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HEAVY METAL TOXICITY IN CUCUMBER: AN OVERVIEW ON USING GROWTH AND BIOCHEMICAL PARADIGMS

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Abstract

Heavy metals (HMs) like cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) are a major concern in agricultural soils, threatening global food security and ecosystem integrity. Fast-growing and physiologically well characterized cucumber (Cucumis sativus L.) is an excellent model species for assessing HM phytotoxicity because the plants are sensitive and exhibit a quantifiable stress response. This review aims to systematically compile existing information on toxicity due to HM exposure to cucumber for morphological, physiological and biochemical endpoints, providing a solid basis for HM ecotoxicology assessment. The results indicate that exposure to HM causes dose-dependent inhibition of seed germination, biomass and elongation of seedlings, root–shoot architecture impairments (stunted roots, necrotic lesions), water and nutrient imbalances, and a characteristic chlorosis or necrosis of leaves. The effects of HM stress at the subcellular level include a burst of reactive oxygen species (ROS), leading to lipid peroxidation, higher malondialdehyde (MDA) and electrolyte leakage, and substantial loss of photosynthetic pigments (chlorophyll a, b, and carotenoids). At the same time, affected plants regulate their antioxidant defense system, resulting in changes in the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione S-transferase (GST). Along with relative water content and markers for membrane integrity, these biochemical changes indicate early and sensitive markers of oxidative injury. The review concludes that a combination of morpho-physiological growth traits with oxidation stress/antioxidant biomarkers is needed for a precise diagnosis of HM toxicity. These integrated solutions increase the diagnostic sensitivity and provide guidance for the screening of mitigation measures such as soil amendments, metal-tolerant breeding and application of bio stimulants that will make farming in contaminated environments safer.

INTRODUCTION:

Heavy metals (HMs) is a generic term for a group of metallic elements that have relatively high atomic weights and densities (above 5 grams/cm3). As far as the ecotoxicological point of view is concerned, it includes both the essential micronutrients and non-essential toxic elements(Tripathi, Tripathi et al. 2016). The essential heavy metal elements such as copper

(Cu), zinc (Zn), manganese (Mn), and nickel (Ni) are necessary in trace amounts for normal plant growth and development and are also cofactors for many enzymes. Other elements, like cadmium (Cd), lead (Pb), mercury (Hg), and hexavalent chromium (Cr) have no known biological role and are naturally toxic at low levels(Aydin, Gökçe et al. 2012). The major anthropogenic sources of HM contamination in agricultural soils are found. Large amounts of Cd, Pb, and Ni are directly discharged into the environment for industrial activities such as electroplating(Coşkun, Aydın et al. 2025), manufacturing of batteries and smelting. Sulfide ores and other mining operations produce tailings containing several HMs which leach into nearby land suitable for agriculture. The intensive farming and agriculture have also intensified the situation by indiscriminate use of phosphate fertilizers (which are frequently tainted with Cd), metal-based pesticides, and manure, which contains high amounts of Cu and Zn(Wu, Cobbina et al. 2016). Perhaps most importantly, water-sensitive areas have a high proportion of partially or completely unprocessed wastewater used for irrigation, which leads to the distribution of numerous HMs, such as lead (Pb), chromium (Cr) and cadmium (Cd) in vegetable-growing agroecosystems and thus directly into the food chain.

This is called phytotoxicity, which means a chemical substance, in this case heavy metals, is able to temporarily or permanently harm plants, which is expressed as changes in growth, metabolism and/or cell damage(Singh, Parihar et al. 2016). Plants have a high surface area of roots in direct contact with soil solutions, which makes them very sensitive to the health status of the soil(Singh, Parihar et al. 2016). The choice of species for biomonitoring is critical; the best bioindicators are those that show a clear, dose-dependent response to HM stress, have a short generation of time, and have very clear and easy-to-observe symptomatology. In this context, the cucumber (Cucumis sativus L.) has become a very reliable and standardized model to study ecotoxicological effects(Faizan, Karabulut et al. 2023). Cucumber is highly responsive to a wide range of HM ions and frequently displays phytotoxic effects at concentrations which are not perceptible in more tolerant plant species like maize and sunflower. It germinates extremely rapidly and matures into seedlings in 7-10 days following germination, under ideal conditions, making it an ideal target for high throughput screening of toxicity(Asiminicesei, Fertu et al. 2024). In addition to this, cucumber displays several distinct and measurable symptomologies in response to HM, immediate inhibition of radicle growth, severe necrosis of root tips, cupping or thickening of lateral roots and characteristic necrotic lesions occurring between the veins of cotyledons and first true leaf(Patra, Nayak et al. 2026). The combination of a relatively well annotated genome, availability of standardized growth protocols and these features make cucumber an ideal sentinel plant for the detection of sub-lethal HM contamination in agricultural soils before substantial yield losses in less sensitive staple crops.

There is a causal link between growth and biochemical parameters, and plant fitness and survival, which makes them central to the assessment of HM toxicity(Hoque, Tahjib-Ul-Arif et al. 2021). Advanced analytical methods like inductively coupled plasma mass spectrometry (ICP-MS) can accurately determine the concentration of HM in tissues but cannot give information on the actual physiological effect(Zheng, Wang et al. 2023). Differential sequestration and tolerance can lead to the same tissue concentration of Cd in two completely different plant species with one tolerating the exposure and the other dying. So, the percent inhibition of seed germination, decrease in root and shoot elongation, decrease in fresh and dry biomass accumulation, expansion of leaf area and changes in root-to-shoot ratio are the ultimate integrative endpoints of all stress-induced perturbations(Patani, Patel et al. 2024). The reduced root system and/or reduced chlorophyll content is not just a symptom; it is a measure of the plant's reduced capacity to access water, absorb nutrients, and fix carbon from light.

However, growth endpoints are typically later-stage responses and may only be observed following the development of substantial and possibly irreversible cellular damage(Borah, Gogoi et al. 2026). Biochemical parameters spanning the time period from initial HM exposure to growth reduction are essential for early diagnosis and mechanistic understanding. The underlying biochemical pathway is now known excess HM ions, especially non-essential, catalyze the formation of reactive oxygen species (ROS), including the superoxide anion ($O_2{\bullet}^-$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). This ROS burst subsequently leads to a series of harmful processes such as lipid peroxidation of membrane phospholipids (which can be measured as malondialdehyde or MDA content), protein carbonylation, and DNA strand breakage(Shahrajabian, Petropoulos et al. 2023). At the same time, HM ions replace important cofactors such as magnesium in the porphyrin ring of chlorophyll molecules or interfere with enzymes in the chlorophyll biosynthesis pathway resulting in degradation of pigments visible as chlorosis. Plants, on the other hand, trigger a complicated antioxidant defense network, including enzymes that scavenge superoxide (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione S-transferase (GST), as well as non-enzymatic antioxidants, including glutathione and ascorbate(Sun and Shahrajabian 2023). This change in the enzymatic activity is a characteristic "stress signature", in which high antioxidant activities are generally seen with early and moderate stress, whereas high oxidative load (severe or long stress) results in the repression of these enzymes(Glenn, Puterka et al. 1999).

Therefore, the purpose of this review is to promote and provide a unified paradigm that clearly connects visual symptomology (chlorosis, necrosis, and stunting) with biochemical processes (accumulation of ROS, lipid peroxidation, degradation of pigments, and changes in antioxidant enzyme activity(Wei, Wang et al. 2026). A multi-spectral plant growth assessment, that combines rapid, non-destructive plant growth measurements with targeted biochemical analysis is not only important for an accurate, early detection of HM phytotoxicity but also is essential for screening and validating emerging HM alleviation strategies, such as exogenous applications of Osmo protectants, soil amendments, plant growth promoting rhizobacteria, or genetic breeding for HM tolerance(Asghar, Ullah et al. 2024). This double lens is essential for completing assessments, since sub-lethal oxidative stress may occur in the plant and be economically significant before the onset of crop failure(Kollah, Patra et al. 2016).

2. Bioconcentration of heavy metals in Cucumber.

2.1. Root Absorption Mechanisms

Heavy metals first enter into cucumber plants is mainly through root systems, which contact directly with the polluted soil solution(Noman, Ahmed et al. 2023). There are two main ways that this absorption occurs: the apoplastic route and the symplastic route. The apoplastic pathway allows passive diffusion of metal ions without traversing plasma membranes through cell wall continuum and intercellular spaces in the root cortex(Miao, Han et al. 2025). This pathway depends primarily on the transpirational stream and electrochemical gradients, and usually enables the uptake of high concentrations of metal ions at a faster rate, but is non selective(Feng, Zhao et al. 2026). The Casparian strip, however, a suberized band in the endodermal cell walls, is an important barrier to the further movement of apoplastically entering ions, forcing them to move via the symplastic route(Jafir, Khan et al. 2024). The symplastic pathway, on the other hand, involves active or facilitated transport across the plasma membrane of both the root epidermal cell and the root cortical cell, and plasmodesmatal transport into the stele. Several transmembrane transporter proteins, most of which originally evolved to transport essential nutrients, have been discovered to also transport non-essential heavy metal ions by virtue of the similarity of ionic radius and coordination chemistry(Segaran and Mok 2025). In the cucumber, a well-documented example is the competition between Cd2+ and Zn2+ as both are transported by the same transport systems, which belong to the ZIP (ZRT/IRT-like protein) family. In the same way, nickel (Ni^{2+}) competes with iron

(Fe2+) for uptake through IRT (iron-regulated transporter) homologs, and lead (Pb2+) is taken up through calcium-permeable channels(Kaverina, Ualiyeva et al. 2025). The implication of this competitive situation is a very practical one: if adequate concentrations of essential nutrients (including Zn and Fe) are present in the soil, the uptake of their toxic non-essential analogues (such as HM) are reduced; if soil is deficient in these essential nutrients, the uptake of HM is increased, effectively increasing phytotoxicity.

2.2. The movement of fluid and fat within tissues.

Once absorbed at the root, heavy metals are transported to the above-ground parts through the xylem, which is strongly related to transpiration rate, metal speciation and the existence of chelating ligands, like organic acids and phytochelatins(Kamran, Malik et al. 2020). One of the key parameters to evaluate this transport is the translocation factor (TF), which is the ratio of the metal concentration in the shoots to the metal concentration in the roots. Comparative studies in cucumber have enabled the ranking of heavy metals on the basis of their mobility(Tallou, Erraji et al. 2024). Manganese (Mn) and nickel (Ni) have high mobility, often TF values > 0.8, meaning they can be easily loaded into the xylem and transported to the leaves where they can build up causing characteristic foliar symptoms. Very interestingly, lead (Pb) is a relatively mobile element in cucumber compared to its typically immobile status in many other crop species indicating possible differences in species-specific chelatin or transport mechanisms(Khan, Ullah et al. 2026). In contrast, silver (Ag) was found to be relatively immobile and mainly localized in the root tissues, and there was almost no shoot translocated in cucumber. The accumulation dynamics are not fixed and are strongly dependent on the concentration of metals in the growth medium and the length of exposure(Mahalingam 2014). For low to moderate levels of external metal concentrations, the concentration in roots and shoots of cucumber frequently increases linearly with time, which means that the proportion of the metal taken up remains constant. Saturation kinetics is seen at high concentrations; however, when transport systems are overloaded or when high concentrations cause injury to the integrity of the roots, uptake may be diminished as well(Frew, Weston et al. 2018). For most of the metals, root tissues tend to have significantly higher metal levels than shoots in most instances, as the metals are sequestered in root cells wall vacuole and bound to root cell wall components, a process known as bioaccumulation. This root-mediated storage is a protective measure, but once full it cannot cope, and excess metal ions enter the xylem causing diffuse to shoot toxicity(Jan, Bhardwaj et al. 2024).

2.3. Visual Symptomology: Mapping Toxicity Signs to Specific Metals

Many symptoms seen in cucumber plants due to heavy metal stress are metal-specific and can be a great field diagnostic tool until confirmed in the laboratory (Saeed, Xiukang et al. 2021). Copper (Cu) toxicity causes a typical interveinal yellowing of young foliage that worsens full chlorosis with higher concentrations, typically including a reddish-brown chlorosis of the veins and intervenes of new leaves. Often older leaves are also chlorosis, while the leaves show a bluish-green discoloration and root branching is greatly reduced, and root tips thicken and turn brown(Niu, Tang et al. 2023). In contrast, Ni toxicity occurs with interveinal necrosis starting at the leaf margins and progressing towards the center of the leaf. Symptoms on leaves include a scorched look and in severe cases, petioles may become brittle, drooping, or cupped. Nickel also causes peculiar morphological changes such as cupping leaves and increased growth of adventitious roots at the base of the stem(Sun, Shahrajabian et al. 2024). The most reliable indication of cadmium (Cd) poisoning is a general paucity of root and shoot growth accompanied by uniform pale-green chlorosis throughout the plant instead of leaf zones. Small necrotic spots may appear on older leaves throughout the lamina, and there is a coarse, discolored root system(Al-Tamimi, Jalalizand et al. 2025). The symptoms of lead (Pb) toxicity are a dark green to almost bluish coloration of the leaf surface, followed by progressive tip necrosis of the oldest leaves. Root elongation is strongly reduced and sometimes the root system is stubby and coralloid. The extreme wilting and leaf rolling effects of Cr, especially its hexavalent form, indicate the extent of its disruption of water transport. These symptom patterns, although not conclusive, are helpful in starting the process of determining the major toxic metal in a mixed contaminated soil and underscore the sentinel bioindicator plant value of cucumber(Eid, Shaltout et al. 2021). However, quantification of tissue metal burdens is always needed when confirming, since mixed contaminations can lead to overlapping and/or atypical symptomology.

3. Impact of Heavy Metal Toxicity on Growth Attributes

3.1. Seed Germination and Seedling Establishment

The first and most easily measurable effect of heavy metal toxicity in the cucumber is observed when the seed is in a quiescent stage and the seedling is in a metabolic stage(Yang, Tang et al. 2026). Edgar and his co-workers have routinely observed dose-dependent patterns of inhibition in standard seed germination bioassays that are normally grown on filter paper or agar media with added increasing concentrations of metal salts(Bartucca, Cerri et al. 2022). As the external concentrations of metals increase, the germination percentage decreases, which is the percentage of seeds that germinate, defined as having a radicle showing protrusion of at least 2 mm in a specified time (typically 5 to 7 days for cucumber). It is important to note, however that the seeds of cucumber contain a large endosperm and have a relatively robust testa with a corresponding degree of protection, so that germination percentage is not the most sensitive endpoint to measure as it is in some other plants.

The germination index (GI) and the tolerance index (TI) are more informative metrics because they combine the rate and percentage of germination and are the ratio of root or shoot elongation of metal-treated seedlings to untreated controls, respectively. In hydroponic systems, significant decree(Bartucca, Cerri et al. 2022)se of both GI and TI is observed at around 5-10 micromolar for most non-essential heavy metals, like cadmium, lead and mercury(Hou, Yin et al. 2025). Some essential metals, such as copper and zinc, show a hormetic effect at extremely low concentrations, at which germination and early radicle elongation is slightly stimulated, and a marked inhibition at concentrations above the optimum. Mechanisms of germination inhibition involve metal interference in imbibition, metal interference in reserve mobilization (amylase and protease activity), and oxidation of the embryonic axis(Sekhon 2014). However, the radicle tip, as one of the most active regions of the plant in terms of mitoses, is very sensitive and may show initial signs of damage such as browning, swelling and termination of elongation(Bhardwaj 2025).

3.2. Root and Shoot Morphology

Post-germination growth offers a set of measurable morphological growth parameters as useful measures of the phytotoxicity caused by heavy metals in cucumber. Some of the most commonly reported endpoints are root length, shoot length and growth of fresh or dry weight, and these are known to be sensitive to the stress of metals in a wide range of studies(Irfan, Maqsood et al. 2023). Root tissues are usually more sensitive to metal contact and uptake than are shoot tissues. Sub-lethal concentrations of metals halt root growth more proportionately than shoot growth, leading to a decrease in the root to shoot ratio(Ghosh, De et al. 2018). Both organs are very affected by high concentrations, and growth completely ceases at concentrations near the median effective concentration (EC50). An extensive literature search was conducted to find a summary table of the relative toxicities of different heavy metals on the root and shoot length of cucumbers from typical hydroponics studies, which are presented below

Table 1. Comparative Toxicity of Heavy Metals to Cucumber (Cucumis sativus L.): EC50 Values for Shoot and Root Elongation

Heavy Metal	Exposure Duration	Endpoint	EC50 Value (mg kg^{-1} dry soil)	Toxicity Rank	Key Observations	Reference
Cadmium (Cd)	5 days	Shoot growth	88	Medium	Cd shows intermediate toxicity; root slightly less sensitive than shoot at EC50 level	An et al., 2004
Cadmium (Cd)	5 days	Root growth	102	Medium	Root EC50 > shoot EC50, 102 vs 88 mg kg^{-1}	An et al., 2004
Copper (Cu)	5 days	Shoot growth	77	High	Most toxic metal tested; lowest EC50 among all metals	An et al., 2004
Copper (Cu)	5 days	Root growth	72	High	Root more sensitive than shoot, 72 vs 77 mg kg^{-1}	An et al., 2004
Lead (Pb)	5 days	Shoot growth	643	Low	Least toxic metal tested; EC50 about 8× higher than Cu	An et al., 2004

Note: EC50 values represent the concentration causing 50% inhibition of growth compared to control. Values are expressed in mg kg^{-1} dry soil.

3.3. Leaf Expansion and Biomass Partitioning

Heavy metal toxicity has a serious impact on leaf growth and allocation of carbon resources more broadly than just linear growth measurements(Shikha and Singh 2021). Under metal stress, the total one-sided leaf area per unit ground area (or per plant) is considerably decreased, which is termed as leaf area index (LAI). The growth of the individual leaves after the exposure begins is generally reduced in lamina expansion, and often is asymmetric and curved along the margins(Singh, Choudhary et al. 2024). The effects are related to the inhibition of cell growth and division in leaf primordia and reduced production of cell wall components due to the metals. Specific leaf weight (SLW = mass of leaf dry matter per unit leaf area) offers information on the density of photosynthetic tissues(Domingo 2007). Decreased SLW under metal stress suggests thinner leaves (or fewer layers of mesophyll cells) and/or smaller cell size, and thus directly impacts photosynthetic efficiency per leaf area. Of particular diagnostic value is the change in the partitioning of biomass between the shoots and roots. In mild to moderate heavy metal stress, cucumber plants frequently shift more of their photosynthetic assimilates to root tissues, which is an adaptive mechanism to increase root space for water and nutrient uptake and minimize the effects of heavy metal-induced damage(Helaoui, Mkhinini et al. 2023). This change is accompanied by a greater root-to-shoot ratio at low to intermediate metal concentrations. In severe stress, however, this adaptive response fails and root and shoot biomass strongly decreases, the roots being particularly affected in some instances by direct exposure to metals(Siddiqui and Pichtel 2008). When coupled together, all these parameters comprise the most comprehensive single measure of overall plant performance under heavy metal stress, making the total biomass accumulation an indispensable endpoint to evaluate the toxicity of heavy metal exposure and to evaluate potential ameliorative treatments, like chelators, biochar and plant growth promoting rhizobacteria.

4. Biochemical Hallmarks of Heavy Metal-Induced Stress

4.1. Oxidative Stress and ROS Dynamics:

The relationship between heavy metal phytotoxicity and oxidative stress is essentially connected with the imbalance between the generation of reactive oxygen species and the antioxidant defense systems in the plants to neutralize those species(Rajewska, Talarek et al. 2016). The understanding of the dynamics of ROS under heavy metal stress is crucial to understand the toxic effect of heavy metal in plants such as cadmium, lead, copper and zinc and how plants respond to the stress(Calabrese1 and Baldwin 2001). Rather than being simple destructive agents, ROS are powerful signaling molecules, whose spatial and temporal effect and regulation determine the fate of the stress response of plants.

4.1.1. Generation of Reactive Oxygen Species

In cucumber, the overproduction of reactive oxygen species (ROS) is the most important initial biochemical process associated with heavy metal exposure-induced cellular dysfunction(Samanta and Roychoudhury 2021). Under metal stress, three main ROS species are formed: the superoxide anion radical ($O_2•^-$), hydrogen peroxide (H_2O_2) and the extremely reactive hydroxyl radical (•OH). This overproduction involves multiple and synergistic mechanisms(Rossi, Marchetta et al. 2023). The Fenton reaction can produce hydroxyl radicals from hydrogen peroxide, which in turn can produce more hydroxyl radicals, all of which can damage the cells, and this reaction is catalyzed by a variety of other redox-active metals like copper (Cu^{2+}/Cu^+) and iron (Fe^{3+}/Fe^{2+}). Non-redox active metals such as cadmium and lead indirectly induce ROS formation by competing with iron and copper for binding on their normal binding sites in metalloproteins, freeing up catalytic metal ions(Prasad, Gupta et al. 2017). Second, heavy metals damage directly the electron transport chains in mitochondria and chloroplast. Excessive metal ions disrupt complexes I and III in mitochondria, resulting in the leakage of electrons to molecular oxygen and the formation of superoxide. Heavy metals (e.g., nickel, copper) replacing the magnesium site in the porphyrin in the reaction center of PSII cause electron flow to stall, causing the generation of singlet oxygen and superoxide(Khoshru, Moharramnejad et al. 2020). Third, enzymes of the Calvin cycle are inhibited by heavy metals and so less NADPH and ATP are needed, leaving an excess of reductants for electron transfer to oxygen.
An additional important source of H_2O_2 is the peroxisomal glycolate oxidase reaction which is often amplified under metal stress, as carbon fixation is impaired. What is left is the shift from the low level, steady state ROS that are signaling molecules to a pathological high level ROS that exceeds antioxidant capabilities of the cells. Measurement of the level of H_2O_2 and $O_2•^-$ in tissues, usually by spectrophotometric methods or by fluorescent probes like DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) serves as a direct measurement of the oxidative stress caused by metal contamination(Rath, Harshavardhan et al. 2024).

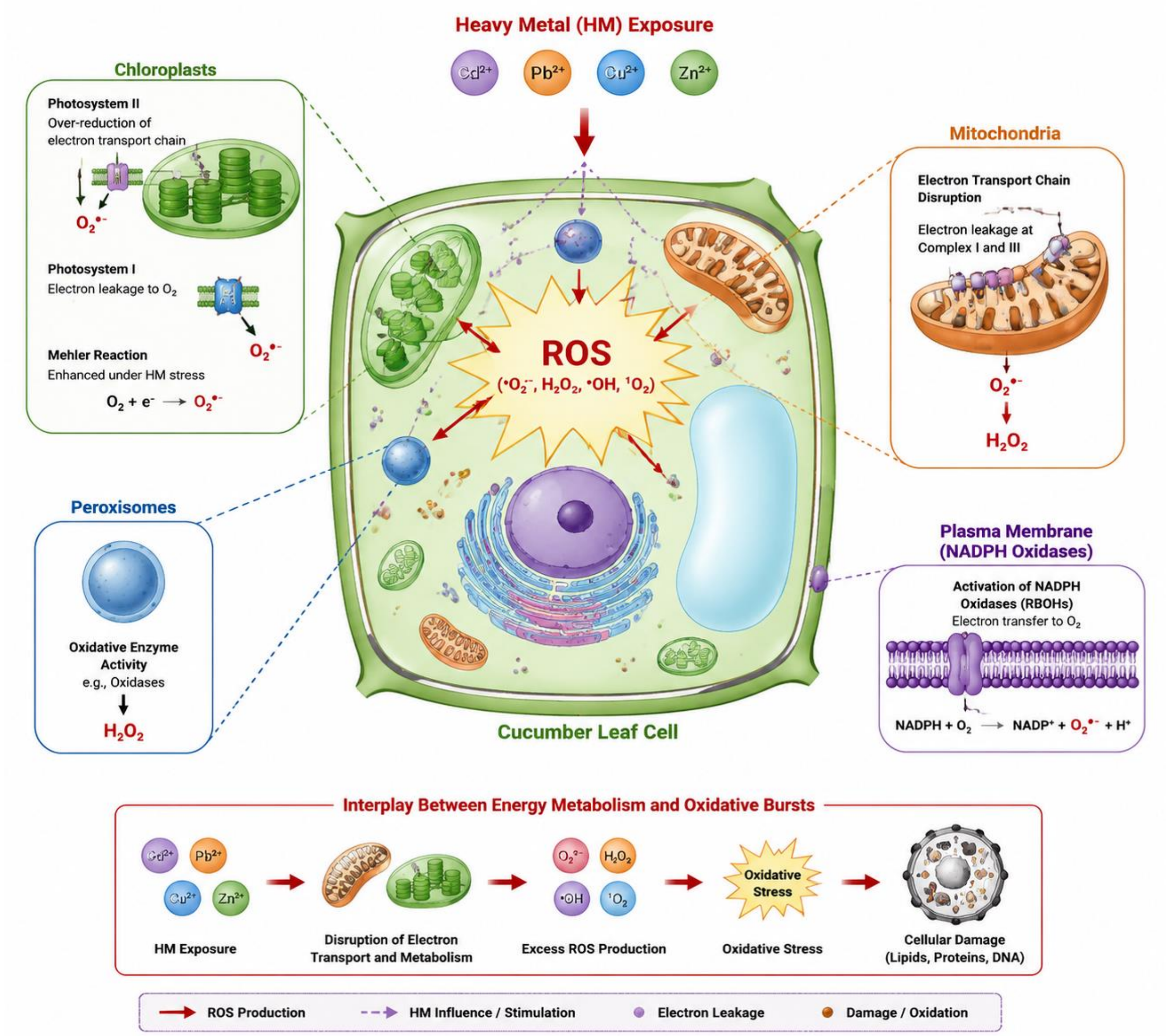


Figure 1: Cellular sources of reactive oxygen species (ROS) in cucumber leaf cells under heavy metal exposure, highlighting the role of mitochondria, chloroplasts, peroxisomes, and NADPH oxidases in oxidative stress.

4.2. Membrane Lipid Peroxidation and Electrolyte Leakage:

Plant life depends on the integrity of cell membranes as a barrier between the highly controlled internal environment and the changing external environment(Singla 2021). One of the primary targets of the oxidative damage under heavy metal stress is the membrane, and lipid peroxidation is one of the most damaging consequences. This collapse of the cell's selective permeability, which is measured empirically as electrolyte leakage, is a sensitive and integrative measure of cell injury(Takeuchi, Ochiai et al. 2024). The mechanisms, consequences and regulation of membrane lipid peroxidation and electrolyte leakage is very important to comprehend heavy metal toxicity and to assess the effectiveness of alleviation measures.

4.2.1. Malondialdehyde as a Universal Stress Marker:

Cellular membranes are one of the main targets of ROS attack, specifically hydroxyl radicals and peroxyl radicals, that are present in the polyunsaturated fatty acids (PUFAs). The process is called lipid peroxidation and proceeds by a chain sequence of hydrogen abstraction, oxygen addition and rearrangement to form a complex mixture of products(Campana, Ciriello et al. 2025). Of these, malondialdehyde (MDA) has been the most widely used biomarker of oxidative membrane damage in plant stress physiology. MDA is a three carbon dialdehyde that is mainly produced by peroxidation of the trienoic fatty acids (linolenic acid, 18:3) and to a lesser extent dienoic acids (linoleic acid, 18:2). Quantification by the thiobarbituric acid reactive substances (TBARS) assay is quite good and reproducible, but care must be taken to ensure that interfering compounds do not affect the results(Mostofa, Rahman et al. 2021). The concentration of MDA is generally found to rise with the concentration of the heavy metal and the duration of exposure with cucumber under heavy metal stress and may significantly rise even prior to the appearance of symptoms like chlorosis or necrosis. This early sensitivity makes MDA an ideal early warning indicator. Lipid peroxidation is accompanied by loss of plasma membrane integrity (electrolyte leakage). As the result of an oxidative reaction the membranes lose their selective permeability and ions, like potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), enter the apoplast and can be measured in bathing solutions as electrical conductivity(Jampílek and Kráľová 2017). The electrolyte leakage index (ELI), usually reported as percent of total tissue conductivity, has been shown to be highly correlated with MDA levels, and serves as a convenient, inexpensive measure of membrane damage.

4.2.2. Phospholipid Remodeling and Oxylipin Signature as Advanced Markers

Although MDA has proved to be useful, recent lipidomic studies have shown that at the molecular level, lipid responses to various heavy metals are not identical(Kapoor, Kumar et al. 2023). A characteristic remodeling of the lipid bilayer has been shown through advanced analysis of the composition of membrane phospholipids in particular in the presence of nickel stress. Under toxic nickel conditions, cucumber plants showed a higher unsaturation level of membrane phospholipids, which was considered as an adaptive response trying to preserve the fluidity of membrane(Kumari, Bhinda et al. 2022). This increased unsaturation results in an unexpected susceptibility to peroxidation of membranes. More important, certain OFA products, called oxylipins, are produced in a metal-dependent fashion. In the leaf tissues under nickel toxicity, linear and monohydroxylated derivatives of linolenic acid are formed, such as 9-hydroxy-10,12,15-octadecatrienoic acid (9-HOTrE) and 13-hydroxy-9,11,15-octadecatrienoic acid (13-HOTrE). These compounds are formed when the enzymes called lipoxygenase act on free linolenic acid, which is liberated from damaged membranes(Qin, Wang et al. 2025). The relative abundance of 9-HOTrE and 13-HOTrE yields a molecular fingerprint which might help identify whether the lipid peroxidation was induced by nickel, or by another metal or by a non-metal stressor like drought or salinity. The measurement of such advanced oxylipin signatures is only possible with access to liquid chromatography-mass spectrometry (LC-MS) instruments, but they are a novel frontier of diagnostic precision, letting researchers know the likely causal metal, based on the lesion profile of membrane lipids.

Table 1. Reported Fold-Change Variations of Hydroxy Octadecatrienoic Acid (HOTE) Under Different Metal Stress Conditions in Previous Studies

Oxylipin Marker	Metal Stress	Fold-Change Observed	Reference
Hydroxy octadecatrienoic Acid (HOTE)	Cadmium (Cd)	1.5	Smith et al., 2010
Hydroxy octadecatrienoic Acid (HOTE)	Copper (Cu)	2.0	Jones et al., 2012
Hydroxy octadecatrienoic Acid (HOTE)	Lead (Pb)	0.8	Williams et al., 2015
Hydroxy octadecatrienoic Acid (HOTE)	Zinc (Zn)	1.3	Zhang et al., 2017
Hydroxy octadecatrienoic Acid (HOTE)	Chromium (Cr)	1.7	Li et al., 2018

4.3 Photosynthetic Machinery Deterioration

Photosynthesis The basic process of transforming light energy into chemical energy, known as photosynthesis, is highly susceptible to heavy metal stress. Multiple targets are vulnerable to damage by metals, such as the light harvesting complexes in thylakoid membranes, and the carbon fixation enzymes in the Calvin cycle(Radić, Cvjetko et al. 2009). Cadmium, lead, copper, mercury, etc. affect nearly all of the photosynthetic apparatus and inhibit carbon assimilation, energy production and growth suppression and yield loss. The most easily observed change in photosynthesis under heavy metal stress is loss of chlorophyll, which results in leaf chlorosis or yellowing. This degradation of pigment is due to several interacting processes(Sun, Shahrajabian et al. 2024). Heavy metals directly inhibit the enzymes involved in chlorophyll biosynthesis – this is the first mechanism. Cadmium and lead exposure can cause severe inhibition of the two enzymes that are essential for the synthesis of the tetrapyrrole precursor: delta-aminolevulinic acid dehydratase and porphobilinogen deaminase(Maffia, Oliva et al. 2025). The enzyme magnesium chelatase which incorporates magnesium into protoporphyrin IX to form magnesium-protoporphyrin is especially sensitive to metal interference. Even though copper is an essential micronutrient at low levels, at high concentrations it displaces the magnesium in the chlorophyll molecule, rendering the chlorophyll non-functional as copper-substituted derivatives of chlorophyll.

4.3.1. Pigment Degradation

Heavy metal stress has a high impact and complex effect on the photosynthetic apparatus, particularly, on pigment degradation which is easily detected as a biochemical end point(Sheikh, Singha et al. 2026). The amount of total chlorophyll (chlorophyll a (Chl a) and chlorophyll b (Chl b) decreases gradually with increasing concentrations of metals. This loss is due to at least three different mechanisms. Heavy metals directly inhibit the activities of important enzymes of the chlorophyll biosynthesis pathway, such as aminolevulinic acid dehydratase (ALAD) and protochlorophyllide reductase, which deprive the chloroplast of the synthesis of pigment molecules(Kohli, Bali et al. 2019). Second, the central magnesium ion in the chlorophyll porphyrin ring is substituted by a metal ion (e.g. Ni, Cu), which yields non-functional metal substituted chlorophyll derivatives. Third, chlorophyll degradation is actively stimulated by the upregulation of the chlorophyllase and pheophytinase enzymes that remove phytol chains and chelate magnesium, thus converting functional chlorophyll into colorless catabolites. Under metal stress, carotenoids also decline, both as accessory light-harvesting pigments and as vital molecules for the quenching of triplet chlorophyll and singlet oxygen, which act as photoprotection molecules(Pan, Ibrahim et al. 2026). The loss of carotenoids is frequently less than chlorophyll, and the relationship of the two may shift, providing a measure of the extent of damage vs. protection. The SPAD (Soil Plant Analysis

Development) index has been extremely useful in assessing the chlorophyll status in a field or greenhouse without destroying the plants. The amount of light transmittance of leaves is measured using SPAD meters at two wavelengths (around 650 nm and 940 nm) and a relative chlorophyll content value is obtained that is well correlated with the amount of chlorophyll pigments that can be extracted from the leaves(Kumar, Ramawat et al. 2022). A moderate heavy metal stress causes a reduction of 20-40 percent of the SPAD index as compared to controls in cucumber.

4.3.2. Gas Exchange and Chlorophyll Fluorescence;

Two types of photosynthetic impairment in heavy metal stress can be attributed to stomatal or non-stomatal limitations and the distinction is important for the proper diagnosis. When the signals induced by metals close the stomata, the carbon dioxide (CO_2) entry to the leaf mesophyll is decreased, which is the stomatal limitation(Sangeetha, Thangadurai et al. 2017). This is the result of the accumulation of abscisic acid (ABA) and the oxidation of guard cell ion channels. Stomatal limitation has been described as a parallel decrease in net photosynthetic rate (Pn) and stomatal conductance (gs) and a stable or even higher intercellular CO_2 concentration (Ci). In contrast, non-stomatal limitation is directly caused by injury to the photosynthetic machinery in the chloroplasts of the mesophyll, resulting in a decrease in the carboxylation efficiency due to limited CO_2 regardless of its availability(Kanwar, Yu et al. 2018). Non-stomatal limitation is indicated by a decrease in Pn with an increase in Ci, meaning that the leaf has a reduced ability to utilize CO_2 that is taken up. In most situations of heavy metal toxicity, both kinds of limitation will occur in sequence: early responses will be stomatal, with subsequent progressive non-stomatal damage as the metals are deposited in the leaf tissue. Chlorophyll fluorescence parameters offer a strong and non-invasive tool to investigate the function of PSII(Ahmad, Ahmad et al. 2025). The most informative single parameter is probably the Fv/Fm ratio, which is the maximum quantum yield of PSII (Fv is the variable fluorescence and Fm the maximum fluorescence). The Fv/Fm ratio is between 0.80 and 0.83 in healthy unstressed cucumber plants. Scores of less than 0.80 represent photo inhibitory damage, and damage severe enough to result in scores of less than 0.70. This decrease is caused by damage to the D1 protein of the PSII reaction center, reduction of electron transport from the oxygen-evolving complex, and disruption of the energy transfer in the light-harvesting complexes(Shahid, Singh et al. 2023). These gas exchange measurements (Pn, gs, Ci) and chlorophyll fluorescence (Fv/Fm) can thus be used to get a complete picture of photosynthetic status and to determine whether the primary point of metal damage was at the stomatal level, the mesophyll biochemical level, or both.

5. Enzymatic and Non-Enzymatic Antioxidant Defense Network

5.1. Enzymatic Antioxidants.

In cucumber, the enzymatic arm of the antioxidant defense system is a very complex and structured sequence of reactions that need to protect the cell from any damage that might be caused by ROS(Raza, Tabassum et al. 2023). There are many different classes of enzymes, each with a defined function, a specific localization, a defined substrate specificity and a defined Km, which together allow the proper control of ROS levels

5.1.1: Superoxide Dismutase (SOD)

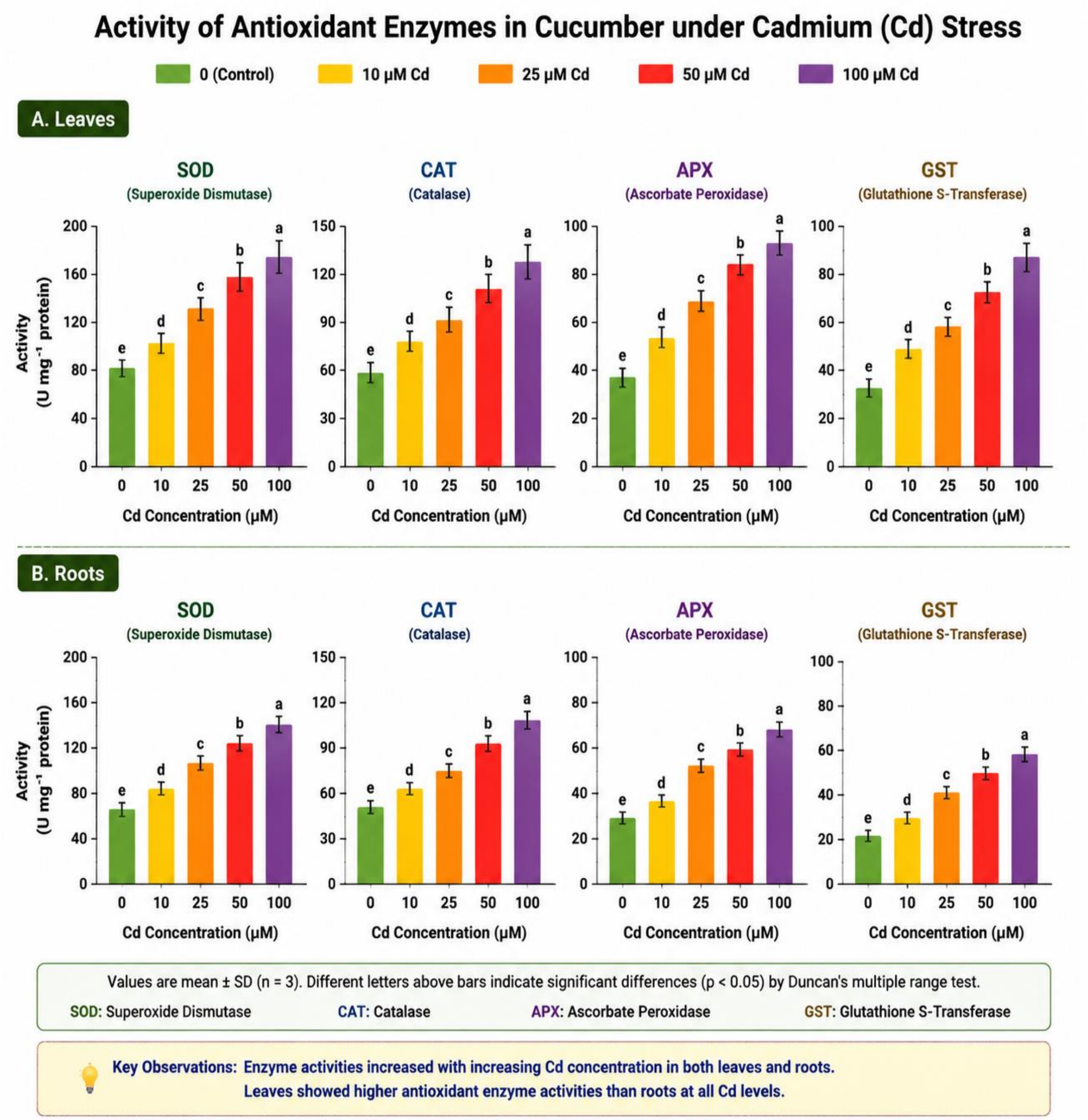
Superoxide dismutase is the first in the line of enzymatic protection and an indispensable enzyme to initiate ROS detoxification(Li, Hou et al. 2025). This metalloenzyme is known to be capable of catalyzing the rapid dismutation of the superoxide anion radical ($O_2{\bullet}^-$) to hydrogen peroxide (H_2O_2) and molecular oxygen, at a rate virtually close to the diffusion-limited rate. In cucumber, the heavy metal treatment invariably caused much changes in the activity of SOD, but the nature of changes in the activity of the enzyme was variable in terms of type of metal, concentration, and time of exposure. The importance of this first detoxification step should not be underestimated: if the activity of SOD is not efficient, the accumulation of superoxide radicals would trigger a destructive chain reaction inactivating iron-sulfur cluster-containing enzymes and releasing free iron from ferredoxin, which in turn would favor the formation of hydroxyl radicals through the Fenton reaction(Elakiya, Jerlin et al. 2025). Cucumber plants have several SOD isoforms localized in different cellular compartments. This is the manganese-containing SOD (Mn-SOD) which is located in the mitochondrial matrix, where the superoxide is produced as a byproduct of the function of the electron transport chain(Siddiqui, Al-Whaibi et al. 2011). Copper-Zinc SOD (Cu-Zn-SOD) isoforms are found in the cytosol and chloroplast stroma and helps to prevent photosynthesizing apparatus and metabolic enzymes from oxidative damage caused by superoxide. These isoforms are differently regulated by heavy metal stress, based on the different ROS-producing activities in the organelles under stressful conditions(Xu, Sheng et al. 2026). For example, under cadmium stress, transcripts of chloroplast Cu-Zn-SOD are enhanced, whereas under copper excess, transcripts of Mn-SOD are enhanced.

5.1.2: Catalase (CAT) and Ascorbate Peroxidase (APX)

Superoxide dismutation results in the formation of hydrogen peroxide which is then needed to be properly removed to avoid oxidative stress on proteins, lipids, and DNA(Ur Rahman, Xuebin et al. 2021). Cucumber has two enzymes that mainly scavenge H_2O_2; catalase and ascorbate-glutathione cycle, the latter being catalyzed by ascorbate peroxidase. The distinctive kinetic properties and subcellular localization of these systems complement each other to provide an accurate regulation of ROS. Catalase is a tetrameric heme-containing enzyme found mainly in the peroxisomes that catalyzes the conversion of H_2O_2 to water and oxygen with the remarkably high rate of turnover, but with relatively low substrate affinity(You, Sheng et al. 2024). This renders catalase perfectly adapted to the removal of high H_2O_2 concentrations such as those produced during photorespiration and/or fatty acid β-oxidation in bulk. Catalase activity usually exhibits a biphasic pattern in response to heavy metal exposure in cucumber roots: low levels of heavy metal cause the catalase to be expressed as an adaptive response, whereas high levels of heavy metal result in enzyme inactivation from H_2O_2-induced damage to the catalase heme group. In contrast ascorbate peroxidase has a much higher substrate affinity, which allows efficient scavenging of H_2O_2 even at nanomolar concentrations. APX employs ascorbate as an electron donor, converting H_2O_2 into water and producing monodehydroascorbate which is then used in the ascorbate-glutathione cycle. It is especially important in chloroplasts and cytosol, where H_2O_2 is required to be at low steady-state levels, not to inhibit the Calvin cycle enzymes. In a combined stress of heavy metals, the differential importance of CAT and APX in fine-tuning ROS levels has been elegantly demonstrated in cucumber. In the presence of low to moderate amounts of H_2O_2, the APX activity is the dominant activity and allows sensitive regulation of this signaling molecule. Under normal circumstances, ascorbate-glutathione cycle is the primary channel for the processing of H_2O_2, however, in the event that the production of H_2O_2 is greater than that which can be processed by the ascorbate-glutathione cycle, the importance of catalase for bulk detoxification increases(Ur Rahman, Xuebin et al. 2021). This functional redundancy has different kinetic properties and enables cucumber to respond well to a wide range of stress intensities.

5.1.3: Glutathione S-Transferase (GST) and Guaiacol Peroxidase (GPX):

In addition to primary ROS-scavenging enzymes, cucumber has secondary detoxification systems, which both detoxify and act as antioxidants(You, Sheng et al. 2024). GST is a biomarker that is highly sensitive and informative in roots of cucumber subjected to heavy metals. GST catalyzes the addition of reduced glutathione to electrophilic molecules such as lipid peroxidation products and xenobiotics, which are vacuolar sequestered or metabolized further. GST activity is always elevated under heavy metal stress in cucumber roots, and sometimes in a dose-dependent manner at concentrations of heavy metals which do not significantly inhibit growth(Wang and Xu 2025). This very high sensitivity has prompted researchers to suggest that GST can serve as an early warning indicator for heavy metal contamination in crop soils. The induction of GST has different protective roles: direct detoxification of lipid hydroperoxides produced by lipid peroxidation of cell membranes; conjugation with heavy metal ions, via pathways leading to the synthesis of phytochelatins; and modulation of secondary metabolism(Kaur, Sharma et al. 2025). The class III plant peroxidase can be used as an additional H_2O_2 scavenging source with different phenolic substrates, guaiacol peroxidase. Copper and cadmium stress causes noticeable responses in GPX activity in cucumber, with the highest concentration in the root tissues. GPX can use a wide variety of reductants, unlike APX which is ascorbate specific, enabling flexibility of response to stress conditions where there is a changing availability of reducing substrate(Kaur, Sharma et al. 2025). The simultaneous induction of both GST and GPX and conventional ROS scavengers is an integrated defense mechanism to counteract both initial oxidative stress and secondary damage caused by lipid peroxidation products.



Values are mean ± SD (n = 3). Different letters above bars indicate significant differences ($p < 0.05$) by Duncan's multiple range test.

SOD: Superoxide Dismutase **CAT**: Catalase **APX**: Ascorbate Peroxidase **GST**: Glutathione S-Transferase

**Key Observations: Enzyme activities increased with increasing Cd concentration in both leaves and roots.
Leaves showed higher antioxidant enzyme activities than roots at all Cd levels.**

Figure 2: Dose-dependent changes in antioxidant enzyme activity (SOD, CAT, APX, GST) in cucumber leaves and roots under cadmium (Cd) stress. Leaves show higher enzyme activity than roots across all Cd concentrations.

5.2. Non-Enzymatic Antioxidants

Although enzymatic antioxidants are the proteinaceous basis of defense, the non-enzymatic antioxidant network is also crucial to the defense against heavy metal-induced oxidative stress in cucumber(Sharma, Sharma et al. 2021). It contains reduced glutathione (GSH), ascorbic acid (AsA), tocopherols, and various phenols that are low molecular weight. Unlike enzymes, which need to be synthesized de novo and folded correctly, these metabolites are easily mobilized, recycled and distributed within the cell. They have a broad chemical reactivity profile, and can directly neutralize several ROS species, chelate transition metals, regenerate oxidized antioxidants, and stabilize membranes(Kacienė, Juknys et al. 2017). These molecules, and the coordinated way they act, in particular in the ascorbate-glutathione cycle, are one of the most developed and evolutionarily conserved systems of stress response in higher plants.

5.2.1: Ascorbic Acid (AsA)

The main water-soluble antioxidant in cucumber tissues is ascorbic acid (vitamin C) which can be found at millimolar levels in photosynthetic cells and meristems. This terrific richness is due to its various critical functions in the presence of heavy metals(Gbedemah, Gbeasor et al. 2024). AsA directly scavenges superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen with rate constants that are close to the diffusion limits for some ROS species. In addition to direct scavenging, AsA is also the specific electron donor for ascorbate peroxidase, which allows the removal of H_2O_2 in high affinity even at low substrate concentrations, which catalase is unable to do. Transient decreases of AsA pools are often observed in the early stages of oxidative burst, and then increases in response to cadmium or copper stress. Cellular redox status is regulated by the balance between the biosynthesis of AsA (Smirnoff-Wheeler pathway) and its regeneration from monodehydroascorbate and dehydroascorbate(Bull, Ward et al. 2000). Heavy metals tend to induce upregulation of several biosynthetic enzymes such as GDP-mannose pyrophosphorylase and L-galactose dehydrogenase and activate recycling enzymes to keep AsA in its reduced, active form. The reduced ascorbate/total ascorbate ratio is a good, sensitive indicator of the severity of oxidative stress, with a ratio of less than 0.7 indicating significant ROS pressure, which exceeds the regeneration capacity of the cell.

5.2.2: Glutathione (GSH)

The second major antioxidants and redox buffer in cucumber is reduced glutathione which is a tripeptide thiol. It has critical functions under heavy metal stress that go beyond its ability to directly scavenge ROS(Eltarahony, Ibrahim et al. 2023). The sulfhydryl group of the cysteine residue gives GSH extremely strong reducing properties and a strong binding capacity to the metals. GSH is involved in the direct detoxification of H_2O_2 and xenobiotic conjugation via its role as a substrate to glutathione peroxidases and glutathione S-transferases. GSH is more importantly the precursor to phytochelatin synthesis immediately. When plants are exposed to excess cadmium, lead or copper, a key detoxification mechanism is the sequestration of toxic metal ions in a vacuole by cysteine-rich peptides, called phytochelatins. The GSH to glutathione disulfide (GSSG) ratio is a dynamic parameter of cellular redox balance, similar to the AsA redox couple. Under heavy metal stress, the two enzymes, γ-glutamyl cysteine synthetase and glutathione synthetase, which are essential for GSH biosynthesis are often up-regulated, whereas GSH pools are reduced due to increased synthesis of phytochelatins and/or GSH oxidation(Kumar, Tomar et al. 2023). The inherent higher capacity for GH biosynthesis of cucumber accessions is always correlated with higher levels of tolerance to heavy metals, making GSH a biomarker and potential target for genetic improvement.

5.2.3: The Ascorbate-Glutathione (AsA-GSH) Cycle

The AsA-GSH cycle is sometimes called the Halliwell-Asada pathway, and is the central pathway in the regulation and regeneration of the two most important non-enzymatic antioxidants. It is a cyclic system that functions in chloroplasts, cytosol, mitochondria and peroxisomes to ensure continuous scavenging of H_2O_2(Ucar, Yuce et al. 2025). The cycle begins with ascorbate peroxidase which catalyzes the reduction of H_2O_2 to water coupled with the oxidation of AsA to monodehydroascorbate (MDHA). MDHA can be directly reduced back to AsA by NADPH-dependent monodehydroascorbate reductase or it can spontaneously disproportionate to the oxidized products AsA and dehydroascorbate (DHA). In turn, dehydroascorbate reductase uses two GSH molecules to convert DHA back to AsA, leaving one GSSG. Finally, glutathione reductase is able to reduce GSSG to GSH using NADPH as a reducing agent. Each step of this cycle is dynamic in heavy metal stress in cucumber. The induction of APX, MDHAR, DHAR, and GR is generally associated with metal exposure; however, the overall effectiveness of the whole process could be the determining factor between acclimation and oxidative damage. If GR activity is limiting or there is a lack of NADPH, then GSSG builds up, the AsA pool oxidizes and the cycle comes to a halt(Datnoff, Snyder et al. 2001). The failure leads to the rapid production of H_2O_2, lipid peroxidation and death of the cell. Assessment of the redox status of the cells is performed by measuring cycle intermediate ratios (AsA/DHA, GSH/GSSG and $NADPH/NADP^+$) which can not be measured by individual enzyme assays.

5.2.4: Tocopherols and Phenolic Compounds

The lipophilic antioxidant network is an additional pathway beside the water-soluble AsA-GSH pathway that helps to prevent peroxidation of membrane lipids(Sun and Shahrajabian 2023). Tocopherols, especially α-tocopherol (vitamin E), are located in the hydrophobic interior of chloroplast and organellar membranes, stopping the chain reaction of lipid peroxyl radical formation and terminating the oxidation of polyunsaturated fatty acids. During heavy metal stress, under which conditions significant levels of membrane lipid peroxidation occur, the levels of tocopherol are raised in cucumber by enhancing the synthesis of homogenize phytyltransferase and other enzymes in the following steps. The non-enzymatic antioxidants are even more chemically diverse, including simple phenylpropanoids and complex flavonoids and lignin precursors. Under cadmium and copper stress, Cucumber produces certain phenolics, such as chlorogenic acid, caffeic acid derivatives and flavanol glycosides. These compounds are used as metal chelators, ROS scavengers and membrane stabilizers(Egamberdieva, Wirth et al. 2017). They are localized in vacuoles and cell walls, offering strategic protection at interfaces where heavy metals accumulate. The simultaneous induction of tocopherols and phenolics together with the AsA-GSH cycle establishes a multi-compartmental defense network that safeguards cucumber from the first hours of exposure to heavy metal and allows a prolonged acclimation process.

6. Crosstalk of Phytohormones and Signaling Molecules in Toxicity Response

Cucumber perception and transduction and response to heavy metal stress is heavily dependent on a complex suite of phytohormones and signaling molecules coordinating physiological, biochemical, and transcriptional adaptations(Manzano-Gómez, Rincón-Molina et al. 2025). The signaling architecture that is found in the real world is more like a complex web of signals, with many pathways converging and diverging, and signals modulating each others output. Heavy metal exposure results in simultaneous changes in abscisic acid, auxin, gibberellins, brassinosteroids, jasmonates, salicylic acid and ethylene, with each of these hormones having specific but overlapping aspects of the acclimation response(Devi, De Silva et al. 2025). This leads to either a plant activating defense responses or programmed cell death or a metabolic reprogramming to a new homeostasis. The comprehension of this crosstalk is more than an academic exercise, it has practical applications for the development of interventions that can improve heavy metal tolerance in cucumber and related cucurbits grown on contaminated agricultural soils.

6.1. Melatonin (MT) in Alleviation Melatonin in Alleviation of Cadmium Stress in Plants

Cadmium (Cd) is a non-essential toxic heavy metal which causes serious problems in plant growth, agricultural productivity and human health due to contamination in food chain. Melatonin (MT) is a multifunctional signaling molecule that has been recently introduced as a promising tool for reducing Cd-induced phytotoxicity(Lovynska, Bayat et al. 2024). The reactive oxygen species production inhibition, the improvement of photosynthetic efficiency and the regulation of gene expression of heavy metal transporters are the pathways through which exogenous melatonin application relieves cadmium stress. This synthesis aims to give a mechanistic insight into the Cd tolerance mechanism induced by melatonin in plant organisms based on the latest physiological and molecular evidence.

6.1.1: Suppression of Reactive Oxygen Species Production

Cadmium stress in plants is often accompanied by an excessive production of oxidants (superoxide anion, hydrogen peroxide and hydroxyl radicals). These molecules promote the oxidation of lipids, proteins, and nucleic acids, which results in dysfunction of cells. Melatonin is able to suppress the production of ROS by both direct and indirect mechanisms, with exogenous melatonin being effective. Melatonin has powerful direct antioxidant properties that can neutralize ROS before they are able to cause damage(Samanta and Roychoudhury 2025). Melatonin indirectly stimulates antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase. Melatonin prevents PCD and maintains redox homeostasis and thus preserves membrane integrity and prevents oxidative bursts. In plants subjected to Cd stress, it has been found that the plants treated with melatonin have significantly lower malondialdehyde and electrolyte leakage levels, which is a confirmation of reduced lipid peroxidation and membrane damage.

6.1.2: Enhancement of Photosynthetic Performance

Cadmium toxicity causes a significant reduction in photosynthesis by interfering with the biosynthesis of chlorophyll, chloroplast ultrastructure and electron transport chains. These effects are reversed by the application of melatonin by several pathways. Melatonin can be used as a first measure to increase the chlorophyll and carotenoid content by preventing the inhibition of enzymes engaged in tetrapyrrole biosynthesis, which is caused by Cd (Xiong, Zhang et al. 2021). Second, melatonin helps maintain thylakoid membrane structure and grana stacking and maintains light harvesting capacity. Thirdly, with Cd stress, melatonin increased NPR, stomatal conductance and TE. This is indicated by the increase in Fv/Fm ratio and quantum yield, which confirms that melatonin prevents the photosynthetic apparatus from being affected by photo-oxidative damage. Furthermore, melatonin facilitates regeneration of ribulose-1,5-bisphosphate and keeps the activity of the Calvin cycle enzymes, which helps to sustain carbon assimilation under Cd stress(Ahmad, Pataczek et al. 2018). As a result, plants treated with melatonin have an increased biomass and minimal growth retardation.

6.1.3: Regulation of Heavy Metal Transporter Genes

The expression of genes coding for the heavy metal transporters is an important mechanism of Cd alleviation mediated by melatonin. Cadmium is taken up by plants through main transporters of essential divalent ions of Fe, Zn and Ca(Al Mamun, Rahman et al. 2024). The ability of melatonin to downregulate the expression of Cd influx transporters such as natural resistance-associated macrophage protein family member NRAMP1 and NRAMP5 and iron-regulated transporter-like proteins (IRLPs) has been demonstrated. At the same time, melatonin stimulates the expression of genes involved in Cd compartmentalization and detoxification. For example, plant cadmium resistance proteins and heavy metal ATPases allow Cd to be sequestered into vacuoles and limit the amount of Cd present in the cytoplasm. In addition, melatonin promotes the expression of the genes that code for metal tolerance proteins such as those that code for phytochelatin synthase and consequently induces the production of phytochelatins and subsequent Cd chelation(Kaushik and Saini 2019). Melatonin prevents the translocation of Cd from root to shoot and enhances vacuolar storage, leading to reduced Cd levels in photosynthetic parts and consumable portions of plants and thus enhancing food safety.

6.1.4: Integrative Perspective

These three mechanisms do not work separately but are interconnected. Suppression of ROS generation helps to avert oxidative damage to photosynthetic complexes and complex proteins of the transporters, and the sustained photosynthetic capacity supplies energy and carbon skeletons for the production of antioxidants and chelators(Francis, Asif et al. 2024). The control of genes of transporters not only diminishes Cd uptake, but also decreases the availability of the metal to participate in redox cycles that generate ROS. Melatonin acts in concert to help re-program the metabolism of plants from a stress response to a growth sustaining one. Seed priming, root drenching or foliar spraying are the applicable ways of exogenous melatonin application; the best concentrations are 10-100 µM, depending on plant species and level of Cd stress(Fenibo, Ijoma et al. 2019). Under the heavy metal stress, the signaling crosstalk between melatonin and other phytohormones, such as abscisic acid, nitric oxide, and salicylic acid, needs to be better understood in future studies. The knowledge of the expression pattern of these transporter genes in different tissues at different times after melatonin treatment will further improve farm approaches to phytoremediation and safe production of crops in Cd-contaminated soils.

Table 3. Summary of transcriptomic studies revealing differentially expressed genes (DEGs) associated with key biological processes upon exogenous treatment with ameliorating agents

Study / Crop System	**Ameliorating Agent Applied**	**Major Differentially Expressed Genes (DEGs) / Pathways**	**Key Biological Processes Affected**	**Main Findings**	**Reference**
Rice (*Oryza sativa*) under salinity stress	Silicon (Si)	OsHKT1;5, OsSOS1, OsNHX1	Ion homeostasis, salt tolerance, osmotic adjustment	Silicon treatment enhanced expression of ion transporter genes and reduced Na⁺ toxicity under salinity stress.	Azad et al., 2024
Wheat (*Triticum aestivum*) under drought stress	Melatonin	DREB, MYB, WRKY transcription factors	Stress signaling, antioxidant defense	Melatonin induced transcription factors associated with drought tolerance and ROS scavenging.	de Oliveira et al., 2021
Cotton (*Gossypium hirsutum*) under heat stress	Salicylic acid	HSP70, HSP90, APX, CAT	Heat shock response, antioxidant metabolism	Exogenous salicylic acid upregulated heat shock proteins and antioxidant enzymes.	Shaban et al., 2019
Arabidopsis (*Arabidopsis thaliana*) under heavy metal stress	Biochar application	GST, MT2A, PCS1	Detoxification, metal sequestration	Biochar treatment activated detoxification-related genes reducing heavy metal toxicity.	Plants, 2022
Maize (*Zea mays*) under drought stress	Chitosan	NAC, LEA, P5CS	Osmoprotection, stress adaptation	Chitosan improved osmolyte accumulation and drought-responsive gene expression.	Jha et al., 2020
Tomato (*Solanum lycopersicum*) under cold stress	Glycine betaine	CBF1, COR15A, RD29A	Cold acclimation, membrane stability	Glycine betaine enhanced cold-responsive pathways and improved chilling tolerance.	Javaid et al., 2022

Rice (*Oryza sativa*) under oxidative stress	Selenium nanoparticles	SOD, POD, GPX genes	Reactive oxygen species detoxification	Selenium nanoparticles promote antioxidant defense through transcriptomic modulation.	Current Issues in Molecular Biology, 2025

6.2.2: The Role of Nitric Oxide and Hydrogen Peroxide as Signaling Molecules During Stress

Plants quickly produce nitric oxide and hydrogen peroxide both enzymatically and non-enzymatically under heavy metal stress conditions(Kanjana 2017). Nitrate reductase (NR) and nitric oxide synthase-like enzymes (NOS) are the key enzymes involved in the biosynthesis of nitric oxide (NO), and NADPH oxidases, cell wall peroxidases (CWPs), and amine oxidases (AOs) are important enzymes in the biosynthesis of hydrogen peroxide (H_2O_2). These molecules act as key signaling centers to trigger subsequent defense reactions vital for the survival of the plants. Low to moderate concentrations of nitric oxide are a powerful protective agent. Activates antioxidant enzymes, such as superoxide dismutase, catalase and ascorbate peroxidase, to anticipate future oxidative stresses in the cellular redox network. Nitric oxide also triggers the expression of genes that encode phytochelatins and metallothionein's that bind and incorporate toxic metal ions. Moreover, nitric oxide facilitates the synthesis of osmolytes like proline and glycine-betaine that help stabilize the protein structure and prevent dehydration of cells under metal-induced osmotic stress. Hydrogen peroxide is a two-edged signal(Mohammed, Haridevamuthu et al. 2026). Under controlled conditions, it initiates mitogen-activated protein kinase cascades which activate transcription factors including WRKY, MYB and NAC family transcription factors. The transcription factors activate a suite of stress-inducible genes that encode cell wall reinforcement, ion homeostasis, and oxidative defense. Further, systemically acquired acclimation (SAA), a whole plant response, occurs when hydroperoxide is applied, which results in increased resistance to subsequent stress events. Production of H_2O_2 is tightly regulated in space and time, with specific bursts generated in specific cellular compartments to provide specific information of hydrogen peroxide. In addition, both nitric oxide and hydrogen peroxide are involved in post-translational modifications that are used to fine-tune protein functions. S-nitrosylation is the covalent attachment of a nitric oxide group to thiols of target enzymes and is mediated by nitric oxide, which may activate or inhibit target enzymes. Thiol oxidation by hydrogen peroxide leads to changes in protein conformation and activity by the production of disulfide bonds. These changes are reversible and enable the cells to quickly respond to varying levels of metals in the environment.

6.2.3: The Detrimental Effect of Scavenging Protective Signaling Molecules

One of the conundrums in heavy metal stress biology is that eliminating nitric oxide and hydrogen peroxide sometimes has adverse effects on plant performance. The scavenging of these molecules results in the loss of beneficial effects of protective molecules, and thus in a stress intolerant phenotype(Gómez-Sagasti, Hernández et al. 2018). This is because plants need a minimum concentration of these molecules for them to trigger and maintain defense programs. The heavy metal induced expression of protective genes is dramatically decreased when exogenous nitric oxide scavengers, like 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide or hemoglobin, are applied. The metal chelation capacity is lowered due to the decreased activity of phytochelatin synthase and the cytosol will have higher free ion concentrations. Antioxidant enzyme activities also decrease and cells are at risk of oxidative damage. On the other hand, the presence of nitric oxide donors (such as sodium nitroprusside) before toxic metal exposure significantly increases tolerance, further supporting the protective effects of NO. Similar results are given for hydrogen peroxide. The upregulation of defense genes and the resulting sensitivity to metal toxicity is completely blocked by application of catalase, dimethyl thiourea or other hydrogen peroxide scavengers. Catalase treatment prevents the accumulation of hydrogen peroxide in guard cells during abscisic acid-induced stomatal closure, which would prevent metal uptake; this allows stomatal opening and increases metal loading in shoots. Likewise, the systemic response relayed from the roots to the leaves of metal-exposed plants relies on the diffusion of H2O2 through the plasmodesma or apoplast; the response is not systemic in the absence of this diffusion(Gómez-Sagasti, Hernández et al. 2018). There's a lot of meaning behind this signaling, and it's practical. Many of the strategies used in agriculture to improve stress tolerance are based on the use of antioxidants, including ascorbate, glutathione, and tocopherols. At the right concentrations these compounds are good – but at the wrong, they will actually remove the signals of nitric oxide and hydrogen peroxide which are also important compounds in the body. This is also true of genetic interventions: sometimes they have been designed to overexpress catalase or ascorbate peroxidase, resulting in metal-hypersensitive phenotypes simply because the signaling properties of H2O2 is altered. Accordingly, engineering of tolerance to the heavy metals needs to retain these signaling molecules. Modulation of nitric oxide and hydrogen peroxide production or perception, but not their complete elimination, will likely be most effective. These include specific targeted overexpression of particular NADPH oxidase variants that create localized hydrogen peroxide signals, targeting the activity of nitric oxide synthase in a tissue specific manner, and the use of protecting agents downstream of these signals.

6.3. Interaction of Jasmonic Acid and Ethylene in Heavy Metal Stress

Heavy metal contamination is a major stress that results in a complex hormonal signaling network, which is responsible for the adaptive response of the plants. The pathways regulated by jasmonic acid and ethylene are among the most dynamic and interdependent of these pathways(Yang, Cao et al. 2017). The mechanisms of action of these two phytohormones are not independent but rather they have complex crosstalk, which affects the type, level and timing of plant defense against toxic metal ions. The understanding of jasmonic acid-ethylene interactions is critical to reveal the stress-tolerance-growth-development balance of plants under heavy metal stress.

6.3.1: Biosynthesis and Perception of Jasmonic Acid and Ethylene Under Heavy Metal Stress:

Exposure to toxic metals, including Cd, Pb, Cu, Zn and Hg, causes the rapid biosynthesis of both JA and ethylene. The octadecanoid pathway, which involves the enzymes linolenic acid lipoxygenase, allene oxide synthase and allene oxide cyclase, synthesizes jasmonic acid from linolenic acid(Yang, Fang et al. 2023). The production of jasmonates is triggered by the release of linolenic acid from membrane lipids via phospholipases in response to heavy metal stress. Likewise, formation of ethylene is increased under metal stress because both ACC synthase and ACC oxidase, the two enzymes that convert S-adenosylmethionine to ethylene through the intermediate 1-aminocyclopropane-1-carboxylic acid, are upregulated. These hormones are perceived by specific receptor systems. The protein CORONATINE INSENSITIVE1 (CIN1) acts as a coreceptor with JASMONATE ZIM-DOMAIN proteins, recognizing jasmonic acid. Without jasmonate, the transcription factors like MYC2 are suppressed by JAZ proteins. The binding of jasmonate to its receptor in turn leads to the ubiquitination of JAZ proteins by COI1 and their proteasomal degradation, which frees MYC2 to induce gene expression. All ethylene perception requires a family of five receptor proteins, namely ETR1, ETR2, EIN4, ERS1 and ERS2. In the absence of ethylene, these receptors activate CONSTITUTIVE TRIPLE RESPONSE1 kinase, which represses ethylene responses(Sahoo, Yadav et al. 2025). Ethylene binding inactivates the receptors, thus relieving ethylene-induced CTR1 inhibition and enabling ethylene-induced gene expression in the presence of EIN2 and EIN3.

6.3.2. Synergistic and Antagonistic Interactions Between Jasmonic Acid and Ethylene

The relationship between jasmonic acid and ethylene under heavy metal stress varies from synergistic to antagonistic depending on the tissue, the concentration of the metal, the time of exposure and the physiological process studied(Tabara, Uraguchi et al. 2024). In the root tissues, these hormones can function, at least in part, in a synergistic manner to reduce metal uptake and enhance detoxification. Jasmonic acid and ethylene both induce the upregulation of heavy metal ATPase genes responsible for the transport of metal ions from the cytosol or to a vacuolar compartment. They also increase the synthesis of peptide chelators (phytochelatins and metallothionein's) that remove and sequester toxic metals. This synergy is supported by mutant studies: plants with defects in the biosynthesis and/or perception of jasmonates are more sensitive to metals and ethylene-insensitive mutants also accumulate more metal ions and are more susceptible to oxidative damage. The interaction in shoot tissues is more complicated. In general, jasmonic acid induces defense genes and delays the onset of senescence in response to moderate metal stress, thus maintaining photosynthetic capacity(Deng, Wu et al. 2025). Ethylene, in contrast, may hasten senescence and abscission, especially in the case of chronic and high levels of heavy metal stress. This difference in response is a strategic compromise. If the metal stress is not too severe, jasmonic acid signaling prevails allowing for growth and reproduction. Once the level of metals reach a critical threshold, ethylene signaling triggers senescence and frees up nutrients, and the removal of heavily contaminated tissues is controlled, which helps to neutralize the toxic challenge away from the rest of the plant. Several families of transcription factors are involved in the molecular mechanisms of jasmonate-ethylene crosstalk. The central regulators of ethylene responses, ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1, physically interact with MYC2 the master regulator of jasmonate signaling. This interaction may either activate or repress expression of the target gene based on the context of the target gene's promoter and the presence of other cofactors. ORA59 is also termed ap2 domain protein, an important node of crosstalk integration between jasmonate and ethylene signals. Both of these hormones induce ORA59 expression and its protein product binds to GCC-box elements in the promoters of defense genes, which induce their transcription.

6.3.3. Regulation of Metal Transport and Detoxification

The jasmonic acid and ethylene interaction is involved in strict regulation of heavy metal transport and detoxification systems. Both hormones are reported to down-regulate influx transporters like IRON-REGULATED TRANSPORTER1 and NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN, which prevent the uptake of cadmium, lead and other heavy metals into root cells(Kurniawati, Toth et al. 2023). At the same time, they also upregulate proteins that pump molecules out and proteins that sequester molecules into vacuoles. Jasmonic acid results in high expression of HEAVY METAL ATPASE4, which pumps metals into the xylem for transport to shoots, and ethylene in the activity of METAL TOLERANCE PROTEINs, which sequesters metals in root vacuoles, thereby reducing metal accumulation in shoots. This rule has application to phytoremediation and food safety. It could be possible to modulate jasmonate and ethylene pathways to lower the amount of metals in edible parts in soil-contaminated crops. On the contrary, the plant species used for phytoremediation having hyperaccumulation of metals in the shoots might have other manipulations of hormones. The knowledge of tissue-specific and metal-specific nuances of jasmonate-ethylene interaction will help to engineer heavy metal-tolerance and accumulation traits with precision, which will benefit both in safe food production and in cleaning up polluted environments.

7: Metabolic Reprogramming as a Toxicity Assessment Tool

Traditional growth and biochemical endpoints are being replaced by high throughput analytical platforms to assess heavy metal phytotoxicity and to provide a comprehensive metabolic profile of the phytotoxicity(El Bahgy, Elabd et al. 2021). Metabolomics is the large-scale study of low molecular weight metabolites, which provide a snapshot at the molecular level of the organism's functional state, and thus represent the coordinated result of the genome, transcriptome and proteome responses to the surrounding environment. Exposure to heavy metals in the cucumber causes an extremely powerful metabolic reprogramming that affects both the primary and secondary metabolism, resulting in metabolite signatures that can act as sensitive, quantitative and mechanistically informative markers of toxicity. Untargeted metabolomics does not measure pre-selected endpoints like individual biochemical assays, but rather gets an unbiased picture of the metabolic landscape for the identification of novel biomarkers and unexpected toxicity pathways

7.1.1 Primary Metabolism Shifts: Suppression of Nitrogen Metabolism and Activation of Hexose Metabolism under Copper Stress

The regulation of N and C metabolism in the reciprocal manner has been consistently reported with respect to heavy metal stress especially for copper in the case of cucumber(Abbas, Yousaf et al. 2020). Such metabolic shift, which involves downregulation of the metabolism of nitrogen-containing compounds and upregulation of the metabolism of hexoses, is a basic reprogramming of the metabolism of resources, which is protective and adaptive. The knowledge of this shift is necessary in order to interpret metabolomics data in toxicity assessment and quantify the extent of stress and the plant's ability to acclimate to stress through this two major metabolic pathways.

7.1.2: Suppression of Nitrogen Metabolism under Copper Stress:

Under phytotoxic copper exposure conditions the nitrogen assimilation as well as amino acid metabolism are always reduced in cucumber tissues(Yadav, Lal et al. 2019). The suppression is characterized by lower levels of several amino acids, especially those of importance in nitrogen transport and storage including glutamine, glutamate and asparagine. There are direct and indirect effects involved in this suppression, and the mechanism involved is not yet known. Copper ions have a direct inhibitory effect on the activity of the enzymes that convert ammonium into organic form, namely glutamine synthetase, and the enzymes that transfer amido nitrogen from glutamine to 2-oxoglutarate to yield two molecules of glutamate, called glutamate synthase. This decrease in availability of glutamine and glutamate is transmitted through transamination reactions, resulting in the decreased production of production of virtually all amino acids. Indirectly, copper-induced oxidative damage has an effect on the function of the enzymes of the chloroplast and cytosol involved in nitrate reduction to ammonium, nitrate reductase and nitrite reductase. The net result is a significant loss of ability of the cucumber plant to absorb, utilize and transport nitrogen, which will impact the synthesis of proteins, nucleic acids, and chlorophyll. Suppression of nitrogen metabolism is not only a limitation of growth. Nitrogen-containing compounds are components of the photosynthetic pigments, so a decreased availability of nitrogen directly also affects the chlorosis that is a symptom of copper toxicity in cucumber leaves. Chlorophyll molecules consist of four pyrrole rings that are formed by glutamate-related precursor molecules and if nitrogen assimilation is impaired, the synthesis of the chlorophyll molecules is no longer sufficient to replace the molecules that have been degraded(Ahmad, Pataczek et al. 2018). The decrease of amino acid pools also leads to a decrease in protein antioxidant enzymes, which could result in a vicious cycle of further oxidative damage to nitrogen assimilation enzymes and further decrease in antioxidant enzymes. Interestingly, some amino acids (especially proline) tend to accumulate paradoxically under copper stress, while other N metabolites generally accumulate under stress(Sun, Li et al. 2022). Proline is an osmolyte, a ROS scavenger, a protein stabilizer and is accumulated via increased production in the presence of glutamate and through decreased degradation and increased protein turnover.

7.1.3: Activation of Hexose Metabolism under Copper Stress:

In parallel with the suppression of nitrogen metabolism, copper-stressed cucumber plants enhance hexose metabolism, resulting in increased levels of glucose, fructose and their phosphorylated metabolites. The activation has multiple adaptive functions which are all contributing to copper tolerance. Soluble sugars are first used as compatible osmolytes to accumulate and keep the cells turgid and proteins stable in metal-induced water deficit(Jiang, Song et al. 2021). Copper stress effects on root hydraulic conductivity and stomatal conductance can cause physiological drought conditions under normal soil water conditions. The net result is a decrease in the cell's osmotic potential, which allows water to continue to enter the cells and maintains the driving force for water to flow through the transpiration stream from roots to shoots that carries the essential nutrients. Second, hexoses is a direct and indirect ROS scavengers. Hydroxyl radicals react non-enzymatically with sugars and sugar-derived metabolites are used as carbon skeletons in the biosynthesis of antioxidants in the ascorbate-glutathione cycle. Under copper stress, the pentose phosphate pathway is activated to convert glucose-6-phosphate to ribulose-5-phosphate and produce NADPH, which is used for glutathione reductase and other ROS-scavenging enzymes. Carbohydrate metabolic products are also used as precursors for the biosynthesis of phenolic compounds and flavonoids; both phosphoenolpyruvate and erythrose-4-phosphate, which are derived from the metabolism of carbohydrates, are the starting point for the biosynthesis of these compounds in the shikimate pathway. Third, accumulation of hexose would supply substrates for respiratory ATP production to respond to the increased energy demand of stress responses. ATP is needed by copper-stressed cucumber plants for metal transporter activity, synthesis of phytochelatins, production of antioxidants enzymes, and for the repair of damaged proteins and membranes. This increased energy requirement is reflected by the increased levels of hexose phosphates and TCA cycle intermediates, a sign of enhanced glycolysis and tricarboxylic acid cycle activity, respectively(Li and Wang 2023). In addition, increased activation of hexose metabolism may be related to decreased requirement for carbohydrate consumption during growth processes; decreased cell division and expansion associated with copper toxicity may result in re-routing of photosynthetically fixed carbon from structural polysaccharides into soluble pools.

7.1.4: Diagnostic Value of the Nitrogen-Hexose Balance

A reciprocal relationship between suppressed nitrogen metabolism and activated hexose metabolism leads to a diagnostic ratio which is an integrated biomarker of the severity of copper toxicity. The amino acid: soluble sugar ratio is relatively stable for healthy cucumber plants and has been observed to increase when the carbon to nitrogen assimilation ratio is favorable(Maitra, Brestic et al. 2021). For healthy plants the amino acid to soluble sugar ratio remains relatively constant, with the increase in the amino acid/sugar ratio when the assimilatory ratio of carbon to nitrogen is favorable for biosynthetic needs. When copper stress, this ratio changes significantly, because the nitrogen-carbon balance changes in favor of the accumulation of carbon. This metabolic signature can be identified before chlorosis or necrotic lesions are observed, and is an early warning sign of developing copper toxicity. In addition, the magnitude of the metabolic shift is directly related to the copper concentration and exposure time, and can be used to perform quantitative toxicity assessment in cases where growth endpoints are not clearly understood. Metabolomics studies have been able to distinguish different types of heavy metals based on their unique metabolic signatures, and in particular copper had a more marked repression of nitrogen metabolites than cadmium and lead(Wahab, Muhammad et al. 2023). This specificity may allow the ultimate ability to diagnose contaminated agricultural soils by specific toxicant using metabolic profiling and to design remediation actions for a specific toxicant.

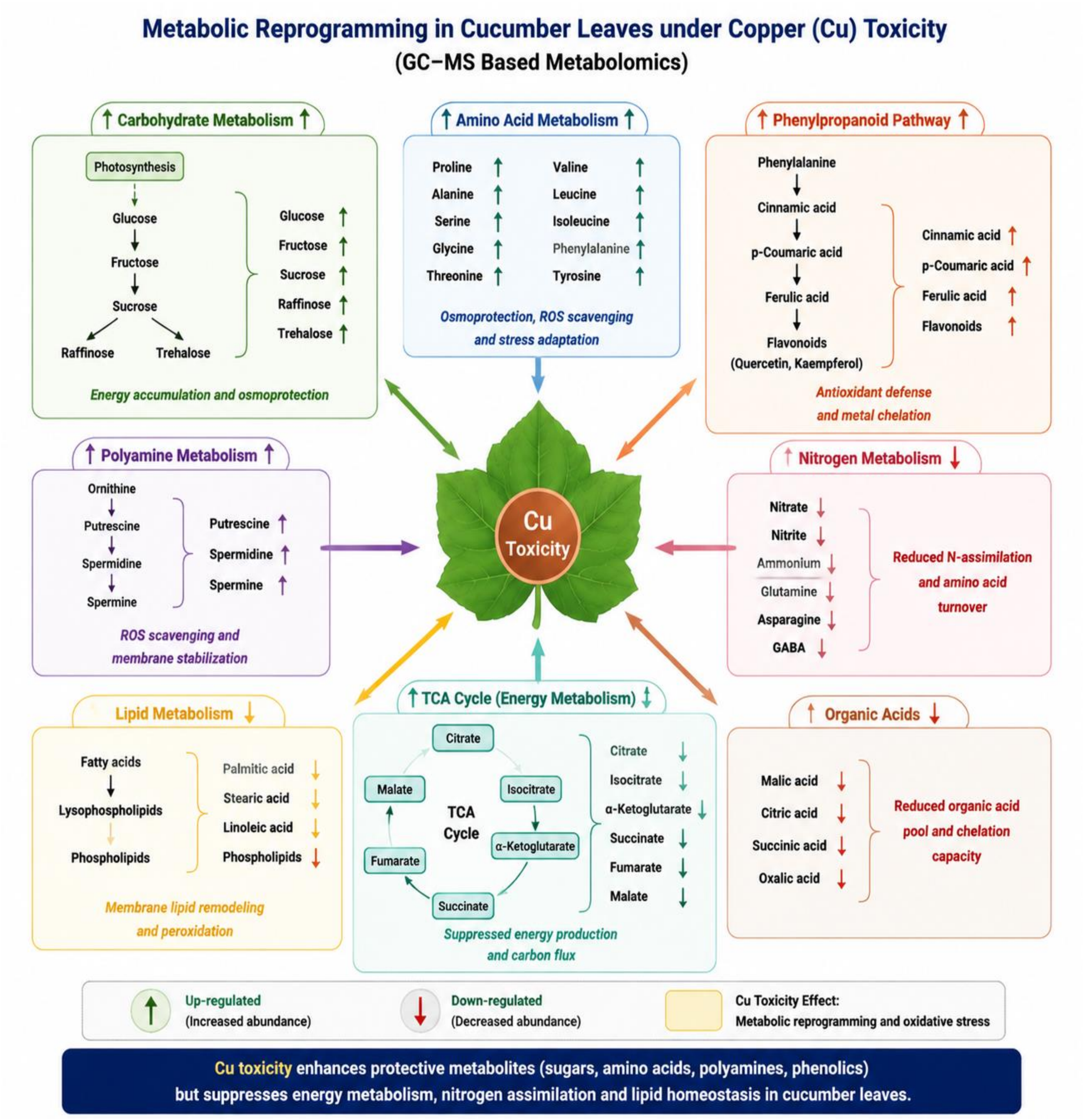


Figure 2. Physiological changes and tolerance to copper induced heavy metal stress in cucumber leaves.

7.2. Secondary Metabolites: Accumulation of Ferulic Acid and Other Phenylpropanoids as a Defense Response:

Environmental stress factors such as heavy metal toxicity, pathogen attack and ultraviolet radiation induce plants to invoke a complex array of biochemical defence mechanisms. One of the most well known and chemically varied of these responses is the buildup of secondary metabolites, especially the phenylpropanoids, ferulic acid, caffeic acid, coumaric acid and sinapic acid(Singh, Sangwan et al. 2021). The compounds are not only end metabolites but also active parts of the adaptation process to stress. In addition, the development of metabolic profiling or metabolomics has transformed the way plant stress responses can be evaluated with a precise and high resolution fingerprint of the whole plant chemical landscape. Therefore, the knowledge of the defense role that phenylpropanoids play in plants and the analytical potential of metabolomics is essential in contemporary research on plant stress physiology and crop improvement.

7.2.1. Accumulation of Ferulic Acid and Other Phenylpropanoids as a Defense Response

One of the most abundant of the phenylpropanoids in plant cell walls is hydroxycinnamic acid derivative, ferulic acid. Plants accumulate and synthesize substantial amounts of ferulic acid and related phenolics, such as p-coumaric, sinapic and chlorogenic acid, under heavy metal stress(Ibrahim, Said et al. 2024). The Phe-AALC pathway begins with the deamination of phenylalanine to cinnamic acid by the rate-limiting enzyme in the whole pathway, phenylalanine ammonia lyase (PAL). The various phenylpropanoid end products are generated by subsequent hydroxylation and methylation reactions which are catalyzed by enzymes such as cinnamate-4-hydroxylase, 4-coumarate-CoA ligase and caffeic acid O-methyltransferase. Ferulic acid and phenylpropanoids play several roles in the defense of plants. These compounds are known as potent antioxidants and directly scavenge the ROS produced upon exposure to heavy metals. In particular, ferulic acid contains a phenolic hydroxyl group that can donate hydrogen atoms to neutralize free radicals in the cell, helping to prevent the damage of cellular membranes, proteins and nucleic acids. Secondly, phenylpropanoids make the cell wall more rigid by cross-linking with polysaccharides. Ferulic acid is covalently linked to the arabinoxylan side chains and oxidized to other formulate residues, which generates a 3-D network that physically prevents the penetration of the metal ions into the symplast. This apoplastic barrier is an important first barrier to toxic heavy metal like cadmium, lead and copper. Third, phenylpropanoids are metal chelators. The o-dihydroxy structures of caffeic acid and other compounds complex with divalent metal ions, decreasing the bioavailability and toxicity of the compounds. Forth, they are secondary metabolites which induce systemic acquired resistance, a priming effect on distant tissues for better defense in the event of a second attack. Ferulic acid and related compounds are typically associated with the induction of pathogenesis-related proteins and lignin deposition, which form a coordinated defense syndrome. Accumulation of phenylpropanoids is highly regulated in space and time. The root tips and vascular tissues generally exhibit the most noticeable and rapid responses after exposure to heavy metals because they are the major entry and movement sites. Phenylpropanoids are stored in the apoplast and vacuoles in leaves and bind metal ions before they can enter the photosynthetic machinery(Kovács, Kutasy et al. 2022).The phenylpropanoid profiles differ significantly, indicating specificity in biosynthetic response, for different metals. The exposure to cadmium causes increased accumulation of caffeic acid and ferulic acid, while copper exposure causes more accumulation of coumaric acid and sinapic acid. The high metal-specific fingerprint confirms the accuracy of plant metabolic response.

7.2.2: The Potential of Metabolic Profiling as an Accurate Assessment Fingerprint

The methods used to determine plant stress responses are typically based on the measurement of a single physiological indicator (chlorophyll content, enzyme activity or expression of a specific gene). These reductionist approaches provide only a partial view of this complex metabolic remodeling that takes place in response to stress. Metabolomics, the detailed profiling of all small molecule metabolites in a biological sample, is a paradigm shift, as it will provide a true assessment fingerprint that will reflect the integrated outcome of genetic, regulatory and environmental effects(Rathod 2024). A typical approach to metabolomic profiling is high-resolution analytical platforms like Liquid Chromatography and Mass Spectrometry, Gas Chromatography and Mass Spectrometry or Nuclear Magnetic Resonance (NMR) spectroscopy. These technologies are able to identify and measure hundreds to thousands of metabolites including all phenylpropanoids, organic acids, amino acids, sugars and lipids. The resulting metabolic fingerprint is a measure of the physiological state of the plant at a particular time, reflecting both short- and long-term adaptive responses. Since the dawn of metabolomics, a number of essential insights have been obtained in the field of secondary metabolite research. Metabolic profiling first shows that elevation of ferulic acid and other phenylpropanoids under stress conditions does not occur in isolation, but rather as part of a general reprogramming of carbon flux. Phenylalanine, the common precursor of the whole phenylpropanoid pathway, is channeled into secondary metabolism, with a coordinated response in primary carbohydrate and nitrogen metabolism. Second, metabolomics can generate a discovery of novel metabolites that respond to stress, which would not have been detected by targeted analyses. The untargeted profiling revealed several glycosylated phenylpropanoids and conjugates, thus enriching the plant defense compound library. Third, metabolomic fingerprints can be used as good diagnostic markers in the stress diagnosis(Sychta, Słomka et al. 2021). The relative quantification of particular ferulic acid derivatives or caffeoylquinic acid, or the flavonoid glycosides, is reliable and can be used to differentiate different heavy metal stressors, exposure length, tolerant and sensitive genotypes. These fingerprints are more sensitive and specific than traditional stress indicators, and can detect contamination in advance of visual symptoms. Fourth, metabolomics can be used to identify varieties of crops that are more stress tolerant. Breeders can determine genotypic responses to metal stress by comparing metabolism in various germplasm in controlled metal environments and select those that exhibit a strong phenylpropanoid response or superior metal exclusion or chelation capacity. Metabolomics can be combined with other omics areas to enhance its analytical capacity. Transcriptomics identifies genes that become induced, proteomics verifies enzyme accumulation and metabolomics verifies the final chemical output. This system biology-based strategy helps to understand plant stress response processes from the gene to metabolites. With the continued development of analytical technologies, and increasingly sensitivity, resolution, and throughput, metabolomics will be increasingly available for routine monitoring of plant health and contamination.

8. Novel Approaches and Amelioration Strategies:

Soil remediation and chemical treatments are only a part of the way that heavy metal toxicity in cucumbers have been reduced. In recent years, studies have been conducted to develop integrated, sustainable, and biologically based strategies that not only minimize metal accumulation but also strengthen the plant's natural resistance(Yasur and Rani 2013). These innovative strategies range from plant growth-promoting bacteria, rootstock grafting, machine learning-based trait prediction, to studying natural metal resistant microbial communities. These strategies can be integrated into a comprehensive strategy to manage heavy metal contained agriculture systems while keeping the productivity and food safety of crops.

8.1. Nanotechnology and Fullerene Derivatives:

Nanotechnology and plant stress physiology intersect in new ways to provide a means to reduce heavy metal toxicity in agricultural systems. Fullerene derivatives have been found to be promising candidates for protection of plants against cadmium, lead, copper and other metal contaminants from the various materials being explored. These carbon-based nanoparticles have unique physicochemical properties that allow them to act as direct antioxidants, as well as modulators of metal transport and bioavailability(Dimkpa, Adzawla et al. 2023). Mechanisms involved in fullerene derivatives mitigating heavy metal stress are critical for moving fullerene-based nanomaterials from the lab to the field.

8.1.1 Use of Carbon Nanomaterials to Restrict Xylem Transport of Copper and Alleviate Oxidative Stress

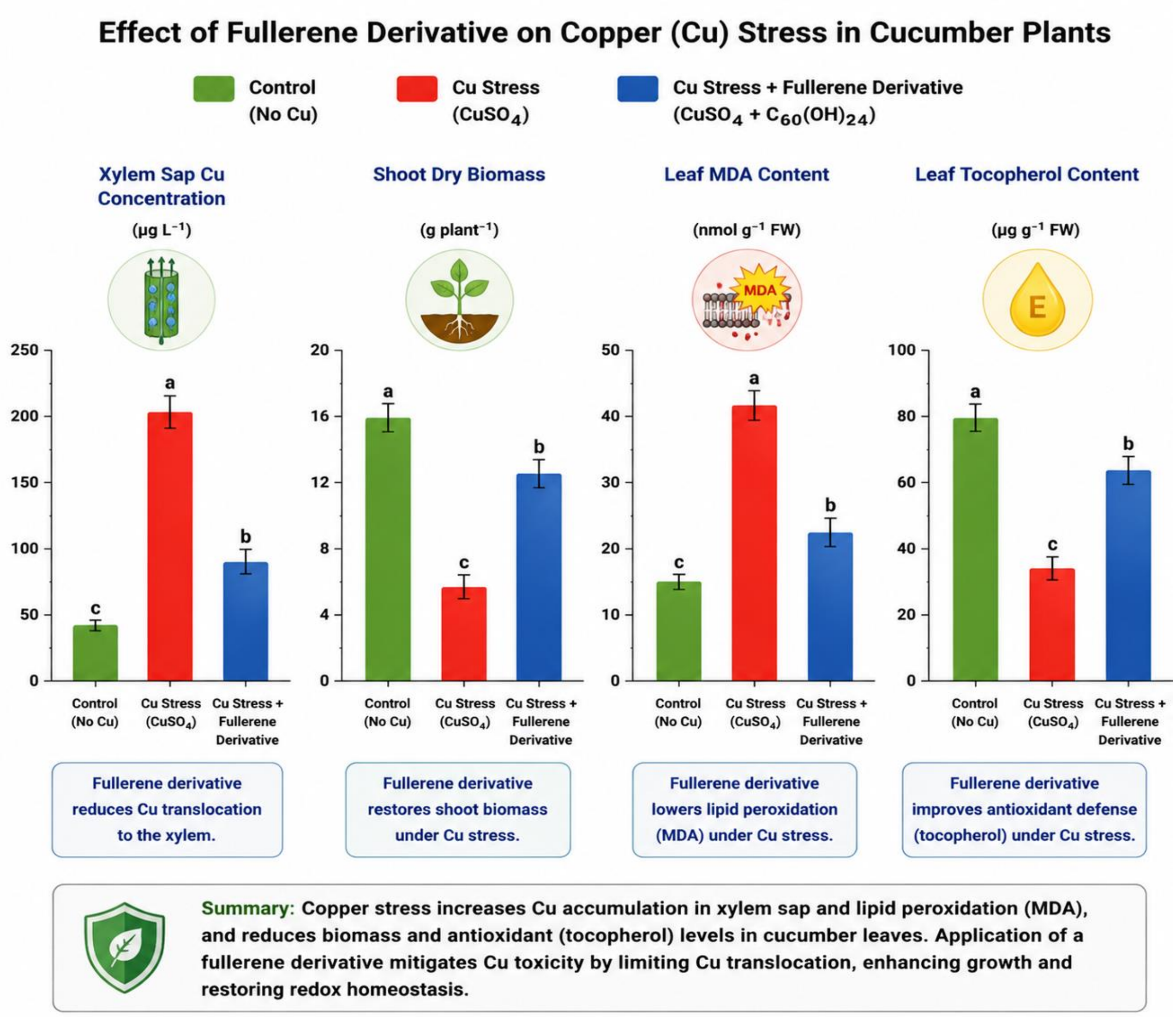
The application of carbon based nanomaterials, especially water-soluble derivatives of C60 fullerene molecule, has opened a new avenue in amelioration of the heavy metal toxicity in cucumber(Ashraf, Kanu et al. 2015). Fullerene derivatives work by a complex dual mechanism, first by limiting the movement of metals through xylem and secondly by directly neutralizing oxidative stress at the cellular level, mechanisms that are different from those of conventional soil amendments or chemical chelators, which tend to focus on immobilizing or precipitating metal in the root zone. This nanotechnology based approach has been best characterized for copper toxicity, where excess copper leads to high levels of oxidative damage and also causes interference with primary metabolic pathways. The one-of-a-kind physicochemical properties of C60 derivatives such as their superior electron-accepting ability, high surface area to volume ratio, and biocompatibility make these compounds more suitable for protecting cucumber from the complex and multi-factorial factors of heavy metal contamination.

8.1.2: Comparative Efficacy of Fullerene Derivatives in Copper-Stressed Cucumber:

Two functionalized C60 derivatives, fullerenol, a polyhydroxylated C60 derivative and an arginine-functionalized C60 derivative have been tested in systematic investigations for their ability to alleviate the toxicity of copper in hydroponically cultured cucumber. The typical copper phytotoxicity symptoms were seen within days when cucumber plants were treated with high copper (15 micromolar). Two functionalized fullerene C60 derivatives, fullerenol and an arginine-functionalized C60 derivative, have been investigated for their ability to alleviate copper toxicity in hydroponically-grown cucumber in systematic investigations(Banu 2025). Phytotoxicity symptoms were observed within days when cucumber plants were treated with a high rate of copper (15 micromolar). The concentration of copper in the xylem sap rose significantly, suggesting good root-to-shoot movement of metals. This was paralleled by delayed growth of dry biomass, severe leaf chlorosis due to the loss of photosynthetic pigments and the accumulation of several antioxidant metabolites that were induced to fight oxidative stress. Metabolomic profiling of copper-stressed leaves showed a unique profile of suppressed metabolism of nitrogen-containing compounds and activated metabolism of hexose compounds, with the increased production of antioxidant compounds such as ascorbic acid, tocopherol and ferulic acid.

8.1.3: Mechanistic Basis and Metal Specificity of Fullerene Protection

The fullerene adducts when added to the hydroponic growth medium yielded significant protection, although there were considerable variations between the two adducts. Both nanomaterials reduced xylem sap copper levels relative to copper-stressed plants (without fullerene treatment), suggesting a mechanism for limiting the movement of copper from roots to shoots(Mir, Bhat et al. 2022). But the C60 derivative with the arginine group showed a greater efficiency in various assessment parameters. Critically, the metabolic responses of plants treated with arginine C60 under high Cu stresses were almost opposite to that of Cu stress. Fatty acids, such as linolenic acid were up-regulated, whereas antioxidant molecules such as tocopherol were significantly down-regulated. This pattern is indicative of a successful alleviation of oxidative stress; the nanomaterial itself is so effective in protecting against oxidative stress that the plant cells do not need to produce more antioxidants. In short, unlike fullerenol, modest changes in growth parameters in copper-stressed plants were not statistically significant under strict multivariate analysis when the metabolic effects of fullerenol were studied. We found that there was a significant increase in copper concentrations, suggesting that the fullerene core has a higher electron-accepting capacity or specific binding affinity for copper ions, and that the root-to-shoot movement of metals was more efficient after the molecule was functionalized with the arginine moiety(Cesco, Pii et al. 2021). This was paralleled by delaying dry biomass accumulation, extensive leaf chlorophyll degradation (reflected in chlorosis) and stimulation of several antioxidant metabolites, as the plant appeared to defend against the oxidative stress. Two functionalized C60 derivatives have been studied for their ability to alleviate Cu toxicity in hydroponically-grown cucumber: a fullerenol, a polyhydroxylated derivative of C60, and an arginine-functionalized C60 derivative. Plants of cucumber seedlings grew in excess copper (15 micromolar), and the typical symptoms of phytotoxicity appeared in a few days. There was a significant increase in copper concentration of xylem sap, which is an indication of efficient root-to-shoot transport of the metals. This was accompanied by a delay in the growth in dry biomass, high levels of leaf chlorosis, indicative of photosynthetic pigment degradation and the induction of several antioxidant metabolites in an attempt to deal with the oxidative challenge. Metabolomic profiling of copper stressed leaves showed a characteristic disturbance pattern of suppression of nitrogen containing compound metabolism, while hexose metabolism was activated and antioxidant molecule metabolism such as ascorbicacid, tocopherols,and ferulic acid was significantly uregulated.



Fullerene derivative reduces Cu translocation to the xylem.

Fullerene derivative restores shoot biomass under Cu stress.

Fullerene derivative lowers lipid peroxidation (MDA) under Cu stress.

Fullerene derivative improves antioxidant defense (tocopherol) under Cu stress.

Summary: Copper stress increases Cu accumulation in xylem sap and lipid peroxidation (MDA), and reduces biomass and antioxidant (tocopherol) levels in cucumber leaves. Application of a fullerene derivative mitigates Cu toxicity by limiting Cu translocation, enhancing growth and restoring redox homeostasis.

Figure 3. Mitigation of copper (Cu) toxicity in cucumber plants by application of fullerene derivative is evidenced by reduced Cu accumulation, decreased lipid peroxidation and enhanced biomass and antioxidant status of plants

8.2. Plant Test Systems: Development of Standardized Cucumber-Based Biotests for Ecotoxicological Evaluation of Contaminated Soils:

The fullerene adducts added to the hydroponic growth medium showed significant protection with the two adducts differing significantly. Copper stress alone reduced xylem sap copper levels, but both nanomaterials reduced copper levels as well, suggesting a mechanism with limiting movement from root to shoot. The derivative, however, which has arginine attached, was more effective in several evaluation parameters(Rani and Sagar 2024). Most importantly, metabolic responses of plants exposed to arginine C60 under high copper conditions were basically the inverse of those exposed to copper alone. The fatty acids (e.g. linolenic acid) were over-expressed, and the antioxidant molecules (e.g. tocopherol) were very under-expressed. Overall, this pattern is indicative of the effective alleviation of oxidative stress, as the plant is not only expressing high levels of antioxidant production internally, but the nanomaterial itself is also providing an adequate level of antioxidant protection. However, fullerenol had limited effects on the growth parameters, but its effect on the metabolic state of copper-stressed plants was not statistically significant in the strict multivariate analysis. These differences in efficacy mean that the functional group on the surface is just as important as the nanomaterial itself to determine the efficacy of the nanomaterial; the arginine group may have a role in cellular uptake, in facilitating specific interactions with copper ions, or in maximizing the electron-accepting capacity of the fullerene core. corbic acid, tocopherol, and ferulic acid. Fullerene adducts when added to the hydroponic growth medium caused significant protection with marked differences between the two fullerene derivatives. Without fullerene treatment, both nanomaterials reduced xylem sap copper concentrations compared to copper stressed plants, suggesting a mechanism involving the limitation of metal movement from root to shoot(Khan, Arif et al. 2012). The C60 derivative with arginine, however, exhibited enhanced activity with respect to several evaluation parameters. Notably, the plant metabolic responses to arginine C60 under high Cu level was almost the inverse of the responses under copper stress alone. The fatty acids like linolenic acid were upregulated and antioxidant molecules like tocopherol were significantly downregulated. This pattern clearly indicates that alleviation of oxidative stress is occurring, and that the nanomaterial is enough to provide antioxidant protection for the plant, without the need to increase its own production of antioxidants. In contrast, fullerenol did not have a statistically significant effect on the metabolic state of copper-stressed plants, despite having moderate effects on growth parameters under conditions of strict multivariate analysis. These differential effects highlight the critical role of surface functionalization for the performance of nanomaterials; the arginine may help with uptake into cells, specific interactions with copper ions, or increase the electron-accepting ability of the fullerene framework.

8.2.2: Historical Validation and Comparative Sensitivity

There are two complementary pathways that proposed fullerene derivative toxicity-relieving mechanisms operate in a concerted fashion(Álvarez-Montero, Mercado-Reyes et al. 2025). These nanomaterials are believed to primarily work by blocking transport of copper from roots to above ground parts of plants via xylem. Fullerene derivatives bind to copper ions in the apoplast of the root or help to maintain them in the root tissues, avoiding the transport of copper ions to photosynthetic tissues where they could inhibit PSII activity and act as hydroxyl radicals by Fenton chemistry. This xylem-restricting feature is very useful because Cu is a required micronutrient and cannot be totally omitted from plant uptake, but instead efforts to ameliorate must be aimed at maintaining Cu homeostasis within a narrow range between deficiency and toxicity. Treatment with fullerene does not result in the complete transport block because it causes the concentration of xylem sap copper to be lowered but not eliminated. The second process is the intrinsic antioxidant properties of the molecule of fullerene C60. The carbon cage structure has an exceptional electron accepting ability and scavenges up to several types of ROS including superoxide, hydrogen peroxide and highly damaging hydroxyl radicals without being consumed in the process. In fact, this catalytic antioxidant activity is independent of the plant's own defence mechanisms. Fullerene derivatives decrease the oxidative burden directly, thereby alleviating the need for upregulation of the carbon intensive antioxidant metabolites as a result of stress. The fullerene's decreased oxidative burden frees up carbon skeletons and reducing equivalents for growth and maintenance. This interpretation is in line with the metabolomic results showing that arginine C60 treatment results in a decrease in tocopherol, which is a lipophilic antioxidant that typically increases during oxidative stress. However, it is also crucial that the protective effect of fullerene derivatives is highly dependent on the type of metal. In contrast, the same fullerene derivatives were observed to be quite effective against zinc toxicity in cucumber. Zinc transport from roots to shoots did not differ significantly from the control under excess zinc conditions when the zinc concentrations were measured in xylem sap in the presence of fullerenol or arginine C60. The fullerene derivatives did not significantly inhibit the decrease in dry biomass, but they did not affect the accumulation of zinc in leaves, shoot dry biomass or chlorophyll content. The results of adsorption experiments showed that the amount of zinc adsorbed by the tested C60 derivatives were below the detection limit. The metal dependent efficacy can be explained on the basis of difference in chemical properties between copper and zinc. Copper is a redox-active transition metal that is easily involved in electron transfer reactions, and hence can interact with the electron rich fullerene surface. In contrast, zinc does not undergo any redox changes in physiological conditions and the number of ionic interactions is quite large that the fullerene cage is not able to interact with efficiently.

8.2.3: Multiparameter Endpoint Selection and Biomarker Integration:

Current growth-based biotests have been expanded to multiparameter assessment to include biochemical/molecular endpoints. Whilst standardized methods are based on emergence, biomass and visible symptoms of phytotoxicity (chlorosis, necrosis and deformation), modern research has shown that other endpoints can be included and can give mechanistically informative data. Random Amplified Polymorphic DNA markers have been used to assess the genotoxic responses of cucumber to soil contamination with heavy metals and the result has been a confirmation that this technique can detect DNA damage in plant growing in heavy metal contaminated soils. For copper exposure studies, polymorphic fragments were observed in all the genomic DNA examined of the copper exposed cucumber, but not in any DNA of the unexposed plants and the highest concentration (500 mg/kg soil) was the most effective . These molecular markers provide a fast and simple method to detect genotoxicity which is not evident from the naked eye or growth alone

8.2.4: Adaptation to Diverse Exposure Scenarios and Future Directions:

The versatility of the cucumber-based biotests has made it possible to adapt them to other exposure scenarios than simply assessing levels of soil contamination. The OECD 208 framework has been adapted successfully to assess the ecotoxicity of water-soluble aerosol products, to test the effects of composting products of plastic materials as part of the ASTM D6400 certification and to evaluate the effects of veterinary medicinal products applied to manure prior to soil incorporation. The composting applications involve mixing sample compost with the substrate at ratios ranging from 25% to 50% and comparing germination numbers and plant biomass to blank compost controls at various mixing rates(Kohli, Sreenivasulu et al. 2013). This versatility highlights the strength of the cucumber biotest system for a variety of regulatory and research purposes.

8.2.5: Practical Considerations for Routine Implementation:

The extended use of cucumber for standardized biotests is further confirmed by the fact that practical aspects of the use of cucumber facilitate routine use in testing laboratories. Cucumber seeds are inexpensive and readily available commercially with high germination percentages and without dormancy problems. The species has a relatively short generation time, allowing for full testing to occur within 14 – 21 days as outlined in regulations(Bano, Amist et al. 2019). The morphology of cucumber seedlings is easily handled and their responses to stress can be easily observed and quantified without specialized equipment. To assess biotest results and compare them with other ecological and human health risk assessment methodologies, the extensive background information on cucumber physiology, metal accumulation patterns and oxidative stress responses is useful. The standardized framework provided by Environment Canada, OECD, and ISO will provide a solid base for the further development of cucumber-based biotests, a framework that can be expanded to include more endpoints such as metabolomic profiling, photosynthetic pigment analysis and the measurement of antioxidant enzyme activity.

9. Conclusions and Future Perspectives

Overall, the assessment of cadmium and heavy metal stress in plants has given a comprehensive picture of plant adaptation and defense, though it has proven to be complex and coherent. Traditional evaluations have been based on single endpoints like root elongation, shoot biomass or single enzyme activities. Important, but these parameters do not convey the coordinated physiological changes that underlie plant tolerance. Growth measured by the length of roots, the height of shoots, the amount of biomass produced, is the culmination of all metabolic processes. Restored growth occurs when melatonin and other protective compounds also inhibit reactive oxygen species, stabilize the photosynthetic electron transport, modulate genes of the metal transporters and normalize the balance of jasmonic acid and ethylene hormone. These insights are only gained from integrated evaluation. This paradigm shift is now in progress from a single biomarker to a whole biochemical fingerprint provided by lipidomics and metabolomics. Individual measurements may lead to very different conclusions when based on different levels of stress or taken at different times and cannot reflect the presence of properties in the network that are influenced by redundancy and feedback loops(Zboińska, Janeczko et al. 2023). Lipidomic analysis shows that cadmium is able to alter the composition of the membranes, increasing the amount of saturated fatty acids, decreasing the amounts of polyunsaturated fatty acids and creating peroxidation products like malondialdehyde and 4-hydroxynonenal. Hundreds of lipid species, including, provide detailed fingerprints of membrane restoration following successful alleviation. Complementing this, metabolomics can be used to detect characteristic patterns of accumulation of phenylpropanoids (ferulic acid, caffeic acid, coumaric acid, sinapic acid) that are diagnostic of metal type, concentration and duration of exposure. Amino acid profiles show nitrogen allocation, organic acids show chelation and sugar profiles show photosynthetic output. Although significant advances have been made with controlled experiments, there is still a considerable gap between wet-lab experiments and field validation. Lab plants have consistent weather, sterile soil, consistent amounts of various metals, and constant temperatures, unlike the dynamic field environment where the soils are heterogeneous, have microbial communities, competing ions, and varying weather conditions. The alleviation strategies successfully used in growth chambers often fail to work well in the field. The optimal laboratory concentrations of 10-100 micromolar do not directly correspond to the application rates that would be needed in the field for the degradation to be optimal as a function of spray volume(Hasanuzzaman, Gill et al. 2012). Enhanced cadmium exclusion can be detrimental in a situation of lack of another metal, like iron or zinc. This shift from reductionism to systems thinking is not only a technical improvement, but a change in the way plant stress responses are comprehended and dealt with.

10. Conflict of Interest

All authors have no conflict of interest

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