death. The plants have mechanisms to avoid metal toxicity which can be divided into two processes: (1) by avoiding metal elements entering into the cell involving metal-cell wall binding or preventing metal insertion by the chelation mechanism facilitated by organic acid or active exclusion pump and (2) by producing compounds that are able to neutralize the damage when the metal element enter the cell through phytochelatine or metallothionein compounds as well as antioxidant mechanisms before being deposited into vacuole. Ultrastructure changes and cell wall thickening and lignin formation are among the cellular responses that have been observed in many species, while the other phenomena including the increase in the number and size of vacuoles and vesicles inside the cells containing crystalloid-metal elements were also detected.

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## Conflict of interest

We declare that they have no conflict of interest.

## Nomenclature

MDA	Malondialdehyde
ROS	Reactive oxygen species

CAT	Catalase enzyme
GR	Glutathione reductase
POD	Peroxidases enzyme

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