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Chapter

Copper Toxicity in Plants: Nutritional, Physiological, and Biochemical Aspects

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Abstract

Copper (Cu) is an essential micronutrient for plants because it participates in several redox reactions and the structural constitution of the Fe–Cu cluster. Although it is required in small concentrations at toxic levels, Cu triggers physiological and biochemical disorders that reduce plant growth. In higher plants, the normal range of Cu concentration is in the range of 2–20 mg Cu kg⁻¹ DW. Above the upper limit of this range, Cu toxicity may occur if the plants are not tolerant to the stress caused by toxic levels of Cu. In view of the growing agricultural and industrial activity that are the main sources of Cu addition in nature, understanding the physiological and biochemical mechanisms of Cu toxicity in plants constitutes an important tool for the selection of more tolerant genotypes based on biochemical and physiological indicators to heavy metal stresses. In this chapter, we propose a systematic review of plants grown under toxic levels of Cu, based on the responses of physiological, biochemical, and nutritional variables. Understanding these responses will contribute to improving the understanding of the basic mechanisms of stress tolerance by toxic levels of Cu in higher plants, providing valuable information for the improvement of genotypes resistant to toxic levels of Cu in the plant culture medium.

Keywords: nutritional disorder, gas exchange, micronutrient, plant growth

1. Introduction

The micronutrient copper (Cu) is a transition metal with atomic number 29, an atomic mass of 63.5 g mol⁻¹, and a density of 8.96 g cm⁻³. It is the 25th most abundant chemical component in the Earth's crust and the third most used worldwide [1].

Cu occurs naturally in soils with contents ranging from 60 to 125 mg kg⁻¹ [2]. It is an essential micronutrient for plant development and, under physiological conditions, it exists in the form Cu⁺ and Cu²⁺. Cu acts as a structural element in regulatory proteins and participates in the electron transport chain of photosynthesis and

respiration, oxidative metabolism, cell wall metabolism, and hormonal signaling [3, 4]. Among the proteins, ascorbate oxidase, Zn/Cu superoxide dismutase, and Cu amino oxidase are those that have more than one Cu atom in their structures (8, 2, and 2 Cu atoms, respectively) [5].

Cu is absorbed in the form of Cu^{2+} or Cu chelate and, despite being poorly mobile in plants, it can be translocated from old leaves to new leaves. Its concentration in the dry mass of plants is small and generally ranges from 2 to 20 mg kg^{-1} . However, concentrations in the dry mass of plants varying between 20 and 100 mg kg^{-1} are toxic to most plants [6].

Soil contamination by Cu is mainly caused by human action represented by industrial, mining, and agricultural activities. Intensive use of Cu-containing agrochemicals or swine manure is the main source of Cu entry into the agricultural soils [7, 8]. This scenario is worrying because the world population is expected to reach nine billion inhabitants in 2050 [9], which suggests an increase in the area of agricultural crops to meet the world demand for plant and animal foods with the consequent increase in the consumption of Cu-containing agrochemicals to phytosanitary purposes.

At toxic levels in the soil, Cu reduces the absorption of water and mineral nutrients [10], promotes oxidative stress [11] and affects photosynthesis [12], causing reduced growth [13] and plant production [14].

This chapter aims to address the impact of copper toxicity on plant growth, emphasizing key physiological, biochemical, and nutritional variables in studies of heavy metal toxicity in higher plants.

2. Copper absorption mechanism

Mineral nutrients are absorbed from the soil matrix by plants through the cells of the root epidermis and then transferred to its center through the parenchyma, endoderm, and xylem. This unidirectional pathway of transition-metal absorption is supported by different metal transporters that act in coordination with other metal transport molecules that sequester/chelate so that adequate absorption and transport of ions occurs in all plant tissues throughout the metagenesis of plants [15].

Cu absorption occurs through three types of transporters present in the plasma membrane of root cells—P-type ATPase copper transports, COPT copper transports, ZIP family transports, and NRAMP family transports. P-type metal transporters (P-type ATPase copper transports) are responsible for the transmembrane transport of toxic metals, such as divalent cations (Cu^{2+} , Zn^{2+} , Cd^{2+} , and Pb^{2+}). These transporters use ATP to pump charged molecules across cell membranes [15, 16]. The family of proteins responsible for the transport of Cu in the reduced form is the COPT transport protein (copper transporter protein). These proteins are formed by five members, that is, COPT1, COPT2, COPT3, COPT4, and COPT5 [17]. The zinc–iron-regulated transporter-like protein (ZIP) are involved in the absorption of Cu ions. Depending on the concentration of Cu in the plant-growth medium, ZIP2 or ZIP4 can act as a Cu carrier [18]. The natural resistance-associated macrophage protein (NRAMP) is responsible for the reallocation of ions, such as Fe, Ni, Mn, Zn, and Cu, from the root and shoot against cellular and vacuolar membranes [19].

After being absorbed by the roots, Cu can be transported in the xylem to the shoot in the form of Cu^+ and Cu^{2+} . But usually, the transport of Cu from the root to the shoot is done in the form of Cu^- complex. In plants, the xylem is the main source of

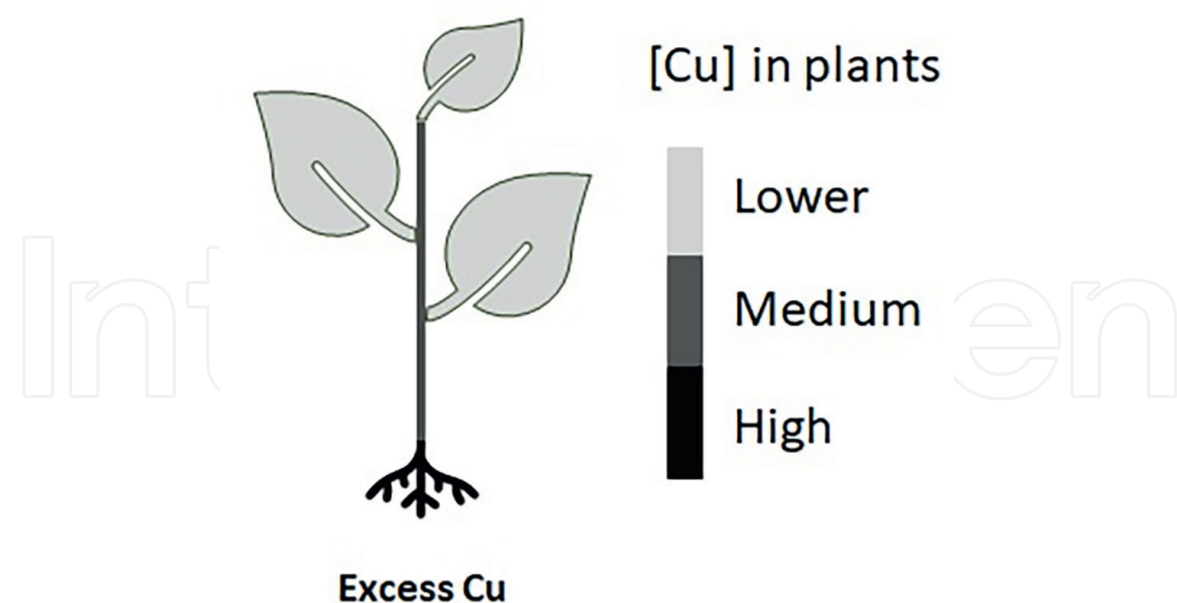


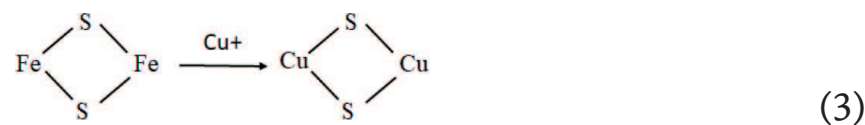
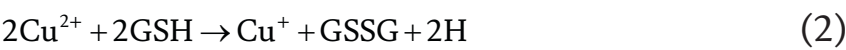
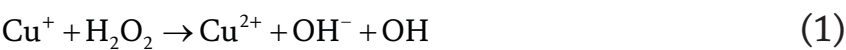
Figure 1.
Variation in Cu content in different plant structures. Because it is not very mobile in plants, Cu accumulates to a greater extent in the root, stem, and leaves.

Cu for the shoot [20, 21]. However, Cu is poorly mobile in plants, accumulating to a greater extent in the root system compared to the shoots of plants [3, 22] (**Figure 1**).

3. Effect of copper toxicity on antioxidant metabolism

Cu is an essential micronutrient for plants because it is a component of several enzymes that act in electron transport and catalysis of redox reactions in mitochondria and chloroplasts [3]. However, at toxic levels, Cu generates oxidative stress that damages cellular structures and molecules, such as DNA, proteins, and lipids. Cu has specific chemical characteristics that generate oxidative stress through the catalysis of oxidation–reduction reactions that form reactive oxygen species, such as singlet oxygen (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These reactive oxygen species promote lipid peroxidation [23].

The biochemical process of free radical formation triggered by Cu involves three mechanisms—participation in Fenton-type reactions (1), reduced glutathione depletion (2), and substitution of Fe in the Fe–S cluster (3) [24].



However, the production of free radicals can be potentiated by the Haber–Weiss reactions (4) and (5) in which superoxide and hydroxyl radicals are formed [25].



Plants under heavy metal stress conditions have enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase) and nonenzymatic (reduced glutathione and ascorbate) mechanisms that minimize the negative impact of oxidative stress triggered by free radicals.

Cu toxicity (100–500 μM Cu) increases the activity of important antioxidant enzymes, such as superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase [26, 27], because, in excess, Cu increases the production of O_2^- and H_2O_2 by modulating the Haber–Weiss reaction [25]. In addition, nonenzymatic mechanisms are involved in the attenuation of oxidative stress triggered by Cu toxicity, such as reduced glutathione (GSH) and ascorbate. GSH is a tripeptide widely distributed in plant tissues. It reacts with harmful oxidants to protect thiol groups of proteins [28, 29]. The concentration of GSH can be reduced in plants treated with toxic levels of Cu because it is used to neutralize free radicals directly or indirectly [28, 30].

Iron–sulfur (Fe–S) groups are versatile cofactors formed by inorganic iron and sulfide atoms because various metabolic pathways and proteins require Fe–S groups for proper functioning [31–33]. The main function of Fe–S-containing proteins is the transfer of electrons to produce redox potential in chloroplasts and mitochondria [34]. Under Cu toxicity conditions, iron (Fe) can be replaced by Cu in Fe–S groups, impairing the functioning of the electron transport chain in mitochondria and chloroplasts and, consequently, reducing plant growth [5].

4. Effect of copper toxicity on gas exchange

Cu is an essential micronutrient element in the transport of electrons between photosystems II and I because it enters the structural constitution of plastocyanin, an important component of the electron transport chain along photosystems [3–5]. However, under toxic levels of Cu, photosystem II is the most sensitive site of Cu action [5, 35]. The most apparent toxic effect of Cu is the inhibition of the oxygen evolution complex and the quenching of photochemical variables [36–38]. Cu^{2+} ions inhibit both the acceptor and the donor site of photosystem II. However, the oxidizing site of photosystem II is more sensitive to toxic Cu [39, 40].

Cu at toxic levels affects photosynthesis because it decreases Rubisco enzymatic activity and stomatal conductance, in addition to increasing the intercellular concentration of CO_2 in plants [12, 41, 42]. Therefore, Cu can affect photosynthesis due to stomatal (reduction in stomatal conductance) and non-stomatal factors (damage to Rubisco and the electron transport chain). For example, toxic levels of Cu (100–1000 mg L^{-1}) impose a stomatal limitation on photosynthesis because the decrease in photosynthetic activity and intercellular concentration of CO_2 occurs in response to the decrease in stomatal conductance caused by Cu [43, 44]. On the other hand, Cu toxicity can promote non-stomatal limitation of photosynthesis due to a decrease in photosynthesis and stomatal conductance in parallel with an increase in the intercellular concentration of CO_2 [12]. This suggests biochemical damage to enzymatic

components of photosynthesis, such as Rubisco. Toxic Cu (700 mg kg^{-1}) negatively affects photosynthesis, transpiration, stomatal conductance, and internal cell concentration in plants [45]. Toxic levels of Cu (800 mg kg^{-1}) cause a considerable reduction in photosynthesis and stomatal conductance, with deleterious effects on plant height and stem diameter [13].

5. Effect of copper toxicity on nutritional status

Depending on the concentration and plant species/genotype, heavy metals can induce toxicity that manifests itself through a decrease in chlorophyll concentration, reduced nitrate reductase activity, nutritional disorder, and, consequently, reduced plant growth [11, 46]. Cu at toxic levels promotes changes in root cell membrane permeability, expression of phosphorus membrane transporters (P), volume, and root area, which result in lower P absorption [47].

The negative impact of toxic levels of Cu on the mineral metabolism of plants may originate from morphological changes in the root system that decrease the surface for nutrient uptake. Thus, there is a close relationship between nutritional disorders and plant root growth. Toxic levels of Cu ($50 \text{ }\mu\text{M}$) reduce the diameter, length, area, and root biomass, coinciding with lower levels of P, Ca, Mg, Mn, and S in plants [47].

Toxic levels of Cu negatively affect the nitrogen metabolism of plants by decreasing the reduction of nitrate and its assimilation into organic compounds. The toxicity of $10.3 \text{ }\mu\text{M}$ reduces the activity of nitrate reductase in roots and leaves, contributing to a decrease in root nitrate content [48]. High concentrations of Cu ($5\text{--}20 \text{ }\mu\text{M}$) inhibit the activity of nitrate reductase and genes encoding the synthesis of low-affinity nitrate transporters (NRT.1), resulting in lower nitrogen uptake and accumulation in plants [42]. Cu ($20\text{--}100 \text{ }\mu\text{mol}$) considerably reduces the activity of nitrate reductase and the accumulation of nitrate in leaves and roots of seedlings, with negative repercussions on plant growth [49].

Regarding sulfur metabolism in plants, Cu toxicity ($5\text{--}10 \text{ }\mu\text{M}$) induced an increase in total sulfur and glucosinolate levels as a defense against Cu-induced stress (antioxidant role and/or Cu chelating agent) [50]. The concentration of thiols and the activity of the enzyme O-acetylserine (thiol) lyase are increased in the aerial part of plants in the presence of $20 \text{ }\mu\text{M}$ of Cu [51]. These changes suggest that Cu at toxic levels modulates changes in the concentration and activity of components of sulfur metabolism in plants to minimize the deleterious effects of Cu.

Despite the toxic effect of Cu, root architecture can be remodeled by plants. For example, the total density of lateral roots, the density of lateral roots less than 0.3 cm , and the density of lateral roots greater than 0.3 cm show an increase in their values when subjected to toxic levels of Cu ($10\text{--}75 \text{ }\mu\text{M}$). Despite this, root remodeling lacks molecular studies to highlight the mechanisms involved in this process. Lateral root elongation and primary root mitotic activity are inhibited in the toxicity range of $50\text{--}75 \text{ }\mu\text{M}$ Cu. These changes in the root system occur together with a reduction in the concentration of essential nutrients, such as P, K, Ca, Mn, and Fe, in the aerial part of plants [52].

Plant roots show a reduction in the concentration of essential nutrients (Ca, K, and F) and a decrease in root length and biomass in the presence of toxic levels of $4\text{--}80 \text{ }\mu\text{M}$ of Cu [53], suggesting that morphological changes in roots and shoots induced by Cu are linked to nutritional disorders in higher plants exposed in the medium and long term to toxic levels of Cu.

Specie	Root Cu concentration	Shoot Cu concentration	References
<i>Oryza sativa</i>	91–3380 $\mu\text{g g}^{-1}$ DM	175–508 $\mu\text{g g}^{-1}$ DW	[46]
<i>Solanum lycopersicum</i>	0.6–3.5 $\mu\text{g g}^{-1}$ DM	0.18–0.4 $\mu\text{g g}^{-1}$ DM	[11]
<i>Cucumis sativus</i>	89.91–5575.10 $\mu\text{g g}^{-1}$ DM	20.10–81.65 $\mu\text{g g}^{-1}$ DM	[47]
<i>Brassica pekinensis</i>	50–500 mg kg^{-1} DM	20–40 mg g^{-1} DM	[48]
<i>Swingle citrumelo</i>	20–270 mg kg^{-1} DM	4–7 mg kg^{-1} DM	[43]
<i>Zea mays</i>	—	0.15–0.25 $\mu\text{g g}^{-1}$ DM	[42]
<i>Amaranthus tricolor</i>	—	8.3–43.5 mg kg^{-1} DM	[28]
<i>Cannabis sativa</i>	10.0–470 $\mu\text{g g}^{-1}$ DM	5.0–20 $\mu\text{g g}^{-1}$ DM	[51]
<i>Arabidopsis thaliana</i>	14.7–205.3 mg kg^{-1} DM	7.0–10.6 mg kg^{-1} DM	[52]
<i>Spinacea oleracea</i>	33–4727 $\mu\text{g g}^{-1}$ DM	25–729 g^{-1} DM	[53]
<i>Citrus grandis</i>	8–31 $\mu\text{g g}^{-1}$ DM	17–690 $\mu\text{g g}^{-1}$ DM	[41]
Grapevine (“14/ rute/1”)	0.012–1372 mg g^{-1} DM	0.016–0.101 mg g^{-1} DM	[12]
<i>Spinacea oleracea</i>	50–1700 mg g^{-1} DM	nd*–40 mg g^{-1} DM	[13]

*Not detectable.

Table 1.
Ranges of Cu concentration in roots and shoots of plants grown under copper toxicity.

The Cu is poorly mobile in plants, with higher levels in the root, stem, and leaves, respectively. This pattern of Cu accumulation is evident in studies of Cu toxicity in plants, in which the highest ranges of Cu contents are observed in the root system followed by the shoot (Table 1).

6. Effect of copper toxicity on growth

Cu is an essential oligonutrient for plant growth, but it is lethal when it exceeds the permissible limit, leading to poor plant-growth performance with loss of production. Thus, the reduction in plant growth induced by heavy metals is a final consequence of changes that initially occur at the biochemical, physiological, and mineral levels of plants.

The physiological functions of essential plant nutrients are disturbed when the concentration of these nutrients is below an adequate limit due to the presence of toxic levels of Cu in the culture medium. Thus, the reduction in the concentration of Fe, Zn (in leaves), and Mg (roots) modulated by toxic levels of Cu coincides with the decrease in leaf area, root length, and SPAD index in plants. This suggests that Cu at toxic levels affects the physiological functions of these nutrients, consequently decreasing plant growth [54]. Natural populations of plants not tolerant to the toxicity of 100 μM of Cu (*Rumex japonicus*) have a reduction in shoot and root dry mass together with a decrease in P, Mg, and Fe contents in the root and P and Fe in the shoot [55]. Thus, disorders in the mineral metabolism of plants are accompanied by a reduction in plant growth and/or production when the plant culture medium has toxic levels of Cu.

In the context of net carbon assimilation, photosynthesis is a vital process, because it allows the carboxylation of CO₂ and the synthesis of phosphate trioses that will constitute the different structural and nonstructural components of plants. However, this process is affected under toxic concentrations of heavy metals due to damage to the photochemical and/or biochemical apparatus, culminating in reduced plant growth. Cu toxicity (700 mg kg⁻¹) has a negative impact on gas exchange (photosynthesis, transpiration, stomatal conductance, and internal cell concentration), chloroplast pigments (chlorophylls a and b, and carotenoids), and photochemical parameters (Fv/Fm, qP, and ETR). These changes promoted by Cu contribute decisively to the reduction of vegetative growth [45]. Furthermore, Cu has a specific action in inhibiting the oxygen evolution complex in photosystem II, which is associated with the oxidation of cytochrome b559 [5, 38]. In soils naturally rich in Cu (3050 g g⁻¹), height and seed production are strongly reduced under wheat-growing conditions. These results were accompanied by lower photosynthetic activity and lower concentration of chlorophylls [14]. All these disorders in the photochemical apparatus of photosynthesis have the ultimate effect of reducing shoot growth, roots, and plant production.

7. Morphological symptoms of copper toxicity in plants

Toxicity due to toxic levels of Cu manifests itself in the root system, which tends to lose its vigor with longer exposure to Cu, acquiring a dark color and thickening, culminating in reduced growth. In the aerial part of the plants, the morphological symptoms of Cu toxicity are evidenced by the chlorosis of the leaves and a marked reduction in growth (leaf area, height, and stem diameter). In advanced stages, leaf edges may become necrotic [10, 56]. **Figure 2** shows the general aspects of Cu toxicity

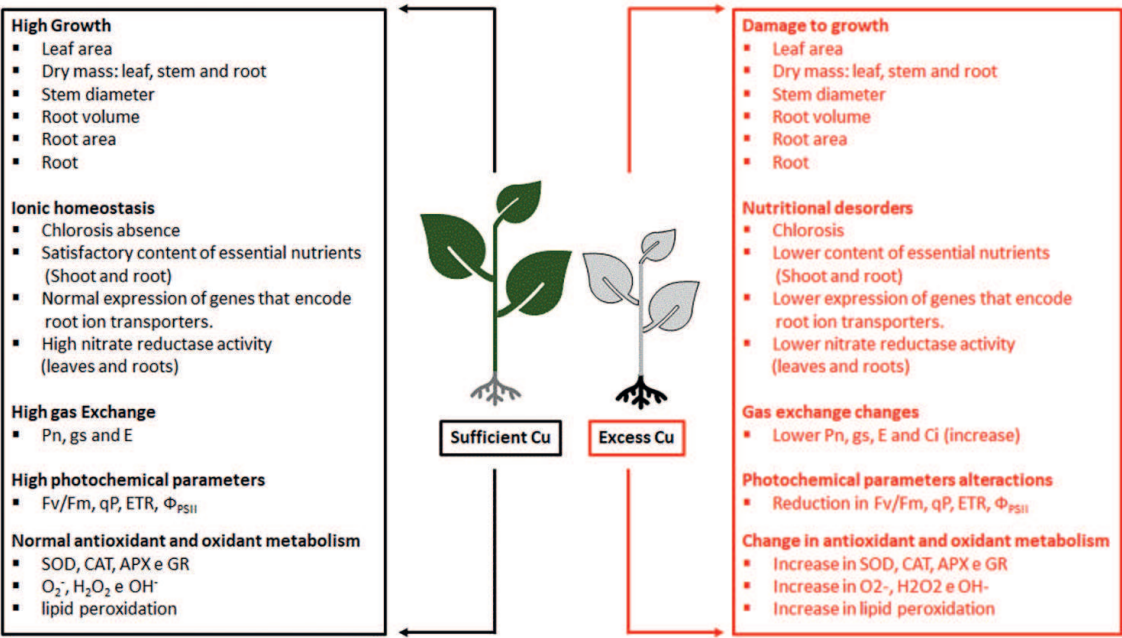


Figure 2. Overview of copper toxicity in plants at the level of gas exchange, oxidant-to-antioxidant metabolism, mineral metabolism, and growth.

in plants, which affects photosynthetic, antioxidant, and mineral metabolism, culminating in reduced plant growth.

8. Conclusion

Contamination of soil and plants with trace elements is one of the most severe ecological problems in many industrialized countries due to industrial, mining, and agricultural activities. However, important progress has been made in understanding the biochemical, physiological, nutritional, and morphological mechanisms of Cu toxicity associated with higher plants. Cu toxicity reduces plant growth because Cu at toxic levels acts as a prooxidant, increasing the production of free radicals that cause damage to cellular and subcellular structures, causing protein oxidation and lipid peroxidation. In another toxic mechanism, excess Cu can replace S in F-S groups, forming Fe-Cu, which affects electron transport in chloroplasts. In addition, Cu toxicity affects the oxygen evolution complex and cytochrome b559. These changes together imply a reduction in the synthesis of phosphate triose and, consequently, in the production of dry matter in plants. In the context of mineral metabolism, Cu has a strong impact on the reduction of P, Ca, and Fe nutrient concentrations. Despite the existence of numerous studies involving the toxicity of Cu, little is reported in the literature about the prooxidant role of Cu at the physiological and molecular levels. For example, plants accumulate most of Cu in the root, but there is a need for further understanding of whether enzymatic and nonenzymatic antioxidant mechanisms in the root contribute to the tolerance of accumulator plants to toxic levels of Cu. Furthermore, understanding how toxicity modulates sulfur metabolism is crucial because sulfur is a key element in the antioxidant activity of glutathione in plants. This suggests the need for further studies to demonstrate the toxic role of Cu and its relationship with the production of oxidative stress in plants because antioxidant metabolism is one of the key mechanisms in inducing tolerance to trace element toxicity.

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