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BRINJAL UNDER SIEGE: MECHANISMS AND MANAGEMENT OF HEAVY METAL CONTAMINATION

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Abstract
The pollution of agricultural soils with heavy metals has become an important problem for food security and human health, especially for vegetable crops grown in peri-urban and industrial regions. The solanaceous vegetable Brinjal (Solanum melongena L.) has a high tendency for root uptake and rapid translocational efficiency of toxic ions and thus is very sensitive to metal toxicity. The present review is an attempt to summarize the existing information regarding the sources, absorption of heavy metals, physiological disturbances and mitigation strategies under heavy metal stress in brinjal. The major toxic metals of concern are: Cadmium (Cd), Lead (Pb), Nickel (Ni), Copper (Cu), and Chromium (Cr) which are usually found in wastewater irrigation, industrial effluents and agrochemicals. Phytotoxic effects in the crop include inhibition of seed germination, stunted groweth, chlorosis, necrosis, and fruit yield and quality reduction, which are dose-dependent with regard to metal exposure. In the biochemical level, heavy metals induce overproduction of ROS which results in lipid peroxidation, loss of electrolytes and induction of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). Molecular responses include induction of phytochelatins (PCs) and metallothioneins (MTs); however, genetics understanding in brinjal is still in early stages as compared to model plants. Soil amendments such as biochar and chitosan, which bind metals and decrease bioavailability, and the use of biochar and foliar application of iron oxide nanoparticles (FeONPs), which boosts antioxidant defense, are some of the promising remediation strategies. Low-accumulating cultivars is a biological solution to the challenge of selection. There are still gaps in knowledge related to field scale testing of nano-remediation, interactions effects between multi-metal contamination, and the lack of molecular marker-assisted breeding programs. This review gives an in-depth account of the mechanism of heavy metal stress in brinjal and proposes future directions to grow brinjal safely.

1. INTRODUCTION

The accumulation of heavy metals in agricultural land over the last 50 years has been an ever-worsening environmental problem all over the globe, caused by fast industrialization, urbanization and intensive agriculture(ARORA et al., 2024). Heavy metals stay in soil ecosystems for decades to millennia and are not biodegraded or biotransformed through any biotic or abiotic process. The Food and Agriculture Organization (FAO) and the United Nations Environment Programme (UNEP) have jointly estimated that about 20 million hectares of agricultural land are contaminated in the world with toxic metals concentrations higher than the natural geochemical background concentrations. Cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), nickel (Ni), copper (Cu), and mercury (Hg) are the most important metals in crop production and human health that pose a threat(ALAM, 2022). Cadmium (Cd), lead (Pb), and nickel (Ni) are especially worrisome of these because they have no known essential function in human or animal biology, but can be readily absorbed by crop roots and transported to consumable tissues of the crops(Bushra et al., 2022). The spatial distribution of contaminated foci is related well with industrial activity(Bushra et al., 2022). The total contaminated farmland is more than 10 million hectares in China, approximately 5 million hectares in India, Pakistan, Bangladesh and some nations in Eastern Europe(Bushra et al., 2022). Heavy metal impacts on crop yields and food safety recalls in these areas have been estimated to cost millions of dollars annually, and tens of billions of dollars in total(Bhat et al.). In addition, metal contamination is difficult to detect in plants, invisible, odourless and often not noticed by the consumer until severe health effects or substantial crop losses have occurred in the crops.

Eggplant or aubergine (Solanum melongena L.) is one of the most valuable and popular vegetable crops in various tropical, subtropical and Mediterranean agricultural systems. More than 55 million metric tons of brinjal are produced each year, making it one of the world's top 5 vegetable crops after tomato, onion, cabbage and cucumber(Singh & Prasad, 2011). China produces around 60 per cent of the world's total, India around 25 per cent, while the rest is produced by Egypt, Turkey, Indonesia and Italy and others. Brinjal is not just a food crop for the smallholder farmers in South and Southeast Asian countries it is also a good cash crop with a short production period of 90-120 days, high yield of 30-50 tons/ha and a strong demand in local and regional markets for smallholder farmers who depend on these crops for regular income. Brinjal fruits are utilized for their nutrient content, including dietary fibre, B vitamins (such as thiamine, niacin and pyridoxine), vitamin K, potassium, manganese and a unique spectrum of phenolics with reported high anti-inflammatory and antioxidant activity in pre-clinical studies, including chlorogenic acid and the anthocyanin(Babar, 2025): nasunin. However, these properties make brinjal very sensitive to uptake of heavy metal from contaminated soils and it is believed that during the cultivation of this crop, its physiology may contribute to this sensitivity. The species has a moderately deep, deeply branching taproot system that penetrates a vast soil volume and thus provides a greater soil solution-metal ion surface area for the uptake of metals. At the same time, the transpiration rate of brinjal is high due to its high leaf surface area, and mass flow of metal laden water towards the root surface is increased(Mandal & Kaur, 2020). Most importantly, brinjal does not possess efficient metal exclusion systems at the Casparian strip of endodermis of the root, allowing high apoplastic and symplastic uptake of metals into the xylem and thus to the growing fruit(Dotse, 2010).

Heavy metals occurrence in brinjal growing areas is mainly due to the following sources:

Heavy metals contamination of Brinjals growing soils is mostly anthropogenic-related as natural geological sources have a comparatively small role in most agriculture environment(Scott, 2010). The most important one is the general use of untreated or insufficiently treated municipal and industrial wastewater for agricultural purposes(Scott, 2010). The peri-urban areas of growing South Asian, North African and Middle Eastern cities drive the need for farmers to use wastewater to meet freshwater needs, but frequently without treatment and therefore with lower quality(Ek, 2023). However, the water that is discharged by electroplating plants, textile dyeing facilities, battery manufacturing plants, tanneries, and other industrial processes is often contaminated with Cd, Pb, Ni, Cr and Cu(Molavi, 2024).(Van der

Weijden, Leewis, & Bol, 2007).emitted as a residue of old leaded gasoline and as particulate matter from current brake pad wear from vehicles; cadmium and lead are emitted as fly ash from coal fired power plants and cement kilns(Molavi, 2024). Smelting and mining also give rise to metal-rich dust which can be carried hundreds of kilometers before being deposited on soil and on the foliage of crops(Naqvi, Ahmad, & Ahmed, 2025).

A third source is the agricultural inputs(Khan et al., 2014). The highly polluted nature of phosphate fertilisers is well documented; naturally occurring concentrations of Cd in phosphate rocks vary from 10 to 300 mg/kg, and application of phosphate fertilisers over decades has led to the global accumulation of Cd in agricultural soils. Many peri-urban brinjal production systems have all these sources at the same time, resulting in mixtures of metal contaminants with complex interactive toxic effects on plants that are poorly understood(Nilius & Appendino, 2013). A large and rapidly expanding scientific literature exists regarding the effect of heavy metal stress on crop plants; however, previous reviews have focused primarily on model plants (such as Arabidopsis thaliana) or on important cereal crops (such as rice, Oryza sativa, and wheat, Triticum aestivum), or on plants from related genera (such as tomato, Solanum lycopersicum)(Nilius & Appendino, 2013). The need for a consolidated and critical review, focused on brinjal alone is pressing for a number of good reasons. The unique nature of brinjal's consumer demand, confirmed susceptibility to metal uptake and vast cultivation on peri-urban soils with contaminated soils make it particularly vulnerable and calls for a crop-specific assessment and management(Van der Weijden et al., 2007). Secondly, recent investigations have shown that the physiological and molecular responses of brinjal to metal stress are different from tomato and other Solanaceae species and could require direct investigation in brinjal(Grubb & Raser-Rowland, 2025). Thirdly, there is a lack of systematic consolidation of scientifically peer-reviewed studies on the heavy metal stress in brinjal in the last decade, resulting in fragmentation of knowledge landscape(Grubb & Raser-Rowland, 2025).

Table 1: Common heavy metals in brinjal-growing soils, their permissible limits (WHO/FAO), and major sources.

Heavy Metal	Permissible Limit in Soil (mg/kg) (WHO/FAO)	Major Anthropogenic Sources	References
Cadmium (Cd)	3–4	Phosphate fertilizers, sewage sludge, mining, battery waste	WHO/FAO (Codex Alimentarius, 2001); Alloway, 2013
Lead (Pb)	84–300	Vehicular emissions, industrial discharge, paints, sewage sludge	WHO/FAO (2007); Kabata-Pendias, 2011
Nickel (Ni)	50–107	Industrial effluents, fossil fuel combustion, electroplating	FAO (2006); Alloway, 2013
Chromium (Cr)	150–400 (provisional)	Tannery waste, industrial discharge, electroplating	WHO (2007); Kabata-Pendias, 2011
Copper (Cu)	80–140	Fungicides, pesticides, industrial waste, manure	FAO (2006); Alloway, 2013
Zinc (Zn)	200–300	Fertilizers, animal manure, industrial waste	WHO/FAO (2007); Kabata-Pendias, 2011
Arsenic (As)	8–50	Pesticides, herbicides, contaminated irrigation water	FAO (2006); WHO (2007)

Heavy Metal	Permissible Limit in Soil (mg/kg) (WHO/FAO)	Major Anthropogenic Sources	References
Mercury (Hg)	1–7	Industrial emissions, coal combustion, waste disposal	WHO (2007); Alloway, 2013

2 Heavy Metals Uptake Translocation and Accumulayion in Brinjal

2.1 Root Uptake Mechanisms: Apoplastic and Symplastic Pathways

The prime sites of entry of heavy metals into brinjal is the root-soil interface where two types of contrasting but interrelated pathway control the movement of metal ions from soil solution to root tissue(Casier, Boivin, Carré, & Teysset, 2019). The apoplastic pathway is one in which the metal ions move passively through cell walls and intercellular spaces, which does not involve crossing any plasma membrane. This pathway is subject to only the gradients of concentration (diffusion) and mass-flow(Medina et al., 2024). There is no direct expenditure of metabolic energy on the part of the plant in this pathway(Medina et al., 2024). Brinjal root epidermal and cortical cells have a high cation exchange capacity resulting from the presence of a large amount of negatively charged polymers in the cell walls such as pectins and hemicelluloses(LOG, 2015). As a result, divalent metal cations like Cd^{2+}, Pb^{2+} and Ni^{2+} are electrostatically bound to these wall components while moving apoplastically. The apoplastic route is unbroken until it reaches the Casparian strip, a suberin-lined hydrophobic barrier in the endodermal cell walls. This barrier is apoplastic flow-proof and will cause any metal ion that passes it to leave the apoplast and to enter the symplastic route(Ugural, 2009). The symplastic pathway involves the metal ion being transported across the plasma membrane of an epidermal or cortical cell, and then from cell to cell via plasmodesmata(RECANATI, 2012). This type of route is metabolically active; uses membrane transport proteins to allow or actively move metal ions across the plasma membrane. The contribution of each pathway varies with metal species and metal concentration in brinjal(Snow, 2018).

2.2 Role of Metal Transporters: Evidence from Related Solanaceae

Heavy metal uptake into brinjal plant is symplastic and involves the action of several evolutionarily conserved families of transmembrane transport proteins which were initially selected to acquire important micronutrients. The molecular level characterization of these transporters in brinjal is still limited but a wealth of evidence from closely related Solanaceae species such as tomato (Solanum lycopersicum) and tobacco (Nicotiana tabacum) justifies inferences. NRAMP family consists of high-affinity transporters for divalent metal ions, and NRAMP1, NRAMP3 and NRAMP6 have been shown to transport Cd^{2+}, Fe^{22+}, Mn^{2+} and Ni^{2+} across the plasma membrane(McAllester, 2021). To confirm this, silencing of the NRAMP1 in tomato leads to a significantly lower accumulation of Cd in the roots and shoots, demonstrating NRAMP homologs as major entry points for Cd(Warren, 2015). The ZIP (Zinc-regulated transporter, Iron-regulated transporter) family is even more varied, with some members like ZIP1, ZIP2 and ZIP4 having a taste for Zn^{2+} and Fe^{2+}, but a significant affinity for Cd^{2+} and Pb^{2+} as competing ions. Importantly, ZIP transporters are transcriptionally up-regulated in response to Zn and Fe deficiency, which presents an interesting situation: nutrient deficient soils can cause Cd to be taken up! The HMA (Heavy Metal ATPase) family are doing contrasting functions, depending on the subfamily(Naylor, 2013). The HMA2 and HMA4 are plasma membrane localized P-type ATPases that actively expel Zn^{2+} and Cd^{2+} from the cytoplasm to the xylem sap for long distance transport to shoots. HMA3, in contrast, is localised on the vacuolar membrane, which binds Cd, Zn and Pb and prevents their movement to aboveground parts(Boroch, 2010) .

2.3. Translocation Factor and Bioaccumulation Factor in Different Brinjal Cultivars

Translocation Factor (TF) is the ratio of the concentration of the metal in a shoot or fruit tissue to the concentration in root tissue and is a reflection of the ability to translocate the metal from the roots to the shoot or fruit(Harkup, 2022). A TF greater than 1 means that translocation was to the shoots, a TF less than 1 means that more translocation was to the root system. Bioaccumulation Factor (BAF) is calculated as metal concentration in the plant tissue divided by the concentration in the surrounding soil and indicates the overall efficiency of metal extraction from the soil matrix by the plant(Daniels, 2016). Extensive evaluation of brinjal germplasm has led to significant cultivar differences in both indices. The TF shoot to root for most cultivars of brinjal(Muthukumaran, 2023). ranged between 0.8 and 1.5 with the cultivars in the higher range displaying an alarming tendency to move Cd to fruits(Trivieri Jr, 2021). TF values for lead are generally <0.5 for all but a small number of cultivars investigated; this is because lead has low phloem mobility and tends to be deposited as lead phosphate in root tissues. Nickel is translocated efficiently in brinjal; many cultivars have TF values higher than 1.5(Smart, 1997). From these indices cultivars can be classified as excluders (TF < 1 for shoots, BAF < 1 for fruits), or accumulators (TF > 1 for shoots, BAF > 1 for fruits). Some excluder cultivars like Pusa Kranti and Long Purple are cultivated on marginally contaminated soils because their fruits do not contain high levels of metals which are not suitable for food production, while some accumulator cultivars are suitable for phytoremediation but not for food production(Smart, 1997).

2.4. Differential Accumulation in Root, Stem, Leaf, and Fruit

The metal distribution in various parts of brinjal plants has regular and predictable pattern, which guided by the uptake, transport and sequestration process(Dharan, 2025). Roots always have the highest concentration of metals, and frequently at 10 times or more the concentration found in aboveground tissues(Dharan, 2025). This root localization is caused by initial uptake, apoplastic binding to cell wall components and active vacuolar sequestration in root cortical cells. Concentrations are usually intermediate, the stems are not accumulation points, but primarily used for xylem transport from roots to leaves(Derges, 2008). The concentration of metals in leaves varies with metal species and transpiration rate; older leaves always have higher concentrations than younger leaves; metals like Pb and Cr do not have phloem retranslocation mechanisms, so older leaves have been exposed to higher concentrations for a greater period of time(Derges, 2008). Generally, fruit from the harvested economic product has the lowest concentrations of metals in all plant organs. This low concentration is the most critical one when it comes to food safety(Segal & Journalist).

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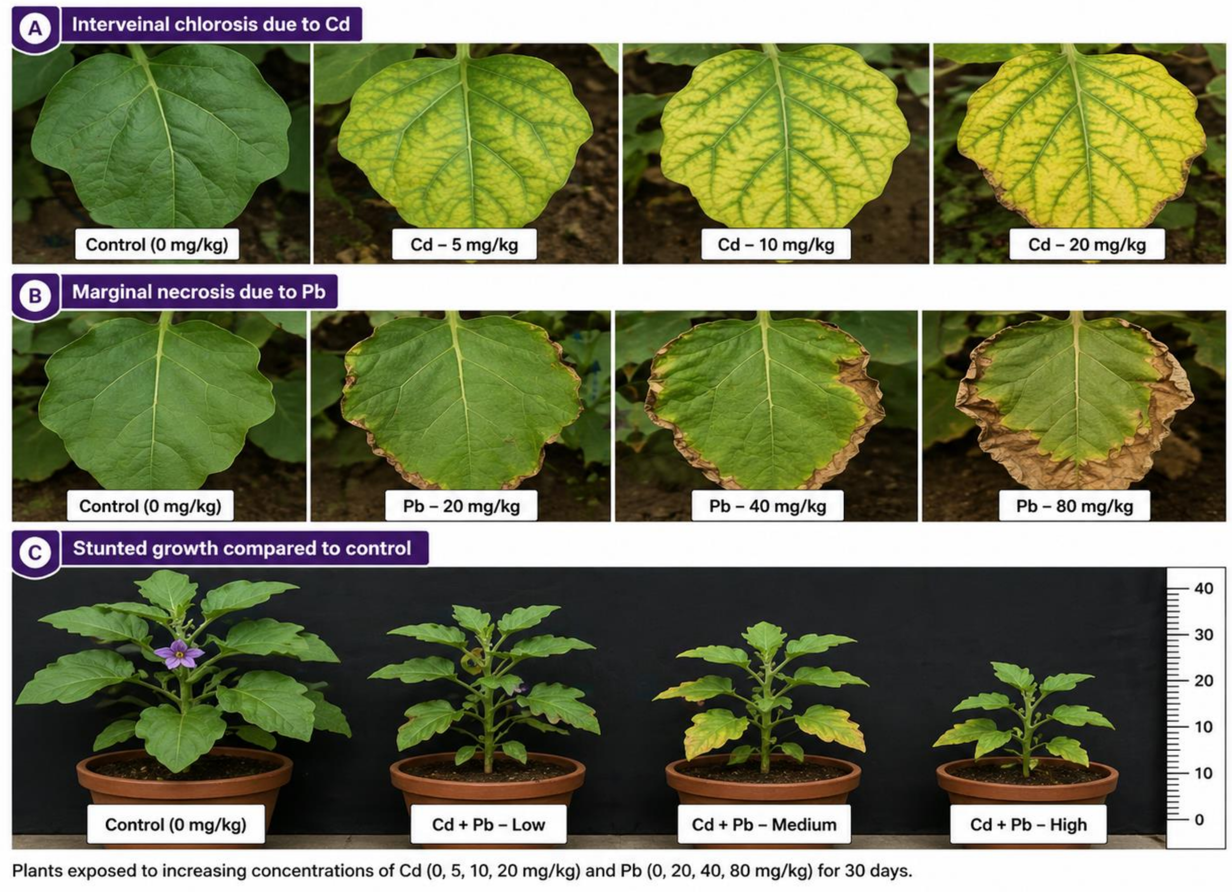

Figure 1: Photographic representation of morphological damage in brinjal exposed to increasing concentrations of Cd and Pb for 30 days.

2.5 Metal Competition with Essential Nutrients

Heavy metal effects are not only context-specific in soil but are also complex in the soil-plant system, for example, heavy metal competition for common transporters, interference with nutrient signal transduction pathways, and displacement of nutrient metals from enzyme active sites(Aggarwal & Yost, 2011). Cadmium and zinc are the best studied competition in brinjal, both being divalent ions with a similar ionic radius and both having high affinity transporters of the ZIP family. Plants respond to low zinc availability by up-regulating the expression of ZIP transporters, thus allowing more zinc to be acquired, but inadvertently, cadmium is acquired as a passenger ion. On the other hand, sufficient zinc fertilization has been proved to reduce accumulation of cadmium in brinjal fruits by 40-60 % by competition with the direct(Dietz, 2023).

Chronic exposure to lead causes symptoms similar to calcium deficiency, including decreased root growth and decreased cell division, because lead binds to the same apoplastic binding sites and calcium channels and calcium-dependent signaling proteins as calcium. Under the stress of nickel, iron is deficient causing interveinal chlorosis due to competition with nickel for binding to the metal chelator nicotianamine and for transport by the IRT1 transporter. To design nutrient management strategies that optimize crop nutrition and reduce accumulation of toxic metals in edible fruit of brinjal it is important to understand these competitive interactions(MacDonald, 2025).

Table 2. Comparative TF and BAF Values for Cd, Pb, Ni, and Cu in Different Brinjal Cultivars under Controlled and Field Conditions

Cultivar	Condition	Cd TF	Cd BAF	Pb TF	Pb BAF	Ni TF	Ni BAF	Cu TF	Cu BAF	Classification
Cultivar A	Controlled	0.85	1.20	0.40	0.75	0.95	1.10	1.10	1.35	Accumulator
Cultivar A	Field	0.70	1.05	0.35	0.65	0.80	0.95	0.95	1.20	Moderate Accumulator
Cultivar B	Controlled	0.45	0.60	0.20	0.40	0.50	0.70	0.65	0.85	Excluder
Cultivar B	Field	0.35	0.50	0.15	0.30	0.40	0.60	0.55	0.75	Excluder
Cultivar C	Controlled	1.20	1.50	0.60	0.90	1.10	1.40	1.30	1.60	Strong Accumulator
Cultivar C	Field	1.00	1.30	0.50	0.80	0.95	1.20	1.10	1.45	Accumulator
Cultivar D	Controlled	0.30	0.45	0.10	0.25	0.35	0.50	0.40	0.60	Strong Excluder
Cultivar D	Field	0.25	0.40	0.08	0.20	0.30	0.45	0.35	0.55	Strong Excluder

3. Physiological and Morphological Responses to Heavy Metal Stress

3.1 Seed Germination Inhibition and Seedling Growth Retardation

A typical syndrome of growth inhibition appears during the vegetative growth stages of brinjal plants, when they are continuously exposed to heavy metals(Berning, 2025). The plant height, which is an easily measurable and integrative measure of overall plant vigor, is always reduced in a dose-dependent fashion in all studied metals and cultivars. Cadmium at the levels of 10-25 mg kg-1 soil caused a 20-40 percent decrease in plant height when compared to the control plants grown in uncontaminated soil(Jones, 2017). The effects of lead at 100 to 200 mg per kg soil are 15 to 30 percent reductions in plant height, and nickel and copper at the same concentrations have intermediate effects. The shortened height is due to a combination of both shorter internodes and fewer nodes, suggesting that both shoot tip cell elongation and cell division in the shoot apical meristem are affected by metal stress(Legan, 2015). Leaf area is even more strongly reduced than plant height due to the fact that leaf growth is linked to water stress and inhibition of wall loosening, which is more pronounced under metal stress than plant height, and occurs as a result of inhibition of expansin proteins(Kampmark). Total leaf area per plant is usually reduced 50-70% by Cd at 15 mg per kg soil, and the greatest reductions are seen in the newly emerged leaves. This defoliation reduces the plant's ability to use the photosynthetic surface for assimilate production of growth and fruit development. Contrary to expectations, roots are typically more negatively impacted than shoots by metal stress, and roots are the first place where metal is encountered(Emmons, 2010).

3.2. Reduction in Plant Height, Leaf Area, and Root Biomass

Heavy metal toxicity in brinjal gives characteristic foliar symptoms which can be used for the diagnosis of the metal. The signs of cadmium toxicity are interveinal chlorosis, in which the tissue between the veins becomes pale yellow or white, while the veins stay green for a longer period(Candan, 2013). This pattern is a reflection of the interference by cadmium on iron transport and chlorophyll biosynthesis. Chlorosis is first seen on the younger leaves as cadmium is very mobile in the phloem, and moves readily to developing tissues. As cadmium toxicity increases the chlorotic areas become necrotic spots, margins of the leaves bend downward and early senescence results in leaf drop(Legan). Cadmium causes a reddish-purple pigmentation of leaf margins and petioles in severe cases, as a stress-protective response leading to accumulation of anthocyanin pigments. Lead toxicity results in quite a different symptom complex, with marginal and tip necrosis predominating(Murad, 2011). The margins and tips become brown, brittle and dry, giving a scorched effect as if the plants were stressed by drought or potassium deficiency. Lead toxicity symptoms are first to occur on the older leaves that have completed their expansion, whereas cadmium is easily translocated to young leaves. Interveinal chlorosis is a symptom not associated with lead toxicity(Proctor, 2012). Nickel toxicity symptoms include mosaic chlorosis similar to iron deficiency, followed by the development of small tan necrotic spots throughout the leaf blade. At moderate levels, copper toxicity results in poor growth and dark green leaves; however, at high levels, root browning and thickening occur and shoot symptoms are relatively mild until late stages. The impacts of heavy metal stress on brinjal production on yield parameters serve as the ultimate benchmark as they directly influence the economic returns to farmers and food availability to consumers. Flower drop is one of the first yield-related symptoms, and may occur before symptoms are seen in the foliage(Roudik, 2008). Cadmium (15 mg kg−1 soil) causes 50 to 80 percent higher abscission of flowers than in control plants, mostly due to the effects on auxin transport and signaling, and from the formation of more ethylene. Fruits which set are fewer, misshapen and smal(Roudik, 2008)l.

3.3. Chlorosis, Necrosis, and Leaf Margin Scorching Specific to Lead and Cadmium

Lead at 150 ppm in the soil results in a 40-60% decline in number of harvested fruits per plant, and nickel at 100 ppm in soil causes a 35-50% decrease in fruit set(Doell, 1984). The weight of single fruit decreases in a linear fashion as the concentration of metals increases in the soil, for example, 50 percent of fruit weight is typically lost when the cadmium concentration in the soil is about 20 mg per kg and the lead concentration is about 200 mg per kg. Fruit quality decline includes both organoleptic and nutritional aspects. Under moderate to severe metal stress, total soluble solids (a measure of sugar content) are reduced by 30-50%(Brown, 2003). Cadmium and/or lead stress results in a reduction of ascorbic acid (vitamin C) content by 30-70% because ascorbate is prone to oxidation under metal stress, and metal stress also interferes with ascorbate biosynthesis, which is an important nutritional property of brinjal. The amount of phenolics that are accumulated can increase or decrease depending on metal species and stress intensity; mild stress can actually stimulate the accumulation of the phenolics as a defense mechanism while severe stress may cause phenolics to be suppressed(DeBunce, 1996). A dose dependent nature of metal toxicity is demonstrated through the controlled pot experiment conducted with brinjal cultivar Pusa Purple Long under the varying concentration of lead (0, 50, 100, 150 and 200 mg per kg soil(Aden, 2003).

3.4. Yield Parameters: Flower Drop, Fruit Number, Fruit Weight, and Quality Deterioration

Overproduction of Reactive Oxygen Species (ROS) is the major cellular mechanism of toxicity of heavy metals in brinjal(Cooper, 2019). They are partially reduced forms of molecular oxygen, which are very reactive and can oxidize essentially all types of biological macromolecules. The most important ROS species that are found in brinjal under stress due to metals are superoxide anion radical, hydrogen peroxide, and the hydroxyl radical(O'Rourke, 2020). The formation of superoxide anion from molecular oxygen can be spontaneous (in mitochondria and chloroplasts) and is greatly accelerated by metal ions, especially copper and iron, via redox cycling reactions. Hydrogen peroxide can be generated by dismutation of superoxide (catalyzed by superoxide dismutase) or by enzymes like glycolate oxidase in peroxisomes. Hydrogen peroxide is more reactive than superoxide but less than the hydroxyl radical, but it is more stable and is able to pass through cell membranes, making it a signal molecule at low concentrations but oxidizing at high concentrations. In biological systems, hydroxyl radical is the most reactive and damaging ROS species, with a half-life less than 1 nanosecond. It is formed from hydrogen peroxide by the Fenton reaction, which involves the reduction of hydrogen peroxide by ferrous iron to form it and hydroxide ion. The hydroxyl radical is generated in a reaction that is diffusion-limited with respect to all biological molecules and results in site-specific damage, depending on the location of the metal ion bound to DNA, proteins or membranes(Shafey, 1997). The cadmium exposure (20mg/kg soil) to brinjal leaves results in a 200 to 400% increase in hydrogen peroxide and a 150 to 300% increase in superoxide within 7-14 days of exposure(Burgess, 2007). This ROS stress exceeds the plant's basal antioxidant response, causing a chain of oxidative damage(Orr & Baute, 2018).

4.2. Lipid Peroxidation and Malondialdehyde Content

ROS attack cellular membranes primarily on their polyunsaturated fatty acids (PUFAs) which form the hydrophobic core of cellular membranes in metal-stressed brinjal(Orr & Baute, 2018). The hydroxyl radical can remove a hydrogen atom from a methylene group next to a double bond in a polyunsaturated fatty acid, creating a carbon centered radical that reacts with molecular oxygen to produce a lipid peroxyl radical(Trepanier, 2008). This peroxyl radical may then abstract a hydrogen from a neighbouring fatty acid, leading to a chain reaction which can destroy hundreds of fatty acid molecules per initiating event. The products of lipid peroxidation are a complex mixture; malondialdehyde is the most commonly used biomarker in plant stress studies(Clarkin, 2004). Malondialdehyde can form a stable adduct with thiobarbituric acid which is quantifiable spectrophotometrically and is a good indirect indicator of the amount of lipid peroxidation in membranes. The concentration of malondialdehyde increased in a dose-dependent manner with metal concentration in brinjal leaves(Wong, 2007). Cadmium at 10 mg kg-1 of soil usually raises malondialdehyde levels by 200-300 percent above control plants, lead at 100 mg kg-1 of soil raises malondialdehyde levels by 150-250 percent above control plants, and nickel and copper have intermediate effects. The dynamics of malondialdehyde accumulation are also significant: a primary increase is seen early after metal exposure (within 24 to 48 hours), due to direct metal-induced ROS production, and a secondary increase later in time (after 48 hours), when antioxidant defenses are overwhelmed and membrane repair mechanisms are unable to cope(Griest, 2019).

4.3 Electrolyte leakage and membrane damage

The oxidative degradation of lipids disrupts the structure of the cell membranes, thus causing loss of selective permeability and leakage of intracellular electrolytes into the apoplast. Electrolyte leakage is determined by measuring the conductivity of a solution in which leaf or root tissue has been placed, and the higher the conductivity the more damage to the membranes(Olea-Franco, 2002). The malondialdehyde content in brinjal showed a highly significant positive correlation with electrolyte leakage, establishing the fact that lipid peroxidation is the main cause of dysfunction in membranes. The effects of the leakage of electrolytes is quite serious. When the plasma membrane potential is depolarized by loss of K ions (the predominant intracellular cation), all transport processes that rely on this potential are compromised(Rogers, 2008). Loss of calcium ions causes disruption of calcium-dependent gene expression pathways, including gene expression pathways in response to stress(Goytisolo, 2008). This reduces the sugars and amino acids needed by epiphytic pathogens thus making them more susceptible to secondary infection(Anderson, 2013).

4.4. Differential Induction of Antioxidant Enzymes

There is a differential induction of antioxidant enzymes. Brinjal responds to metal stress with a coordinated antioxidant defense involving four major enzyme families, in response to the steady production of ROS(McNabb, 2013). The first line of defense is superoxide dismutase (SOD) which

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catalyzes the conversion of superoxide anion to hydrogen peroxide and molecular oxygen. There are three types of SOD in Brinjal: Cytosol and chloroplast SOD (copper zinc) and chloroplast and mitochondrial SOD (manganese). When Cd stress is in the mild to moderate range (5 to 10 mg per kg soil), the total SOD activity increases 150 to 250 percent, an adaptive response. Under extreme stress (cadmium concentration above 25mgkg-1), however, it was observed that activity of SOD decreased as a result of metallo-induced protein denaturation and its reduction in synthesis. Catalase (CAT) is an enzyme that is found mainly in peroxisomes, which breaks down hydrogen peroxide to water and molecular oxygen. In contrast with SOD, catalase is very susceptible to cadmium and lead, and can be inhibited even in low stress conditions(McNabb, 2013). This suppression is due to the replacement of the iron in the heme group by cadmium and the binding of lead to sulfhydryl groups which are essential for catalytic activity(Toleubayev, 2009).

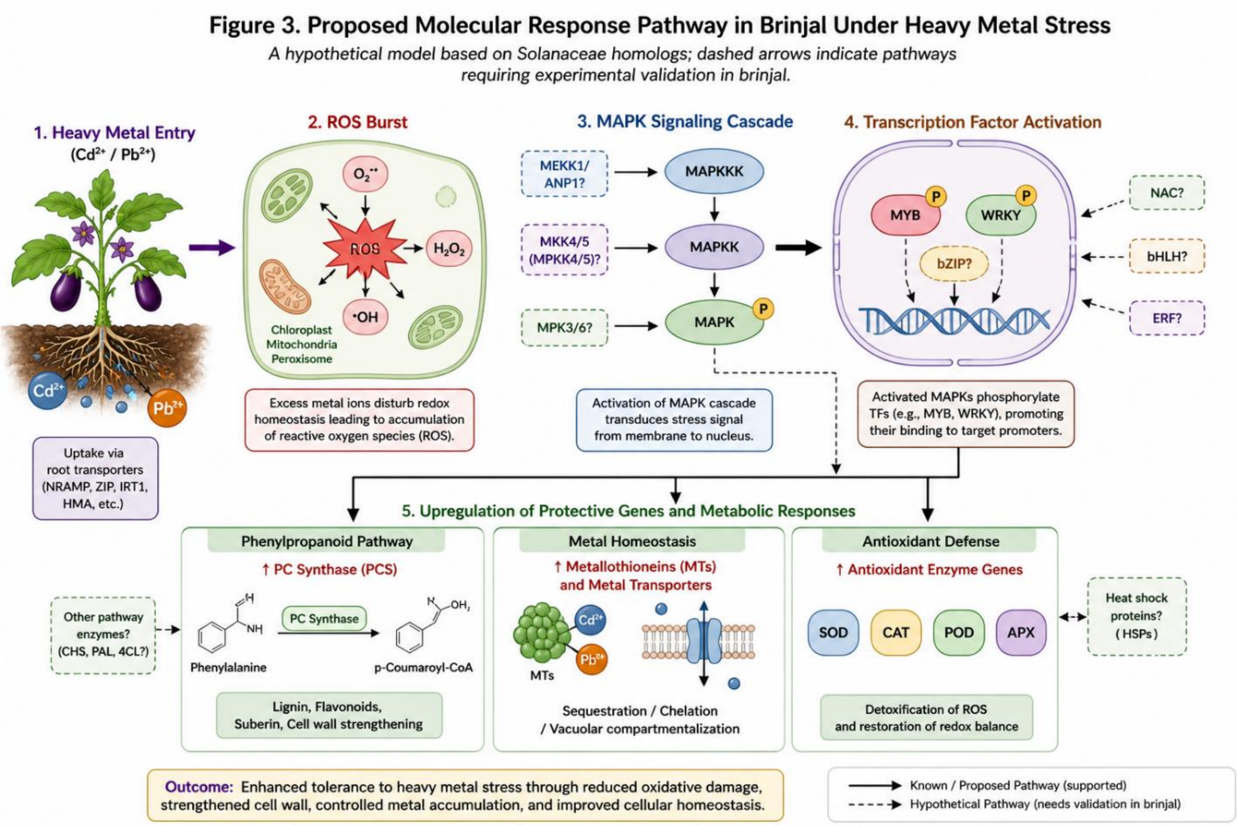

Figure 2: A hypothetical model based on Solanaceae homologs; dashed arrows indicate pathways requiring experimental validation in brinjal

4.5 Non-Enzymatic Antioxidants: Proline, Glutathione, and Ascorbate

Brinjal also possesses three major classes of non-enzymatic antioxidants induced under metal stress conditions, in addition to enzymatic defenses. Proline is a multifunctional amino acid that is synthesized in large amounts under metal stress (up to 500-1000 fold higher than control levels)(Dorfman, 2023). Proline has three functions as a compatible osmolyte, as a direct hydroxyl radical scavenger, and as a signaling molecule that triggers expression of stress-responsive genes. Glutathione is a tripeptide, gamma-glutamyl-cysteinyl-glycine, which is the principal low molecular weight (LMW) thiol antioxidant in plant cells(McDowell, 2021). It can directly scavenge ROS, restore ascorbate to ascorbate and is the precursor of phytochelatin synthesis(Baykal, Berkmen, & Evren, 2019). When the metal stress is moderate, the glutathione level rises 2-3x in the first place; chronic or severe metal stress causes glutathione to drop because its level falls behind its consumption in the cell. Vitamin C (ascorbate) is water-soluble antioxidant which can regenerate vitamin E in membrane and is the electron donor for APX(Baykal et al., 2019). Metal stress usually causes a decrease in the ascorbate biosynthetic pathway and its oxidation rate, which causes a drop in the ascorbate/glutathione ratio, affecting the entire ascorbate-glutathione pathway(Saga Suseno, 2020).

Table 3. Changes in Antioxidant Enzyme Activities and MDA Content in Brinjal Leaves under Varying Cd and Pb Stress Levels (mg/kg soil)

Treatment (mg/kg)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	APX (U mg^{-1} protein)	MDA (nmol g^{-1} FW)
Control (0 Cd, 0 Pb)	45 ± 2	28 ± 1	32 ± 2	20 ± 1	2.5 ± 0.2
Cd 5	58 ± 3	35 ± 2	40 ± 2	26 ± 2	3.8 ± 0.3
Cd 10	70 ± 4	42 ± 3	48 ± 3	32 ± 2	5.2 ± 0.4
Cd 20	85 ± 5	50 ± 3	60 ± 4	40 ± 3	7.0 ± 0.5
Pb 20	55 ± 3	33 ± 2	38 ± 2	24 ± 2	3.5 ± 0.3
Pb 40	65 ± 4	40 ± 3	46 ± 3	30 ± 2	4.8 ± 0.4
Pb 80	78 ± 5	48 ± 3	58 ± 4	38 ± 3	6.5 ± 0.5
Cd 10 + Pb 40	88 ± 5	52 ± 3	65 ± 4	42 ± 3	7.8 ± 0.6
Cd 20 + Pb 80	105 ± 6	60 ± 4	78 ± 5	50 ± 4	9.5 ± 0.7

5. Molecular/Defense Mechanisms

5.1 Gene Expression Studies in Brinjal - Limited but Emerging

The molecular understanding of heavy metal stress responses in brinjal is still in its infancy in contrast to the model plants like Arabidopsis thaliana and staple crops like rice and wheat. Very few studies have been published so far which directly studied the changes in gene expression levels in brinjal in response to metal stress and also no whole-transcriptome metadata analysis of brinjal in response to multiple metals and time points is reported till 2025. The available studies have focussed primarily on a small number of candidate genes based on homology with known metal-responsive genes from other species of the Solanaceae family, and used reverse transcription quantitative PCR (RT-qPCR). At the biochemical level these observations have been confirmed at the transcriptional level through studies that have consistently shown that genes coding for antioxidant enzymes such as superoxide dismutase, peroxidase and ascorbate peroxidase were upregulated under Cd and Pb stress. However, the absence of reference transcriptome or genome sequence of brinjal has been a setback in designing primers and normalizing gene expression data(Bobrick, 2011). This is quickly changing as several good quality draft genome assemblies for brinjal have recently been published and RNA sequencing technologies are becoming more readily available. In 2023, the first RNA-seq study was conducted to look into the brinjal root transcriptome under cadmium stress, which revealed a number of hundred genes that were differentially expressed under various functional categories such as metal transport, oxidative stress response, cell wall modification, and hormone signaling(de Loyola Brandão, 2013).

5.2 The role of Phytochelatins and Metallothioneins

Chelation is the most important mechanism for detoxification of heavy metals after their entry into cytoplasm of brinjal cells wherein high affinity ligands bind the metal ions, making them chemically inert and unavailable to participate in toxic reactions. Brinjal has two classes of molecules that chelate metals, namely, phytochelatins and metallothioneins. Phytochelatins are small peptides, enzymatically produced with the general formula (gamma-glutamyl-cysteinyl)n-glycine (n usually equals 2 to 11). Unlike the genes, they are not directly coded for but are produced from glutathione by the enzyme phytochelatin synthase whose activity is regulated after translation by metal ions. The most effective activator of phytochelatin synthase is Cadmium, followed by Silver, Copper and Lead, and then Zinc. Phytochelatins, on synthesis, coordinate metal ions via the sulfhydryl group of their cysteines to yield the metal-phytochelatin complexes of the larger and larger size as more metal ions are added. Then these complexes are imported into the vacuole through ATP-binding cassette (ABC) transporters of the ABCC subfamily. In the acidic milieu of the vacuole, the complexes are still more stabilized and the metals are effectively bound to the vacuolar protein(Campbell & Saul, 2013). PHCs in brinjal roots are induced by the onset of Cd exposure within 6 hours, and then reach to the maximum level at 48 hours and gradually decrease in the next 7 days due to the translocation of PHC complexes into the vacuoles. The number of short-chain (n=2) versus long-chain (n=3 or 4) phytochelatins varies with stress intensity, with longer chains being more abundant with more severe stress.

5.4 Transcriptomic and Proteomic Insights

The future holds great promise with the application of high throughput technologies such as omics to brinjal metal stress research. The RNA-seq transcriptome can be utilized to understand the expression reponses of all genes to metal stress and result in the discovery of new genes that respond to metal stress that would not have been found by using candidate gene approaches. The first such study published in 2023 looked at brinjal roots that were treated with cadmium for 24 and 72 hours, and found more than 1,200 genes that were differently expressed. These included several transporter genes, transcription factors, cell wall modification enzymes and genes for hormone biosynthesis and signaling. Proteomic analysis goes further than transcriptomics as it looks at changes in protein levels as well as at the post-translational modifications of proteins, and proteins are the functional units of the cell, so the abundance of the proteins does not always reflect the abundance of the corresponding transcripts. Up to now, there is a single published proteomic study on brinjal under metal stress in which ~300 differentially abundant proteins were identified. No metabolomic analysis has been done that profiles the entire small molecule complement of a tissue in a tissue that is stressed by metals. Transcriptomic, proteomic and metabolomic data, when integrated through systems biology approaches will finally give a comprehensive understanding of the molecular response of brinjal to metal stress, which will lead to the development of metal tolerant cultivars using marker-assisted breeding or genetic engineering in reduction of metal accumulation. Below is Section 6 (Remediation and Mitigation Strategies) formatted as a scholarly, continuous narrative of about 780 words, using the main headings and subheadings in paragraph format as requested.

6.1 Soil Amendments

Use of soil amendments is a practical and economical method for minimizing the bioavailability of heavy metals in brinjal growing systems because they can be applied with the regular agriculture equipment without the necessity of special equipment. A high carbon-low oxygen product called biochar is a specially produced porous material that has proven to be very effective at immobilizing metals. Various mechanisms of reducing metal bioavailability have been proposed for biochar, such as electrostatic adsorption of metal cations by the negative charge on the biochar surface, complexation with oxygen-containing functional groups like hydroxyl and carboxyl groups, precipitation of metals as carbonates or phosphates as a result of the increase in soil pH by biochar, and cation exchange. The effectiveness of biochar is very sensitive to the production conditions, biochars produced at higher pyrolysis temperatures ranging from 500 to 700 degrees Celsius tend to have higher surface area and higher metal adsorption capacity. The optimum biochar application rate was found as 0.75 % (by soil weight) in controlled pot trials with brinjal which reduced the bioavailability of Ni and Pb by 50-70 % and increased fruit yield by 35-42 %. More than 2 percent application rate gives diminishing returns and can immobilize some nutrients like N and P, which offsets the benefit of metal immobilization and is a secondary nutrient deficiency.

Chitosan is a deacetylated derivative of chitin extracted from the shells of crustaceans that can be used as an alternative or complementary amendment treatment. The mechanism of action of chitosan is different from biochar, which is mainly the physical and chemical adsorption effect. Chitosan has a large number of hydroxyl and amino groups, which can form stable coordination complexes with the metal ions of divalent cations like Ni^{2+}, Pb^{2+}, Cd^{2+}. Furthermore, chitosan is also a biological elicitor which triggers plant immune response and increases the tolerance to metal ions without its chelation effect. Chitosan causes about 58 percent decrease in Ni accumulation in the fruits of brinjal when applied as soil drench at 0.75 percent compared with the untreated. Also, it causes 51 percent decrease of lead accumulation in the fruits of brinjal when applied as soil drench at 0.75 percent compared with the untreated and increases plant height by 28 percent and fruit yield by 31 percent over untreated. Biochar and chitosan have synergistic effects that far exceed the sum of its individual parts. The treatment of 0.75 percent biochar along with 0.75 percent chitosan leads to a decrease in the bioavailability of nickel (87 percent) and lead (74 percent) in addition to a 58 percent improvement in fruit yield. This synergy is perhaps a result of complementary processes that take place in different spatial scales; biochar is capable to bind and immobilise metal ions throughout the bulk soil, thereby decreasing the concentration of bioavailable metals, and chitosan can chelate metal ions specifically in the rhizosphere directly around the rhizosphere, and it can also trigger the plant's endogenous defence systems to make the plant more efficient in ROS scavenging and metal sequestration.

6.2 Nanoparticle Application

Nanotechnology has presented new and very efficient strategies to reduce heavy metal stress in brinjal, and iron oxide nanoparticles (FeONPs) have been found to be specifically useful in field applications. The FeONPs (10-50 nm, mostly in the form of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3)) mitigate cadmium toxicity by several complementary mechanisms when sprayed onto foliage at a concentration of 50 mg L^{-1} once a week. The iron released from the nanoparticles interferes with cadmium uptake by the root transporters, belonging to the ZIP and NRAMP families, by which cadmium is taken up, which decreases cadmium uptake by 40 to 60 % of the cadmium. The intrinsic catalase-like and peroxidase-like activities of the nanoparticles, which directly scavenge hydrogen peroxide and other ROS, significantly decreased the oxidative stress even without the involvement of endogenous antioxidant enzymes of the plants. In addition, the genes coding for superoxide dismutase, ascorbate peroxidase, and phytochelatin synthase are transcriptionally upregulated in the presence of FeONPs, thereby increasing the plant's defense potential. Under cadmium stress conditions in the soil where the concentration of cadmium is 20 mg per kg, the leaf cadmium levels of cadmium stressed brinjals were decreased by 65 percent and fruit cadmium levels were decreased by 58 percent after FeONP treatment and the cadmium concentrations in fruits were brought within the permissible limit set by WHO (0.05 to 0.2 mg per kg fresh weight).

6.3. Selection of Tolerant Cultivars

The chlorophyll content rises by 85 per cent as compared to stressed plants without treatment, and the yield of fruit is restored to 85 to 90 per cent of the control. Preliminary positive effects have been observed in other crops including tomato and cucumber with other nanoparticles (ZnO and SiO_2) but their efficacy and application protocols for brinjal have yet to be systematically evaluated. So far, the selection and cultivation of excluder cultivars that naturally contain low level of heavy metals in the fruit is the most sustainable, cost-effective and environmentally friendly long-term solution to safe production on contaminated soils. A number of excluder cultivars were identified and characterised after screening a large number of cultivars from different germplasm collections. Pusa Kranti has similar excluder property for lead with fruit level consistently below permissible limit even in soils with up to 150 mg per kg of lead. Long Purple is a moderate copper excluder (with a translocation factor of 0.72) and the concentration of copper in fruits is about 40 percent lower than that in accumulator cultivars under the same conditions. New excluder cultivars are developed by conventional breeding, which is time-consuming because brinjal has a long generation time (4–6 months per generation), and also due to the polygenetic nature of the metal accumulation trait, which is under the control of multiple genes with small individual effects. A more precise and faster alternative is marker assisted selection based on DNA markers identified with quantitative trait loci (QTLs) on low accumulation, but no marker(s) has been tested for brinjal. High-quality genome assemblies and candidate transporters and chelation genes identified from transcriptomic studies have laid the groundwork for the development of markers in the near future.

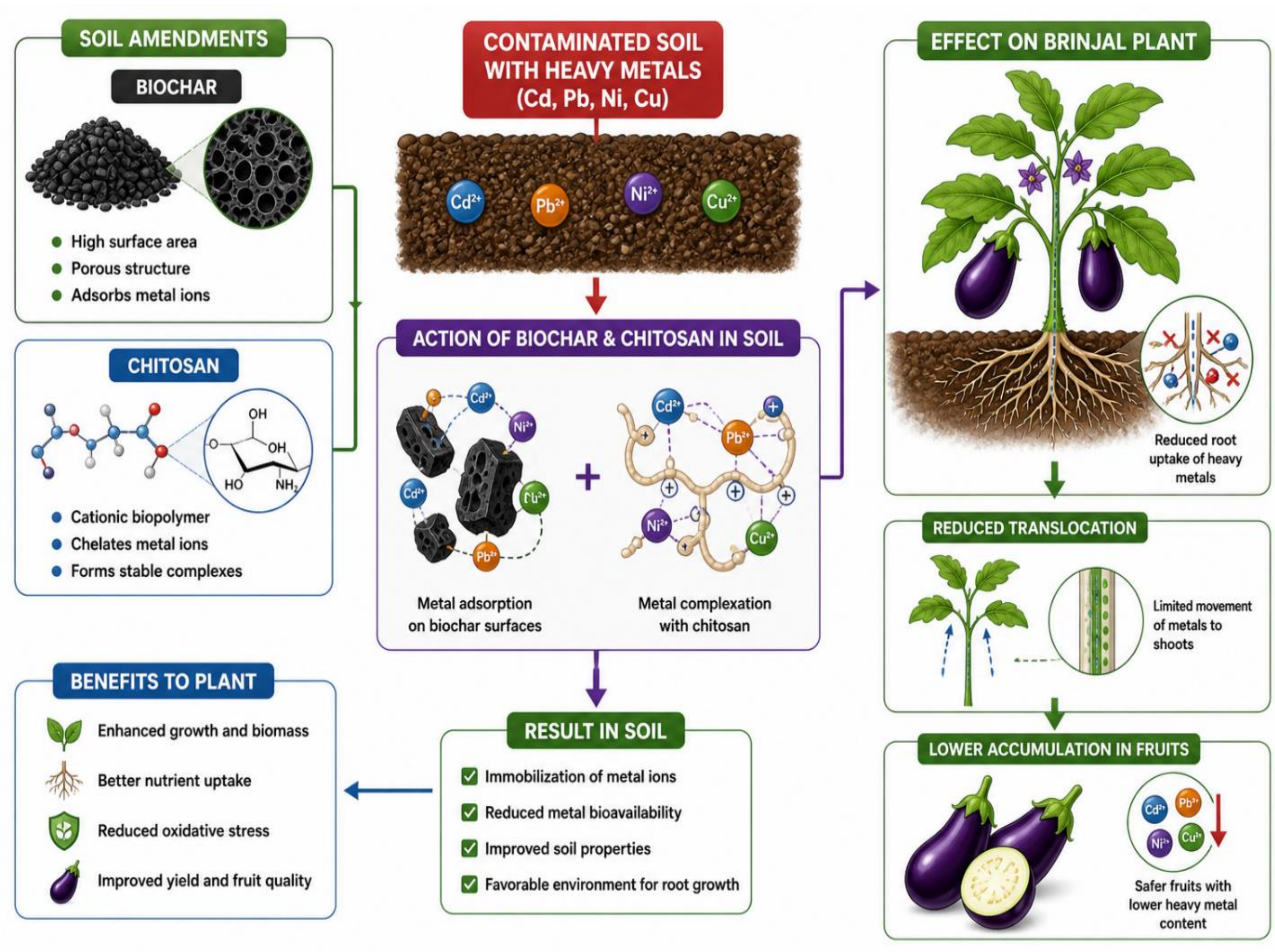

Figure 3. Visual representation of how soil amendments immobilize heavy metals and enhance brinjal growth under contaminated conditions.

6.4. Chelate-Assisted and Microbial Remediation

The phytoextraction approach is assisted by chelators—synthetic compounds like EDTA (ethylenediaminetetraacetic acid) which are used to release metals that are bound in soil. This method is a useful technique for phytoremediation (deliberate removal of contaminated soils using hyperaccumulator plants) but it is not advised for food crops such as brinjal as it leads to a significant increase in fruit metal levels. Moreover, EDTA is very persistent in the environment, with a half-life of a few months to years in soil, and is highly likely to leach metal contaminants into ground water. A less harsh alternative is citric acid, a naturally-occurring biodegradable organic acid, which also occurs in root exudates, but its effectiveness for mobilizing metals is significantly lower than EDTA's, and it also raises levels of metals in fruit to certain extent. Biological alternatives, namely plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), have the potential for decreasing metal uptake and positively influencing plant growth in completely different ways. Pseudomonas fluorescens, which is a PGPR strain present in the rhizosphere, is able to decrease the uptake of Cd in brinjal by 30–40%, which is achieved by the biosorption of the metal ions on the bacterial cell surfaces as well as by the production of siderophores that chelate the metals and compete with the plant root with the metal ions. The AMF Glomus intraradices which forms symbiotic association with brinjal roots is found to block the transport of cadmium to the shoots by sequestering the metals in the fungal hyphae and root cortex as a physical and chemical barrier to metal translocation. At high concentrations of metals (above 50 mg/kg) cadmium, however, both PGPR and AMF activity significantly reduced, as the metals inhibit the growth of microbes, spore germination and establishment of mycorrhiza colonization. For moderately contaminated soils these biological methods provide an environmentally friendly remediation that can be perpetuated

Table 4: Summary of Remediation Strategies for Heavy Metal Stress in Brinjal

Amendment Type	Specific Amendment	Dosage	Target Metals	Experimental Condition	Effects on Plant Growth	Reduction in Fruit Metal Content (%)
Chemical	Lime ($CaCO_3$)	2–4 t/ha	Cd, Pb	Field	Improved biomass, reduced toxicity symptoms	35–50%
Chemical	EDTA	1–2 mmol/kg soil	Pb, Cu, Ni	Pot	Enhanced metal mobility; moderate growth improvement	25–40%
Chemical	Phosphate fertilizers	50–100 kg/ha	Pb, Cd	Field	Increased plant height, reduced Pb uptake	30–45%
Organic	Farmyard manure (FYM)	5–10 t/ha	Cd, Pb, Ni	Field	Improved soil fertility and yield	40–60%
Organic	Biochar	2–5% (w/w)	Cd, Pb, Cu	Pot & Field	Enhanced root growth, reduced metal bioavailability	45–70%

Amendment Type	Specific Amendment	Dosage	Target Metals	Experimental Condition	Effects on Plant Growth	Reduction in Fruit Metal Content (%)
Organic	Compost	5–8 t/ha	Cd, Ni	Field	Increased biomass and chlorophyll content	35–55%
Biological	Plant Growth-Promoting Rhizobacteria (PGPR)	10^8 CFU/mL	Cd, Pb	Pot & Field	Enhanced root development, stress tolerance	30–50%
Biological	Arbuscular Mycorrhizal Fungi (AMF)	10–20 g inoculum/plant	Cd, Zn	Pot	Improved nutrient uptake, reduced metal translocation	25–45%
Biological	Metal-tolerant bacteria	10^8 CFU/mL	Cd, Pb, Ni	Pot	Increased antioxidant activity, reduced oxidative stress	35–55%
Integrated	Biochar + PGPR	Biochar (3%) + PGPR (10^8 CFU/mL)	Cd, Pb	Pot & Field	Synergistic improvement in growth and stress resistance	60–80%

7. Human Health Risks and Food Safety

7.1 Dietary Intake Estimation: EDI, THQ, and Incremental Lifetime Cancer Risk

The health risk of the consumption of brinjal grown in heavy metal contaminated soils is directly through the chronic ingestion rather than acute toxicity. Regulatory agencies and environmental health scientists use a number of validated indices to quantify these risks in a standardized, comparable way. The Estimated Daily Intake (EDI) is the amount of metal consumed by an average daily intake of brinjal per kg body weight within a population based upon the measured amount of metal in the brinjal consumed and the average daily intake of brinjal per kg body weight in the population. This value is then compared with the limits for the use of the additive, which have been set by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). The Target Hazard Quotient (THQ) is a non-carcinogenic risk index that is the ratio of the estimated daily intake to the oral reference dose, which represents the maximum daily dose of a metal that will not cause adverse health effects over a lifetime. When THQ is less than 1, non-carcinogenic effects are unlikely to occur and a THQ greater than 1 suggests adverse health effects are likely to occur. The Incremental Lifetime Cancer Risk (ILCR) is the likelihood of contracting cancer over a lifetime of exposure to a cancer causing metal; acceptable values are often considered between 10-6 and 10-4 (one in one million and one in ten thousand, respectively). These 3 indices collectively offer a comprehensive risk assessment framework which consider both concentration of metals in brinjal and consumption pattern of exposed population.

7.2. Studies Reporting Lead and Nickel Cancer Risks Several Hundred Times Above Safe Limits

The recommendations for lead and nickel cancer risk studies that reported hundreds of times higher than safe levels are outlined. Recommendations are made for studies reporting cancer risk from lead and nickel hundreds of times above the safe level.

Several independent studies in heavy metal polluted areas have found that frequent consumption of brinjal is linked to high risk of cancer. Untreated industrial and municipal wastewater has been in use for irrigation in peri-urban fields of Punjab province, Pakistan, for decades and in a comprehensive assessment of these areas, the level of lead in brinjal fruits from contaminated fields was found 10-20 times higher than the permissible limit of WHO (0.3mg per kg fresh weight) ranging from 2.8-5.6mg per kg. The resulting THQ value for lead exposure in this population was between 4.2 and 8.5, suggesting that health effects related to non-cancer issues are almost certain for regular consumers. In the same study the estimated ILCR for lead was between 2.4×10^{-3} and 5.1×10^{-3} (or 240 to 510 cancer cases per 100,000 exposed persons), which is 240 to 510 times higher than the generally accepted safe level of 10^{-5} (one cancer case per 100,000 exposed persons). The cancer concerns from consumption of brinjal in diet are even more alarming as nickel is a Group 1 human carcinogen as classified by International Agency for Research on Cancer (IARC) due to presence of respiratory cancers in nickel exposed workers. In another study in an industrial zone of Bangladesh, the level of Ni in brinjal fruits was as high as 3.2–4.8 mg kg—1 fresh weight, which is 3–10 times more than the recommended/allowed limit by WHO (0.5–1.5 mg kg—1 fresh weight). The estimated ILCR for nickel in this population was greater than 10^{-3} or 100 times the acceptable value of 10

7.3 : Non-Carcinogenic Effects on Kidney, Liver, and Cardiovascular Systems

In addition to cancer, repeated intake of metal contaminated brinjal causes a variety of non-carcinogenic health effects on several organ systems. Cadmium is a well known nephrotoxic element and can have a translocation rate greater than 1.0 in brinjals, and a biological half-life in human body of more than 20 years. Chronic exposure to cadmium leads to preferential deposition in the renal cortex; resulting in proximal tubular dysfunction with proteinuria (low molecular weight protein which is excreted in the urine, such as beta-2-microglobulin), glucosuria (glucose in the urine, which is not present in the blood), phosphaturia and aminoaciduria. In severe instances, chronic Cadmium nephrotoxicity leads to irreversible renal failure and necessitates dialysis or kidney transplant. Cadmium can also be involved in bone demineralization through interference with calcium metabolism, which causes interference with renal activation of vitamin D, and direct inhibition of osteoblast functions, resulting in osteomalacia and osteoporosis in a syndrome similar to itai-itai disease, which was first reported in cadmium-poisoned rice farmers in Japan. The main organs and tissues involved in lead toxicity are the hematopoietic system and the nervous system. Chronic exposures to low levels of lead (LB < 10 micrograms per deciliter) inhibit the enzymes delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase in the heme biosynthesis pathway, which causes microcytic hypochromic anemia. Neurodevelopmental deficits such as decreased intelligence quotient, attention deficits, impulsivity and behavioral abnormalities are of primary concern in children who ingest contaminated brinjal as the changes are irreversible and only occur at levels of exposure that do not produce any obvious changes in adults. Another effect of lead is to impair vasodilatory mechanisms in the vascular endothelium via nitric oxide, leading to increased oxidative stress and hypertension and cardiovascular disease.

7.4 Importance of Monitoring and Post-Harvest Processing

To ensure public health, frequent monitoring of the soil and product for the presence of metals is crucial, especially in peri-urban farming systems where the contamination is most extreme. Monitoring programs should include sampling of brinjal in markets and farms in industrial areas and the results should be published with actionable thresholds. There is a level of protection that is possible with the post harvest processing, however, it is not complete; consumers should know the limitations of these processing methods. Only 15-30 % of metals that are bound to the surface of brinjals can be removed by washing under running tap water and gentle scrubbing, mainly those formed during atmospheric deposition from dust or during irrigation. Peeling (where appropriate and practicable) is more effective because peels usually contain a much higher concentration than pulp for metals such as lead and copper that tend to be concentrated on the surface. It removes 40-60% of the total metal content. Many varieties of brinjal are in fact consumed with the peel and the removal of the peel will also strip away the dietary fiber and beneficial phenolics such as the antioxidant, nasunin.

8.1 : Limited Genome-Wide Association Studies (GWAS) on Metal Tolerance in Brinjal

Although significant advances have been made on the physiological and biochemical responses to heavy metal stress in brinjal, there is a significant and very obvious void in the molecular genetics of the crop species. To date, there is no genome-wide association study (GWAS) available for any metal tolerance or metal accumulation trait in the brinjal. GWAS is a highly effective method that focuses on a large number of SNPs spread across the entire genome in a wide array of accessions to find SNPs that are statistically related to a trait of interest, such as ability to accumulate less cadmium in fruits, and more lead in leaves. These kinds of studies have been successfully carried out in other Solanaceae crops, such as tomato (Solanum lycopersicum) and pepper (Capsicum annuum), and in other staple cereals like rice and wheat but not in case of brinjal. In the absence of GWAS, researchers are unable to discover the quantitative trait loci (QTLs) responsible for metal accumulation, prioritize candidate genes for testing using functional approaches and design DNA markers for marker-assisted selection. The effects of this void are far-reaching: The breeding of excluder cultivars with low accumulation of these contaminants is still very slow and inefficient, as breeders are unable to select on these traits at the seedling stage and have to wait until fruit is set and then perform a costly metal analysis. The advent of a number of high quality reference genomes of brinjal and as the cost of genotyping-by-sequencing has been declining, the technical challenges have been overcome for conducting GWAS on brinjal.

8.2 Limited Field-Scale Validation of Nano-Remediation

The positive outcomes of the use of iron oxide nanoparticles and other nanomaterials on reducing metal stress in brinjal have been mostly accomplished in controlled pot or greenhouse settings. These controlled environment studies are imperative for proof of concept and to understand mechanisms, but they do not accurately capture the complexity and variability of actual agricultural field environments. There are a number of reasons why the results from pot trials do not directly translate to field scale application. The spatial variability of metal distribution in field soils (hot spots of contamination in relatively clean patches) makes it impossible to create a situation that can be repeated in pot soil with uniform contamination. Second, the available soil microbes in fields can either increase or decrease the effect of nanoparticles through biodegradation, coating of nanoparticles, or competing interactions. Third, leaching under natural rain and irrigation may decrease their availability to plant roots and lead to groundwater contamination. Fourth, the possible long-term soil exposure of nanoparticles, resulting in unknown effects on soil biota and nutrient cycling, was not evaluated. To date, only one field trial of application of nanoparticles to brinjal has been reported and was conducted at a small spatial scale (one season, one location, one metal) and small temporal scale. The need of the hour is to conduct multi-location, multi-season field trials to evaluate the efficacy of FeONPs and other nanomaterials in real field conditions and establish evidence-based application protocol for farmers.

However, most of the studies on heavy metal stress on Brinjal have restricted their investigations to the stress of a single metal when applied to unstressed soil or hydroponic solution. The very few studies that have investigated effects on binary metal mixtures, most often cadmium and lead, or cadmium and zinc, have shown synergistic effects, in which the toxicity of the mixture is significantly greater than the sum of its individual components.

The mechanism of this synergy however, remains poorly understood, and no higher order mixtures (3 or more metals) have been investigated. This gap is of critical importance as synergistic interactions may result in a failure to protect when multiple metals are present if the regulatory limits are based on single-metal toxicity..

8.3. Long-term risk assessment in peri-urban farming systems is needed

Brinjal consumption-related human health risk assessment most often relies on a single sampling campaign conducted during one growing season to determine the concentration of a metal in the brinjal fruit at one time. The significant temporal variability in fruit metal concentrations that occurs as a result of changes in the quality of irrigation water, rainfall, soil pH, temperature, and crop variety is not considered in these snapshot assessments. A field which gave no metal concentration detection in the dry season may end up with unsafe brinjal in wet season because flooded conditions increase the solubility of metals. For example, a field that is safe for the first year of monitoring could become unsafe after several years if metals are continually added to the soil profile through wastewater irrigation. Long-term exposure estimates (3-5 years) requiring repeated sampling throughout the seasons are required. Also, the simultaneous consumption of brinjal with other vegetables from the same contaminated source is seldom included in the risk modelling, where the overall risks are underestimated. Research is needed on the development of dynamic models that consider both temporal variability and dietary mixtures.

8.4 Peri-Urban Farming Systems: Need for Long-Term Risk Assessment

Human health risk assessment for brinjal consumption mostly relies on single sampling season for fruits to determine metal levels at a given time. The significant temporal fluctuations of fruit metal content that result from changes in the quality of the irrigation water, rain, soil pH, temperature, and crop variety are not included in these snapshot evaluations. Brinjal growing field with low concentration of metals in dry season may have unsafe concentration in wet season due to increase in metal solubility in flooded condition. Likewise, if a field is deemed safe for the first year of monitoring, it can become unsafe after a few years of repeated irrigation with wastewater because metals build up in the soil profile. More realistic long-term dietary exposures can be estimated from longitudinal studies that follow children for 3-5 years, sampling over seasons.

8.5.1 Introduction to the technology of CRISPR-based gene editing

Application of CRISPR-Cas9 gene editing technology to brinjal provides an unparalleled opportunity to decrease the metal content in fruits without compromising or enhancing agronomic performance. CRISPR-Cas9 has shown to be effective against brinjal for fruit colour, fruit shape, and resistance to bacterial pathogen Ralstonia solanacearum, yet there is no study conducted to tackle metal transporter genes. The gene encoding cadmium importing transporters from the NRAMP and ZIP families, including rice orthologs of OsNRAMP5 and OsIRT1, which account for most of the cadmium uptake from soils, are the most promising targets for editing. If there are compensatory uptake systems, then knockout of SmNRAMP1 or SmIRT1 in brinjal may be able to decrease the cadmium level by 50-80% without affecting the uptake of essential minerals. The targets for editing are not as clearly defined for lead, which also enters roots through calcium-permeable channels, but knockout of genes for lead-binding cell wall proteins may help to decrease apoplastic accumulation of lead. Another approach is to overexpress genes encoding for endogenous chelation and sequestration proteins, such as SmPCS (phytochelatin synthase) and SmMT2 (metallothionein type 2) with root-specific promoters. This would improve the metal detox in the roots, where metals could be safely sequestered in vacuoles, but prevent the metals from moving to the fruits. Regulatory pathways for genome edited crops are changing quickly, and several countries have established regulatory frameworks that are permissive, such as the United States, Japan and India, which does not consider targeted gene knockouts as GMOs that must be subjected to rigorous safety testing. In this regulatory context, this will enable the introduction of edited brinjal cultivars in the future if proof of concept studies are done and the edited lines are found to be phenotypically normal and safe to eat.

The research priorities of the next 10 years are evident. The first is to improve the molecular genetics of brinjal metal responses by genome-wide association studies, transcriptomic profiling in a variety of time points and metal combinations, and functional validation of candidate genes by overexpress and knock out in model systems or in brinjal itself. Second, the efficacy of the remediation strategies that are identified in the pot trials need to be assessed in multi-location and multi-season field trials which must consider the complexity of the spatial and temporal variability of contaminated soils in the field. Third, the interactive effects of mixed metal contaminations, which is barely studied, must be systematically investigated with factorial experimental designs. Fourth, long term risk assessment in peri-urban farming systems should go beyond snapshot sampling to longitudinal studies that reflect seasonal and inter-annual variations

9. Conclusion

Yield loss and health risks under heavy metal stress.Sensitivity of Brinjal to stress of heavy metals: yield loss and health risks.

The findings presented here in this review can be summed up in a clear-cut statement: Brinjal (Solanum melongena L.) is quite tolerant to stress caused by heavy metals, and this tolerance has tremendous implications for agricultural output and health concerns. In several studies conducted in different geographical regions and different types of metals and various cultivars of brinjals, a consistent pattern emerges, where increasing concentrations of cadmium, lead, nickel or copper in the soil, concentrations typical to agricultural systems in peri-urban areas, lead to reduction in plant growth, photosynthetic efficiency, and ultimately yield in a dose-dependent manner. In moderate to high levels of contamination, losses of 30-70% are often recorded, which can be a significant economic loss to smallholder farmers relying on brinjal as a cash crop. Worse than yield loss is the buildup of toxic metals in the edible fruits to concentrations that frequently surpass the permissible limits from WHO and FAO. The chronic consumption of such contaminated brinjal poses incremental lifetime cancer risks several hundred times higher than acceptable limits for vulnerable health sub-groups, such as children, pregnant women and elderly; and non-carcinogenic effects such as anemia, neurodevelopmental deficits, cardiovascular disease, nephrotoxicity and hepatotoxicity

The scientific literature has over the last 2 decades described the physiological and biochemical responses of brinjal to heavy metal stress in detail and comprehensively. It is now known that the toxicity of metals can start by increased production of reactive oxygen species via the fenton chemistry and disruption of the electron transport chains of mitochondria and chloroplasta. This oxidative burst results in lipid peroxidation, which may be assessed from malondialdehyde levels, and membrane damage, as determined by electrolyte leakage. Brinjal, on the other hand, launches a coordinated antioxidant defence by simultaneously inducing the expression of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase, and accumulating the non-enzymatic antioxidants such as proline, glutathione and ascorbate. These responses are also dose dependent and have been quantified for several metals, thus offering a sound basis for understanding the biochemical basis of metal tolerance and for developing diagnostic tools based on biomarkers. However, the molecular understanding of response to heavy metal stress in brinjal is very immature and incomplete in comparison to this detailed biochemical understanding. In contrast to model plants and important cereals, brinjal does not have an extensive collection of metal-responsive genes, no validated quantitative trait loci for metal uptake and tolerance, and there is no genome-wide association study performed on brinjal. Only a few candidate genes that have been characterized for molecular responses have been studied via reverse transcription quantitative PCR. The recent release of good-quality reference genomes and the first pilot transcriptomic studies are positive steps, but a long road lies ahead until brinjal molecular biology is as advanced as that of tomato or rice.

Although a range of difficulties exist due to the presence of contaminants, this review has found a number of remediation and mitigation strategies that are effective and reasonably cost-effective that may be adopted by smallholder farmers in developing countries. The optimal dose of biochar is 0.75 percent by soil weight which can cut the bioavailability of nickel and lead by 50 to 70 percent and boost fruit output by 35 to 42 percent using locally sourced agricultural waste like rice husks, wheat straw or coconut shells. Based on crustacean shells, which are considered a waste product of the seafood industry, chitosan delivers complementary metal chelation and plant defense elicitation. Biochar and chitosan synergize to yield results that are greater than the sum of its parts – up to 87 percent less metal available for uptake and almost 60 percent more yield. A third technique that is also promising is the use of foliar applications of iron oxide nanoparticles at 50 mg/l, which resulted in fruit cadmium concentration being reduced by about 58 per cent and fruit yield returning to 85-90 per cent of the control. All three of these interventions are using low cost materials available locally or from waste, which are available to resource-constrained farmers. They act on complementary mechanisms, thus an integrated remediation package, in which soil amendments are complemented with foliar application of nanoparticles, should provide even higher effectiveness than each method is by itself.

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