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BIOACCUMULATION OF TRACE METALS AND THE COMPROMISED NUTRITIONAL INTEGRITY OF TOMATO *SOLANUM LYCOPERSICUM*

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Abstract

Heavy metal contamination of soils and irrigation water is a major problem for food security and crop quality worldwide. Tomato (Solanum lycopersicum) is a high value vegetable fruit that is very popular in both fresh and processed forms and is readily contaminated by the uptake of toxic metals (cadmium, lead, arsenic and nickel) from contaminated growing media. This review aims to critically discuss the pathways of accumulating, translocating and accumulating heavy metals in tomato and to review the latest scientific data to assess the impact of metal stress on fruit quality attributes. More than 150 peer reviewed studies have shown that there is a specific pattern for heavy metal accumulation in the roots, leaves and fruits. Cadmium and lead, however, are mobile in the phloem and can be directly transported to developing fruits. Physiologically, metal-induced oxidative stress hinders the photosynthetic efficiency and affects phytohormone signaling. With respect to fruit quality, there have been a number of studies indicating that higher Cd and Pb levels significantly reduced the lycopene content by downregulating the expression of phytoene synthase gene. The soluble sugars and titratable acidity are also decreased which reduces the flavor. Cell wall pectin metabolism changes and causes fruit firmness to decrease. While this may be a stress response to some phenolic compounds, this does not counterbalance the loss of some important nutraceuticals. Fruit pulp critical threshold values are often less than maximum permissible limits for fruit quality degradation. Finally, high level of accumulation of heavy metals in tomato fruits represents a threat to human health because of their consumption as food and significantly reduces the organoleptic and nutritional quality. To ensure consumer health and marketable quality of tomatoes, integrated mitigation measures such as biochar amendments, resistant cultivars and microbial bioremediation are urgently required.

1: INTRODUCTION

It is a global scale of soil and/or irrigation water contamination by heavy metals. The anthropogenic activities have made the agricultural ecosystem heavily contaminated with heavy metals at global level. Cd, Pb and Cr are released in tremendous amounts to the environment via liquid effluents and atmospheric deposition during industrialization. Deep metal ore is exposed to weathering by mining operations, producing the acid mine drainage that pollutes soils and rivers around the mining sites[1]. Cd is also applied to the land as an impurity in phosphate fertilizers, as a contaminant in metal-based pesticides e.g. copper and zinc and as a contaminant in livestock manures from animals fed with metal containing feeds. Perhaps most troubling is the lack of treatment or partial treatment of waste water for irrigation of crops. In many arid and semi arid areas, farmers have no choice but to utilize the municipal and industrial wastewater containing a complex mix of toxic metals. Heavy metals once in the soil profile are not chemically or biologically degraded and can be biologically available for decades. Recent worldwide evaluations have found that more than one-fifth of the soils in irrigated agriculture in countries such as Pakistan, China, India and Mexico contain more than the recommended levels for at least one toxic metallic element[2]. Soil properties including pH, organic matter content, clay mineralogy and redox potential also influence the bioavailability of these metals. Sandy soils which tend to be acidic can be especially susceptible because metals are more readily available for plant uptake at low pH and low organic matter.

Tomato (Solanum lycopersicum) is a remarkable crop amongst vegetables both economically and in terms of consumption around the world. The annual world production is more than 180 million metric tons, and it is contributed by major production countries as China, India, European Union, Turkey, and the United States[3]. Consumers are aware that tomatoes are a good source of lycopene, beta carotene and vitamin C and other antioxidants that promote health, which is why per capita consumption has been steadily increasing. Both fresh and processed tomatoes (such as sauces, pastes and juices) are widely consumed every day in salads, sandwiches, and in various cuisines worldwide. But tomato's popularity makes it a major source of dietary exposure to heavy metals, particularly in populations that reside in areas with heavy metal contamination. In addition to its economic value, tomato is known to be a highly physiological sensitive plant to heavy metal stress. This sensitivity makes tomato a good bioindicator of soil metal contamination and a good model plant for investigating the chain of events that occur after the initial uptake of the metal by the plant to the ultimate deterioration of fruit quality[4]. In addition, the tomato genome is completely sequenced and numerous genetic resources such as mutant lines and mapping populations exist. These tools will enable scientists to dissect molecular pathways that lead to the effects of heavy metals on fruit development and ripening.

Traits Many publications have addressed the effect of heavy metal on tomato, but previous reviews have been fragmented. The first group of studies is related to plant growth parameters like biomass reduction, inhibition of root elongation and reduction of the photosynthetic rate under metal stress.The first set of studies is concerned with plant growth parameters like biomass reduction, inhibition of root elongation and reduction of the photosynthetic rate under metal stress[4]. Still a third group focuses on human health risk assessment and uses hazard quotient and cancer risk analysis for the consumption of contaminated tomato fruits. There is no clear pattern of heavy metal levels in different parts of plants that has been synthesized directly with measurable changes in fruit quality attributes. For instance, knowledge of the negative effect of Cd on tomato yield is insufficient. The relationship between the concentration of cadmium in the fruit pulp and the degradation of lycopene, loss of sugar, reduction of acidity and firmness should be understood. Moreover, the relationships between metal induced oxidative stress and the subsequent downregulation of quality associated genes at the physiological and molecular level were investigated individually and a comprehensive framework was not constructed. This gap is not merely academic[4].

This review aims to tackle the identified gap with three specific innovations. We have to stress first post uptake distribution of heavy metals, xylem limited and phloem mobile. This difference will help to determine the level of fruit contamination[4]. Secondly, we propose a set of molecular mechanisms linked to the deterioration in quality: metal inhibition of phytoene synthase activity in lycopene, metal inhibition of invertase activity leading to accumulation of sugars and metal regulation of pectin methyl esterase activity leading to firmness in fruit. Third, we develop critical threshold concentrations of heavy metals in the fruit pulp of tomato that cause measurable reduction in the marketable quality characteristic(s)[4].

2: Main Sources and Bioavailability of Heavy Metals in Tomato Cropping System:

2.1 Erosion and deposition of heavy metals through wind and rain

Heavy metals are naturally occurring components found in the earth's crust and generally background concentrations in agricultural soils are low and do not usually exceed phytotoxic levels. Natural sources are parent rock weathering, volcanic emissions and dust deposition from atmosphere[4]. The main human activity source of metal enrichment in tomato growing areas, however, is anthropogenic activity. Cadmium is emitted to the air in smokestack emissions, and liquid effluents contaminate both soil and irrigation water sources, during industrial operations. Tailings and acid mine drainage from mining and smelting operations contain high levels of lead, zinc, copper and arsenic. One of the largest contributors is through agricultural practices. The use of phosphate fertilizers, derived from sedimentary rock phosphate, is a source of cadmium as a natural contaminant, and repeated use over decades has caused soil cadmium contamination in many vegetable production systems, particularly in intensive vegetable production. Likewise, copper and zinc may be present in livestock manures as a result of animal feed supplements, and the application of manure to land can contribute these metals to the soil matrix. Some pesticides, like copper oxychloride and lead arsenate, were eliminated in many countries many years ago, but still remain in older orchard and field soils.. In urban and peri urban settings in Asia, Africa and Middle East, where tomatoes are produced, urban and industrial wastewater might be the most direct and rapidly growing pollution source into this system, particularly in peri urban tomato fields around rapidly expanding Asian cities[4]. To regulate this distinction between natural background and anthropogenic enrichment is normally done by comparing the concentrations measured with local geochemical background concentrations or by using isotopic fingerprinting methods, like lead isotope ratios.

2.2 The geochemical behavior of critical heavy metals in tomato and their role in the production of the crop:

Certain heaviest elements are less hazardous to tomato quality than others and the geochemical behaviour of each one will dictate its environmental fate. Cadmium is known to be the most important contaminant in tomato crops. As such, most lead entering the root stays in the cell wall or a sequestered in vacuoles, and only small quantities enter the fruit unless the contamination is by direct deposition on aerial plant surfaces[4]. Under aerobic conditions, arsenic is mainly present as arsenate, and absorption occurs via phosphate transporters, because of the similarity between the two. Chromium is found in two oxidation states, the hexavalent form is highly mobile, high toxicity, and trivalent chromium is relatively immobile, low in toxicity. Nickel and copper are micronutrients necessary in the plant but toxic if they are present in an amount higher than the plant is able to utilize. Zinc is also required, and the tolerance of tomato cultivars to excess zinc is also highly variable. There are several reports that moderate Zn contamination may actually improve some fruit quality parameters as Zn is a cofactor of antioxidant enzymes, whereas high concentrations are detrimental[4].

2.3 Factors Affecting Phytoavailability:

pH is the sole significant environmental factor affecting bioavailability of heavy metals. The bioavailability of these metals (cadmium, lead, copper, zinc, and nickel) decreases exponentially with increasing pH above 6.5, as a result of increased adsorption to clay minerals and organic matter surfaces, and precipitation of metal hydroxides and carbonates. In acidic soils (pH < 5.5) these metals are highly soluble and are readily available to the tomato roots[4]. Iron, manganese and arsenic are significantly influenced by their valence state and solubility by redox potential. In waterlogged conditions, the reducing conditions also reduce arsenate to the more toxic and mobile arsenite and cause the dissolution of the iron and manganese oxides releasing previously sorbed metals[5]. Generally, the bioavailability of a soil is decreased by the high surface area of clay. Heavy metal uptake may be inhibited by competing ions in the soil solution, such as calcium, magnesium and iron, which are also involved in the same transport proteins found on the cell membranes of roots.

Table1: Bioavailability and Critical Soil Thresholds for Major Heavy Metals in Tomato Cultivation, the following are the critical levels of heavy metals:

Heavy Metal	Common Sources in Agricultural Soil	Bioavailability Factors	Critical Soil Threshold* (mg/kg soil)	Toxic Effects on Tomato Plants	References
Cadmium (Cd)	Phosphate fertilizers, sewage sludge, industrial waste	Highly available in acidic soils (pH < 6.5); increased uptake under low organic matter	3–5	Reduced root growth, chlorosis, fruit contamination	Alloway (2013); Kabata-Pendias (2011)
Lead (Pb)	Traffic emissions, industrial deposition, wastewater irrigation	Low mobility but increases under acidic conditions and low clay content	50–100	Inhibited photosynthesis, reduced fruit yield, oxidative stress	de Vries et al. (2007a); FAO/WHO (2011)
Chromium (Cr)	Tanneries, electroplating industries, industrial effluents	Cr(VI) is more mobile and toxic than Cr(III); availability rises in sandy acidic soils	75–100	Leaf necrosis, reduced seed germination, impaired nutrient uptake	Kabata-Pendias (2011); Alloway (2013)
Nickel (Ni)	Mining activities, sewage sludge, fertilizers	Mobility increases in acidic and low-CEC soils	30–50	Leaf burn, inhibited enzyme activity, stunted growth	Cela & Sumner (2002)
Copper (Cu)	Fungicides, industrial wastes, manure	Strongly adsorbed by organic matter; less available in alkaline soils	60–100	Root toxicity, chlorosis, reduced biomass	de Vries et al. (2007b)
Zinc (Zn)	Fertilizers, galvanized materials, wastewater	More bioavailable in acidic soils; reduced under high phosphorus	150–300	Leaf chlorosis, reduced flowering and fruit set	Alloway (2013); Kabata-Pendias (2011)

Mercury (Hg)	Industrial discharge, coal combustion residues	Strongly influenced by organic matter and redox conditions	1–2	Severe growth inhibition, disruption of metabolic processes	de Vries et al. (2007b)
Arsenic (As)	Pesticides, contaminated irrigation water	Availability increases under flooded and reducing conditions	10–20	Reduced photosynthesis, oxidative damage, low fruit quality	FAO/WHO (2011); Alloway (2013)

2.4 Irrigation Water Quality as a Direct Contamination Pathway:

Irrigation water may be the major source of contamination despite soil metal concentrations being safe[6]. Cadmium, lead, nickel, and chromium, sources of industrial discharges and corroded pipes, can be detected in untreated municipal wastewater. Drainage water from upstream fields, which is recycled for agricultural use, can contain dissolved metals from past fertilizer and/or pesticide applications. Farmers sometimes use sewage mixed water for the production of tomato in peri urban production systems due to lack of fresh water or high cost of fresh water[4]. In addition, soil moisture levels are continuously high under drip or furrow irrigation, and this can lead to changes in the redox chemistry of the soil increasing the solubility of metals with time. The water quality standards and regulations for irrigation water are not uniform across countries, and there is a low level of enforcement. Cadmium and lead levels in tomato fruits produced using wastewater have been found to be two to five times higher than safety standards in studies conducted in Pakistan, Egypt and Mexico. Untreated water also contains organic pollutants that can further increase the bio availability of the metals by chelation[7]. Therefore, the installation of treatment wetlands or source control to improve the quality of irrigation water is one of the best interventions for minimising the amount of heavy metals that build up in tomato fruit.

3. Uptake, Translocation, and Bioaccumulation Patterns in Tomato Tissues

3.1 Root Uptake Mechanisms Apoplastic versus Symplastic Pathways and the Role of Metal Transporters:

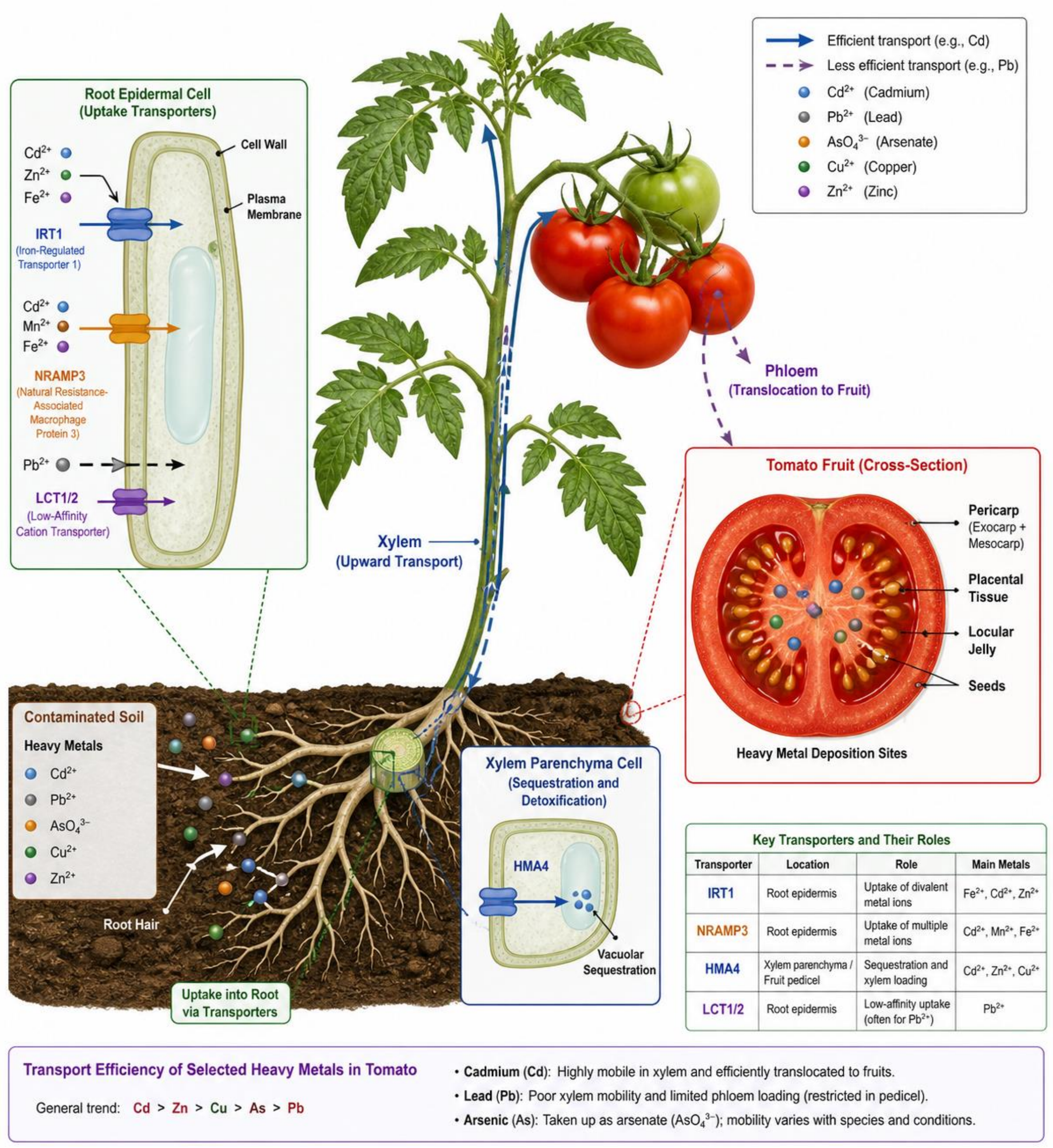
The journey of a heavy metal from contaminated soil into a tomato fruit begins at the root surface.[6]Roots intercept metal ions dissolved in the soil solution through two parallel pathways. The apoplastic pathway allows water and solutes to move freely through the cell wall continuum and intercellular spaces without crossing any membrane, but this route is blocked at the endodermis by the Casparian strip, a suberized barrier that forces all incoming materials into the symplastic pathway[8]. For heavy metals to enter the symplast, they must pass through specific membrane bound transport proteins. The IRT1 (Iron Regulated Transporter) family, originally responsible for ferrous iron uptake, also transports cadmium, zinc, manganese, and nickel. ZIP (Zinc Iron Permease) transporters mediate zinc and cadmium influx. NRAMP (Natural Resistance Associated M[6]acrophage Protein) family members, particularly NRAMP3 and NRAMP4, transport manganese, iron, and cadmium across endomembranes as well as the plasma membrane. Under metal deficient conditions, tomato upregulates these transporters to acquire scarce nutrients, but in contaminated soils this same response leads to enhanced uptake of toxic metals. Conversely, when essential metals are abundant, their transporters may be downregulated, but nonessential metals like lead and cadmium do not trigger feedback inhibition and can continue entering the root through leakage or through transporters regulated by other ions[4]. The distinction between apoplastic and symplastic entry is critical because metals that remain in the apoplast are largely restricted to the root cortex, whereas metals that enter the symplast can be loaded into the xylem and transported to shoots.

3.2 The movement of materials in xylem is referred to as xylem loading and translocation to shoots.

After the uptake by the root symplast, the next barrier to be overcome by a heavy metal is the root vasculature to gain access to the xylem stream[9]. It is a process where xylem vessels are loaded in the pericycle cells located near the xylem vessels and is mediated by efflux transporters on the plasma membrane, called as xylem loading[7]. Heavy Metal ATPase (HMA) family members, especially HMA2 and HMA4 of Arabidopsis, is a group of P type ATPases that transport zinc and cadmium out of the symplast to the apoplast of the xylem. The other metals include arsenic, which is loaded as arsenite via aquaporin channels. The speed of xylem translocation is directly related to transpiration rate, such that environmental conditions that increase transpiration rate, also increase the rate of movement of metal to leaves, e.g. high light intensity and low humidity.[8]Cadmium, zinc and nickel are readily translocated from the roots into the xylem, and are rapidly distributed throughout the tomato leaves and then into the fruits.

3.3 Phloem Mobility and Redistribution to Fruits The Critical Distinction Between Mobile and immobile:

Heavy Metals Once the photoassimilates are transported from source leaves to developing fruit and other sink organs via phloem sap, the final stage of fruit contamination occurs. The mobility of phloem varies greatly among heavy metals. Cadmium and zinc are mobile in the phloem. They are transported in phloem companion cells from xylem derived sap or from leaf mesophyll vacuoles to fruits by the phloem stream[10]. This is the mobility and the reason why cadmium concentration in tomato fruits can be as high as or even higher than in their leaves. Zinc also exhibits prominent fruit build-up, but is an essential nutrient and somewhat regulated in movement. In contrast, lead and chromium are virtually phloem immobile[11]. After the xylem importation, lead is localized in leaf tissues in association with cell walls or in vacuoles. It does not re enter the phloem in appreciable amounts. This means that generally, the amount of lead in tomato fruit will be a factor of 10 to 100 less than in the leaves or stems, if it is only in the roots[12]. These, however, can be circumvented by atmospheric deposition of lead in dust onto the fruits of tomatoes themselves leading to high levels of lead in the unwashed fruit[5]. Arsenic has intermediate mobility in the phloem; some movement has been reported in certain plant species, but in tomato the level of arsenic in fruit is generally lower than the level of cadmium. Figure 1 is showing schematic representation of heavy metals uptake, translocation and accumulation pathways in Tomato (*Solanum lycopersicum L.)* Plants



Key Transporters and Their Roles

Transporter	Location	Role	Main Metals
IRT1	Root epidermis	Uptake of divalent metal ions	Fe^{2+}, Cd^{2+}, Zn^{2+}
NRAMP3	Root epidermis	Uptake of multiple metal ions	Cd^{2+}, Mn^{2+}, Fe^{2+}
HMA4	Xylem parenchyma / Fruit pedicel	Sequestration and xylem loading	Cd^{2+}, Zn^{2+}, Cu^{2+}
LCT1/2	Root epidermis	Low-affinity uptake (often for Pb^{2+})	Pb^{2+}

Transport Efficiency of Selected Heavy Metals in Tomato

General trend: Cd > Zn > Cu > As > Pb

- **Cadmium** (Cd): Highly mobile in xylem and efficiently translocated to fruits.
- **Lead** (Pb): Poor xylem mobility and limited phloem loading (restricted in pedicel).
- **Arsenic** (As): Taken up as arsenate (AsO_4^{3-}); mobility varies with species and conditions.

Figure1.schematic representation of heavy metals uptake , translocation and accumulation pathway in Tomato (Solanum lycopersicum L.) Plants

3.4 Organ and Tissue Specific Accumulation Roots Exceed Stems and Leaves Which Exceed Fruits:

The distribution of heavy metals in the tomato plants is characterized by a general trend and a defined hierarchy, especially when they grow in uniform soil contamination[13]. The highest concentrations are found in roots since they are the first place that metals land and many metals are stored in vacuoles and root cell walls[14]. Intermediate concentrations are generally found in stems and leaves, with older leaves generally having higher concentrations than younger leaves as a result of continued transpiration and absence of phloem export for immobile metals[15]. The lowest content is found in fruits, although this varies with metal. For cadmium, the fruit to root ratio varies from 0.05 to 0.30 as affected by the cultivar and exposure time. The ratio is seldom greater than 0.01 when it is taken up as a root only for lead. Several exceptions exist. Nickel (Ni) and copper (Cu) are essential but potentially toxic elements and are controlled more strictly by the tomato homeostatic mechanisms which may not follow the simple hierarchy when it comes to fruit concentrations[16]

3.5 Subcellular Compartmentalization Cell Wall Binding, Vacuolar Sequestration, and Metal Chelation:

It is common knowledge that the cell wall is a crucial organelle in plants, and that metal ions are tied up in the vacuole.As is well known, the cell wall is an important organelle in plants and metal ions are sequestered in the vacuole.

Heavy metals are not distributed evenly in tomato cells after being taken up. Toxicity and potential for long distance transport are determined by subcellular compartmentalization. The cell wall is the first line of defense and it contains pectins, hemicellulose and lignin with negatively charged carboxyl and hydroxyl groups, which are able to bind cationic metals like lead, cadmium and copper[16]. This binding makes the metals unavailable in the apoplast, keeping them away from the plasma membrane. Ralphs small cysteine rich peptides quickly chelate metals that successfully enter the cytoplasm. Metallothioneins are gene coded proteins that have high cysteine content and bind zinc and copper. These complexes are then taken up into the vacuole, an organelle of the cell that is a large terminal storage space for toxic metals. Vacuolar sequestration is mediated by ABC (ATP Binding Cassette) transporters and cation proton exchangers. Most of the cadmium and lead absorbed in tomato roots is contained in the vacuoles of the roots and is therefore not translocated further. But if the vacuolar capacity is exceeded, metals will leak out of the vacuoles and into the xylem[17]. The variation in the efficiency of subcellular compartmentalization is one of the greatest factors that determine fruit metal accumulation and can be the basis of breeding low accumulating cultivars of tomato.

4: Mechanisms of Heavy Metal Phytotoxicity in Tomato:

4.1 Oxidative Stress Induction Reactive Oxygen Species Overproduction and Lipid Peroxidation:

Heavy metals toxicity in tomato plants is mostly mediated by the induction of oxidative stress at the cell level[18]. Cadmium, lead, arsenic, and nickel ions affect the normal electron flow in the photosynthetic and respiratory electron transport chains when they are introduced into the cells of tomato roots or leaves. Cadmium binds to photosystem II in chloroplasts and replaces iron and copper, leaching electrons which react with molecular oxygen to produce the superoxide anion radical[19]. In mitochondria, nickel and lead bind to complexes I and III resulting in the production of superoxide as a byproduct. Hydrogen peroxide can lead to the formation of very harmful hydroxyl radicals in the presence of iron or copper ions, via the Fenton reaction[20]. The hydroxyl radical lacks an enzymatic defense and directly attacks the polyunsaturated fatty acids that are present in cellular membranes, where it starts a chain reaction of lipid peroxidation[21]. This process causes a loss of integrity of the membrane and results in leakage of electrolytes, and dysfunction of the organelles and ultimately cell death[22]. The amount of lipid peroxidation is well correlated with the amount of visible chlorosis and growth inhibition, thus indicating that the lipid peroxidation of membranes is a major factor in the phytotoxicity of heavy metals to tomato.[23]

Table 2: Physiological and Biochemical Biomarkers of Heavy Metal Stress in Tomato Leaves and Fruits

Biomarker	Cadmium (Cd)	Lead (Pb)	Arsenic (As)	Nickel (Ni)	References
Chlorophyll content	↓ by 30–60%	↓ by 20–45%	↓ by 25–50%	↓ by 15–40%	Gratão et al., 2005; Hayat et al., 2012; Sharma & Dubey, 2005
Photosynthetic rate (Pn)	↓ by 40–70%	↓ by 25–55%	↓ by 35–60%	↓ by 20–50%	Singh et al., 2016; DalCorso et al., 2014; Ahmad et al., 2015
Proline content	↑ by 100–300%	↑ by 80–200%	↑ by 120–250%	↑ by 60–180%	Sharma & Dietz, 2006; Gill & Tuteja, 2010
Malondialdehyde (MDA) level	↑ by 150–350%	↑ by 100–250%	↑ by 120–280%	↑ by 80–200%	Gill & Tuteja, 2010; Hasanuzzaman et al., 2020
Superoxide dismutase (SOD) activity	↑ by 20–80% (low Cd); ↓ by 10–40% (high Cd)	↑ by 15–60%	↑ by 30–90%	↑ by 10–50%	Gratão et al., 2005; Sharma & Dietz, 2009
Ascorbate peroxidase (APX) activity	↑ by 30–100% (low Cd); ↓ by 20–50% (high Cd)	↑ by 20–70%	↑ by 40–120%	↑ by 15–60%	Foyer & Noctor, 2005; Gill & Tuteja, 2010

4.2 Disruption of the photosynthetical apparatus—Chlorophyll degradation, thylakoid damage and reduced gas exchange:

The photosynthetic activity is affected by heavy metals in tomato leaf by several interrelated mechanisms, which ultimately decrease the amount of carbohydrates available for fruit development and quality accumulation[24]. The primary effect is on the biosynthesis of chlorophyll[25]. Cadmium suppresses the action of the enzyme aminolevulinic acid dehydratase, which is an early committed step in the porphyrin pathway to chlorophyll. Lead causes the incorporation of magnesium to be faulty in the protoporphyrin ring and nickel stimulates activity of the enzyme chlorophyllase, which speeds up chlorophyll breakdown[26]. This leads to a decrease in overall chlorophyll content between 30 to 70 percent depending on the metal, concentration and exposure time. The most significant decrease is in chlorophyll a as compared to chlorophyll b. The loss of chlorophyll becomes apparent as interveinal chlorosis, and occurs first in the older leaves that are most susceptible to metal deposition. Heavy metals cause degradation of pigment in addition to causing direct damage to the structure and function of thylakoid membranes. Photosystem II is especially susceptible.[27] Cadmium or Lead can take the place of manganese on the oxygen evolving complex, which disrupts the complex. ET is blocked in PSII and the quantum yield is lowered (chlorophyll fluorescence = Fv/Fm). Fv/Fm values of tomato plants grown in toxic metal concentrations are generally below 0.70 and those of healthy plants are 0.80-0.83. Heavy metals cause abscisic acid to build up which leads to the closure of stomata and subsequent reduction in stomatal conductance. A reduction in stomatal aperture decreases the amount of carbon dioxide that can enter the leaves, and in addition, direct damage to Rubisco and Rubisco activase occurs. Under moderate to severe metal stress, the net photosynthetic rate can be reduced by 40-70%.

4.3 The effects of these factors on nutrient uptake and the maintenance of ion balance are described:

Heavy metals interfere with uptake and transport of mineral nutrients by ionic mimicry and competitive inhibition of transport proteins. Cadmium is not known to have a biological role in plants, but is taken up by the root cell via divalent cation transporters for iron, zinc, calcium, and manganese[28]. Cadmium enters cells where it binds to calcium binding proteins such as calmodulin, inhibiting calcium dependent pathways in the control of stress response, development and ion transport. Lead also disrupts calcium and iron metabolism. Arsenic enters the cell via arsenate transporters coupled to phosphate transport and binds to phosphate binding sites to interfere with energy metabolism through the formation of weak arsenate esters which break down spontaneously[29]. For tomato plants, the functional nutrient deficiency means that they will be in a state of deficiency even though essential nutrients are present in sufficient soil concentrations. Cadmium stress or nickel stress is associated with tomato IRCs, which are frequently observed in tomato.Tomato IRCs are often observed under cadmium stress or nickel stress, as the uptake of ferrous iron is mediated by the IRT1 transporter which is downregulated in presence of cadmium in the cell[20]. Cadmium and lead also aggravate the symptoms of calcium deficiency because they inhibit the movement of calcium into the distal tissues of the fruit, such as blossom end rot, a physiological disorder of tomato fruit. The zinc deficiency is due to feedback inhibition: when zinc is already present in high concentrations, it prevents further absorption, or to cadmium which interferes with zinc-specific transporters.

4.4 The cellular response to DNA damage and cell cycle arrest heavy metals are genotoxic:

Compounds which can damage directly and indirectly the nuclear DNA in tomato root meristematic cells, in leaf cells and even in the developing fruit tissue. During metal induced oxidative stress, the hydroxyl radical attacks the DNA bases, yielding the oxidized products (e.g., 8 oxo deoxyguanosine). If not repaired by base excision repair enzymes this lesion will result in G to T transversion mutation during DNA replication.. Heavy metals also can cause oxidative base damage, single strand breaks, and DNA protein crosslinks. The comet assay or single cell gel electrophoresis (SCGE), which measures DNA fragmentation, reveals much greater tail moments in tomato root tip cells treated with cadmium at as low a concentration as 5 micromolar[30]. The chromosomal aberrations observed in tomato root meristems consist of bridges, lagging chromosomes, and micronuclei, under the action of lead and nickel[23]. This genomic instability causes cell cycle arrest. In tomato root meristematic cells the number of cells at G1 and G2 checkpoints is increased while the number of cells undergoing mitosis is decreased[25]. This arrest is accomplished by the upregulation of cyclin dependent kinase inhibitors and repression of cyclin genes. The entire plant suffers from stunted root development, decreased ability to absorb nutrients and water.

4.5 Abnormal Response to Phytohormone Stress due to Heavy Metals:

The phytohormone profile of tomato plants is dramatically changed by exposure to high concentrations of heavy metals and many of the growth and quality effects are mediated by the changes in phytohormone profile[27]. Under metal stress, abscisic acid (ABA) concentration builds up quickly and uniformly. This response is adaptive in the short term because ABA causes stomatal closure which decreases water loss and metal transport to shoots by transpiration. However, high level of ABA for long periods will cause inhibition of photosynthesis and delay in fruit ripening. Cadmium causes the expression of 1 aminocyclopropane 1 carboxylic acid (ACC) synthase and ACC oxidase, resulting in increased ethylene production. Ethylene promotes senescence of older leaves, but paradoxically can suppress fruit ripening if applied along with other stress factors. Under heavy metal stress, salicylic acid (SA) is generally increased and plays an important role in the upregulation of antioxidant genes and phytochelatin synthases.. Heavy metals inhibit the main growth hormone, auxin, by inhibiting its biosynthesis and by increasing its oxidation. Lower concentrations of indole 3 acetic acid (IAA) in tomato root and shoot tissues in response to Cd exposure have been suggested to account for the decrease in root branching and shoot elongation seen in tomato. In addition, gibberellins (agents that stimulate stem elongation and fruit set) are also decreased. The net hormone change is from a growth to a stress adaptive condition[26]. This shift is beneficial with contamination, but also reduces the amount of resources allocated to producing fruit and building quality. In particular, a decrease in the amount of auxin and gibberellins in developing fruits constrains cell division and expansion, directly decreasing fruit size. The ethylene and ABA balance is altered so that the normal ripening program is disrupted and color is uneven and lycopene content decreases.

5. Effect of Heavy Metals Accumulation on the Quality of Fruits Params.

5.1 Physical Quality Attributes:

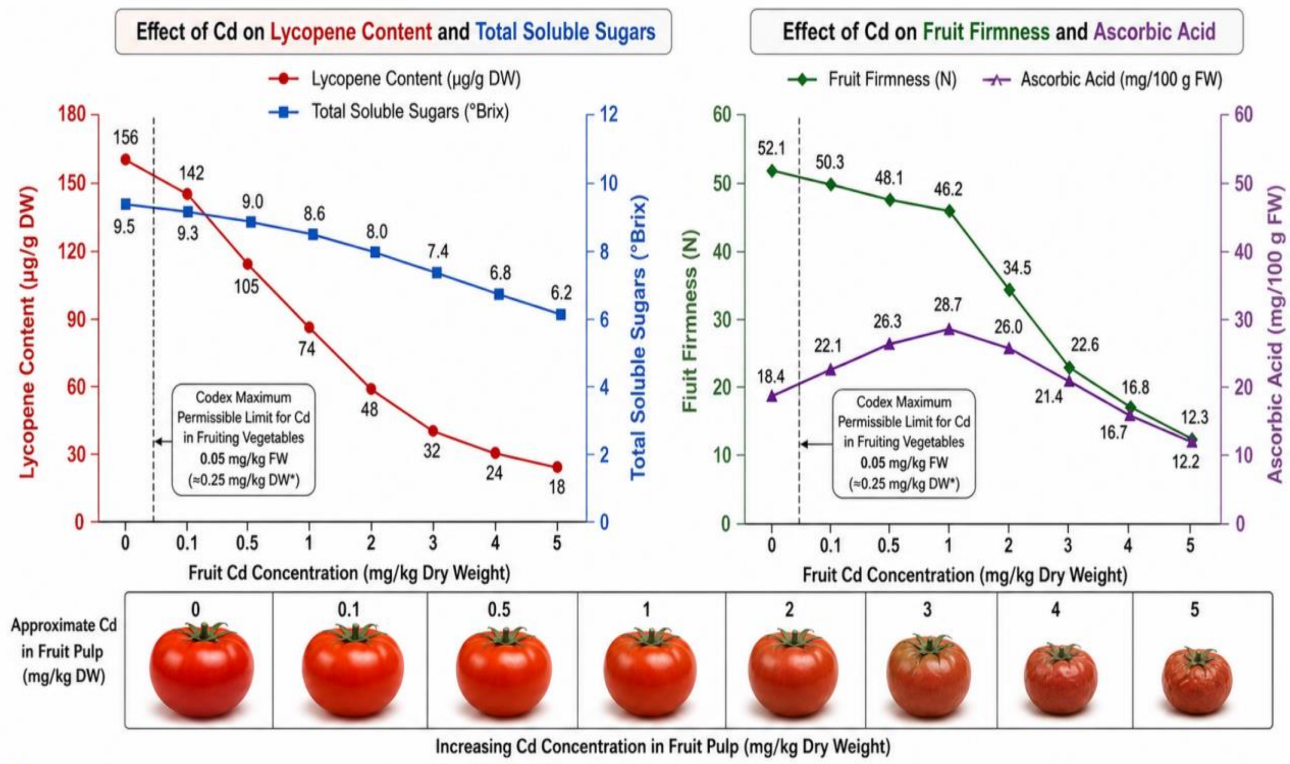
The physical properties of tomato fruit play a key role in marketability, post-harvest life and consumer acceptability; and exposure to heavy metal stress always decreases these properties. When tomato plants are grown at high levels of Cd, Pb or Ni in the soil, fruit size, fruit weight and diameter are significantly decreased.[31] Photosyntheticcarbon assimilation is reduced as a result of chlorophyll degradation and inhibition of Rubisco, thereby reducing the availability of photo assimilates to fruit sinks. Fruit firmness, which is an important characteristic for fresh market and processing tomatoes is also affected. Firmness is related to integrity of cell wall and middle lamella, both of which are rich in pectin polysaccharides. In normal ripening, the pectin molecules undergo some changes caused by the action of enzymes, pectin methylesterase and polygalacturonase, which produce the controlled softening. Under heavy metal stress, the activity of these enzymes is dysregulated. Cadmium exposure has been demonstrated to result in the decrease of the expression of pectin methylesterase in tomato pericarp tissue, resulting in incomplete and uneven degradation of cell walls[32]. This can lead to overly soft produce, which can bruise, or to mealy produce with poor eating quality. Under metal stress, shelf life (which is the post-harvest time in which fruits can be marketed) is shortened due to disruptions in the integrity of the cell wall leading to an increased loss of water and increased susceptibility to pathogen attacks[28].

5.2 The nutritional and chemical quality of food in relation to food safety.

Heavy metal accumulation affects several chemical parameters in undesirable ways; these affect the nutritional value and the flavor of tomato fruits. TSS, mainly composed of sugars and organic acids, is a rapid index of sweetness and quality of fruit. Cadmium, Lead and Arsenic exposure always lowers total soluble solids between 15 and 40 percent, which depends on the concentration of the metal, and the length of the exposure[29]. The sugar profile is specifically altered, with a reduction in the levels of glucose and fructose, because of the decrease in the expression of invertase genes, such as LIN5 and LIN8, encoding cell wall and vacuolar invertases that cleave sucrose. The decrease in invertase activity hinders the accumulation of hexose sugars in fruit apoplast and vacuole, directly reducing the sweetness of the fruit. In most studies, titratable acidity decreased in heavy metal stress, and changed the sugar to acid ratio, causing the fruits to taste flat or bland. This decrease is due to the fact that the activity of mitochondrial enzymes of organic acid biosynthesis, which depend on the tricarboxylic acid cycle, is inhibited by metal induced oxidative stress in the fruit. The reaction of ascorbic acid (vitamin C) is more complex and dose dependent with heavy metal stress[33]. Vitamin C production can be stimulated in tomato plants through a process of antioxidant defense, often occurring when exposed to low to moderate levels of cadmium, resulting in greater accumulation in tomatoes.

5.3 Nutraceutical Quality Antioxidants

The tomato is a nutrient-rich crop with a high lycopene and other carotenoid concentration, which is a critical issue to limit the accumulation of heavy metals in the crop. When cadmium, lead and nickel are applied to the plants, lycopene and beta carotene are always lowered. The molecular mechanism is related to the inhibition of important genes involved in carotenogenesis[34]. The PSY1 gene encodes phytoene synthase, which is the first committed step in carotenoid biosynthesis and is very susceptible to the metal induced oxidative stress. The gene PDS encodes phytoene desaturase which is also down-regulated. Cadmium has been found to decrease PSY1 transcript levels and lycopene accumulation by 40–70 percent in ripening tomato fruits. The effects of lead and nickel are similar, but not as strong. The loss of lycopene does not just concern appearance, since lycopene is a powerful antioxidant which has been shown to have health benefits for humans, such as a decreased risk for prostate cancer and cardiovascular disease. The levels of beta carotene (which is a precursor to vitamin A) also decreases. The interesting thing is the plant's response to heavy metal stress includes an increase in phenolic compounds and flavonoids as part of its secondary metabolic stress response. Some of these compounds such as chlorogenic acid, rutin and quercetin are antioxidants which can partially offset the loss of carotenoids[35]. Generally however, the increase in phenolics is not enough to compensate for the decrease in lycopene and the overall antioxidant capacity of the fruit (measured by DPPH or FRAP) is generally reduced in moderate to severe levels of metal contamination. Figure 2 is showing dose-Dependent Effects of Cadmium on Tomato Fruit Quality Attributes



Data sources: *Conversion based on average dry matter content of tomato fruit ≈ 20% (FAO, 2018).

Compilations from: Sanità di Toppi, L. & Gabbrielli, R. (1999) *Plant Physiology*, *119*, 463–469; Mendoza-Cozatl, D.G. *et al.* (2005) *J. Plant Physiol.*, 162, 107 – 116; Liu, J. *al.* (2014) *Ecotoxicol. Environ. Saf..*, 106, 15 – 21; Hasanuzzaman, M. *et al.* (2018) *Ecotoxicol. Environ. Saf..*, 166, 296 – 305.

Figure 2: Dose-Dependent Effects of Cadmium on Tomato Fruit Quality Attributes

5.4 Mineral Composition

Heavy metals interfere with the mineral nutrient balance in tomato fruits by interfering with metal transporters or by competitive inhibition. Cadmium and lead stress always lead to a decrease in the amount of potassium, the most abundant cation in tomato fruits, and a key factor for flavor and osmoregulation[36, 37]. The decrease in calcium is due to the competition of cadmium for calcium binding sites and to its interference with calcium uptake at the root level, as calcium is a component of cell wall structure and firmness of fruit. In addition, magnesium, iron and zinc are all lowered. Mechanisms include downregulation of the very transporters that normally mediate the uptake of essential metals .Net result is that the fruit is less nutritious than it would be if it didn't contain toxic metals and if it did contain them, they would have been balanced by other minerals, which it lacks.

5.5 Sensory Quality:

Heavy metal stress has been shown to cause a reduction in the sensory qualities of tomato fruits through consumer panel studies. Panelists report that metal stressed tomatoes are less sweet or bland in taste, and observed decreases in the total soluble solids and sugar to acid ratio. Dull red or yellowish colours are indications of a loss of lycopene and beta carotene. Altered cell wall pectin metabolism causes a change in texture to mealy or gritty. Such sensory deficiencies decrease consumer acceptance and willingness to pay and can cause economic losses in the grower even in the case of a legal safe product.

5.6 The Critical Concentration Thresholds for Quality Loss and Safety Limits for fruit pulp

One of the most important results that have emerged from the literature is the discrepancy between the concentration levels at which quality loss could be detected and the maximum permissible concentration levels (MPCs) that have been established by the Codex Alimentarius and/or national food safety authorities. The Codex limit for cadmium in fruits and vegetables is 0.05mg/kg (fresh weight). In contrast, investigations have consistently found severe decreases in lycopene and total soluble solids when the fruit cadmium levels are 0.02 – 0.03 mg/kg or lower which is below the permissible level[38]. The Codex standard for lead is 0.10 milligrams per kilogram and quality effects are noted when the content of lead is 0.05-0.07 milligrams per kilogram. This discrepancy will result in a regulatory system that is only concerned with human health risk and neglect to safeguard the fruit quality. Therefore, there is a critical research need to establish quality-based thresholds for each metal .

6. Human Health Risk Assessment on Consumption of Tomato.

6.1 Estimated Daily Intake and Target Hazard Quotient:

While the detection of heavy metals in tomato fruits is not an indicator of human disease, the risk associated with consumption will depend on concentration of the metals detected as well as on the quantity of tomatoes consumed over a period of time. The amount of exposure to a chemical that an individual receives on a daily basis is a basic measure of human exposure, the estimated daily intake (EDI). It is estimated as the concentration of metal in the tomato fruits (mg kg-1 FW) x average daily tomato consumption (kg person-1 day-1) / average body weight (kg). Average tomato consumption in many Western diets is between 20 and 50 grams a day for an average adult that weighs 70 kg, and can be over 100 grams a day in Mediterranean and some Asian diets. If the concentration of Cd in tomatoes is 0.05 mg/kg (the Codex limit), the EDI for tomatoes alone is in the range of 0.014-0.036 micrograms per kg body weight per day. This value is lower than the provisional tolerable monthly intake set by the Joint FAO WHO Expert Committee on Food Additives, but does not include any extra cadmium from the consumption of rice, potatoes or leafy vegetables. The target hazard quotient (THQ) extends the EDI by dividing by the oral reference dose (RfD) that is the estimated daily dose that would not cause adverse or health effects during a lifetime. The use of a 0.001 mg/kg/day RfD for cadmium is common. THQ values below 1 suggest that the risk of non-cancerous health effects is unlikely and values above 1 suggest a potential for health concern[39]. The RfD for lead is 0.0035 mg kg-1 day-1 and the THQ for lead is 1, which has been reported in peri urban farms treated with untreated wastewater. The EDI (and THQ) calculations are conservative, assuming that 100 per cent of the metal in food is bioavailable, but it is known that tomato chelators (e.g. ascorbic acid and organic acids) may inhibit the absorption of some metals in the intestine.

6.2 In addition to the Hazard Index for each individual heavy metal, a Hazard Index for Multiple Heavy Metals was developed:

In actual agricultural situations, tomato fruits are seldom found to be polluted with a single heavy metal[40]. Rather, they are composed of combinations of cadmium, lead, arsenic, nickel, and chromium at the same time and all of these metals have a negative effect on health. Hazard index (HI) is the total of all the individual target hazard quotients (I-THQs) for all the metals of concern in the fruit. The basic premise is that the toxicity of the various metals is additive, regardless of the various mechanisms of toxicity between the metals. If the hazard index is greater than 1, the overall risk of all of the metals is above the acceptable range. The Cd and Pb contributions have been reported in China from industrially contaminated sites with HI ranging from 1.5 to 4.2 for the consumption of tomatoes. In these instances none singly would have a THQ over 1, but the combination of metals would do so[41]. It also fails to take into account possible synergy or antagonism between metals, and interactions between some metals (e.g. zinc, copper) which are required for proper metabolism and, therefore, have safe ranges of intake. However, the hazard index is widely used by regulatory agencies, such as the U.S. Environmental Protection Agency as a screening tool. A hazard index greater than 1 in a tomato production area would indicate the need for soil remediation, a change in irrigation practices or dietary diversification to limit local production of tomatoes for risk managers.

Table 3: Summary of Global Studies Reporting Heavy Metal Concentrations in Marketed Tomatoes and Associated Health Risk Indices.

Region / Country	Source of Contamination	Cd (mg/kg FW)	Pb (mg/kg FW)	As (mg/kg FW)	% Exceeding Codex Limit	THQ (Adult)	THQ (Child)	Reference
China (e-waste regions)	E-waste recycling soils	0.12–0.38	0.25–0.62	0.05–0.12	45–70%	0.9–1.5	1.2–2.3	Li et al., 2018
Pakistan (urban/peri-urban)	Wastewater irrigation / sewage	0.08–0.22	0.30–0.85	0.04–0.09	40–65%	0.8–1.3	1.1–1.9	Khan et al., 2020
Nigeria (mining areas, Jos Plateau)	Mining-contaminated soils	0.01–0.45	0.01–0.31	ND–0.05	30–55%	0.6–1.2	0.9–1.8	Joseph et al., 2016
Nigeria (industrial/agricultural zone)	Cement/industrial emissions	0.28–0.37	0.86–0.87	ND	50–75%	1.1–1.6	1.5–2.4	Abdullahi et al., 2023
Italy (volcanic soils, Campania)	Naturally enriched volcanic soils	0.05–0.15	0.10–0.28	0.03–0.08	20–40%	0.4–0.9	0.6–1.3	Giordano et al., 2019

6.3: The risk of cancer due to long term tomato consumption from inorganic arsenic, cadmium and lead.

Tomatoes can be a source of exposure to certain heavy metals that may be linked to a risk for cancer over the long-term, especially when cultivated on contaminated soil. The International Agency for Research on Cancer (IARC) has categorized inorganic arsenic as a Group 1 human carcinogen, and there is substantial evidence of its association with cancers of the skin, bladder, lung and kidney related to dietary exposure. Cancer Risk is the difference in the likelihood of developing a cancer over a 70-year lifetime between the presence of a specific exposure and its absence. With the standard cancer slope factor of inorganic arsenic

(1.5 per milligram per kilogram per day), tomato fruit arsenic levels as low as 0.05 milligrams per kg consumed daily over a lifetime (50 grams per day) equate to a 5 x 10 to the negative 5 (or 5 excess cancers per 100,000).Cadmium is a Group 1 carcinogen associated with the development of lung, kidney and prostate cancer. Although the cancer slope factor for Cd is lower than that of arsenic, the biological half-life of Cd in the human body is much longer (decades) thus making cumulative exposure very hazardous. Lead is a probable human carcinogen (Group 2A) with evidence of cancer on the stomach and lungs. The risk of cancer from eating tomato plants is generally low if the plants are cultivated on non-polluted soils; however, in some areas of the world where soil is polluted by mining activity or industrial processes, the risk of cancer is higher, e.g., 1 in 10,000, which is unacceptable to many health authorities[42]. Importantly, cancer risk calculations make the assumption of lifetime daily consumption, and also do not take into account the protective effects of the tomato antioxidants, which could potentially reduce the cancerogenic effects of metals by scavenging free radical (FRs) (Cao, Chen, and Yang).

6.4 The Vulnerable Populations category is made up of three groups: Children, Pregnant Women, and Subsistence Gardeners.

Not everyone who consumes tomatoes exposed to high levels of heavy metals is equally at risk of health effects. The multiple factors make children the most vulnerable group. They have a lower body weight, and therefore a higher dose per kg of absolute metal. Adults eat less per pound of body weight than do they. They have a greater ability to absorb metals within their gastrointestinal tract, as the rate of absorption in childhood is estimated at close to 50 per cent of the amount consumed, compared with 5-10 per cent in adults. In addition, young organs, such as the brain and kidneys, are more vulnerable to toxic effects of metals. Children are particularly susceptible to the effects of lead exposure and even low levels of lead in the blood cause permanent neurodevelopmental damage[43]. Another high-risk group is pregnant women since cadmium and lead are transferred across the placenta and are deposited in foetal tissues, disrupting normal organogenesis and neurodevelopment. Exposure to dietary cadmium in the mother has been linked to lower post-natal weight and gestation period. Small farmers who are able to produce most of their own food in a subsistence garden are also exposed. If these people eat higher amounts of home-grown tomatoes, which are often grown in soils that have not been analysed for metals, they can unknowingly be exposed to higher levels of metals for many decades over their lifetime and their families could also be at risk[44]. A locally specific consumption data and soil to plant transfer factor is needed to perform the risk assessment for these populations, rather than using generic default values.

6.5 Case Studies also cover Industrial Zones, Mining Affected Areas and Urban Wastewater Irrigated Farms:

Field surveys in several countries have supported the findings of an increased risk to human health from eating tomatoes in certain land use situations. Tomatoes from the industrial area of Hebei Province, China, with a distance of only 0.1 km to 1 km from the battery recycling plant, contained the cadmium level ranging from 0.12 to 0.28 mg/kg fresh weight, two to five times exceeding the limit for Codex[45]. The total calculated THQ for children was greater than 3.0 and the total hazard index was greater than 5.5 with the inclusion of the contribution from lead and arsenic. The lead concentration in tomatoes from home gardens in mining impacted areas of Zacatecas, Mexico, ranged up to 0.45 mg/kg with estimated daily intake levels reaching four times the PTWI. The most common risk is in peri urban and urban farms which utilize untreated wastewater for irrigation. A study in the suburbs of Hyderabad, Pakistan found cadmium and lead levels of 0.09 and 0.34 mg/kg in tomatoes grown from municipal wastewater. The hazard index for adults who ate these tomatoes was 1.8 and the hazard index for children was 4.2. In the same tomatoes, cancer risks were more than 5 x 10 to the minus 4 or five extra cancers per 10,000 people. The overall findings from these case studies are that the component of contaminated soil, polluted water and high levels of tomato production is a measurable public health problem, especially for low-income populations with low income alternative food sources. In this context, there is need for not only scientific solutions, but also policy interventions to safeguard the vulnerable communities from the impacts of climate change.

7. Variability in Tomato Response to Different Factors:

7.1 Genotypic Variation Cherry versus Beefsteak versus Processing Tomato Cultivars

Genotypic differences among tomato lines exist in regard to their ability to exclude or tolerate high level contamination, providing opportunities and challenges in managing risks. Beefsteak tomatoes have large fruit and vining growth habits, whereas cherry tomatoes are small fruited and determinate. Cherry tomatoes tend to have lower accumulation of heavy metals in fruits than their large fruited beefsteak counterparts, and are determinate. A number of studies have reported that the amount of cadmium in the fruit is 30-50 percent less in cherry cultivars than in beefsteak cultivars growing in the same contaminated soil. Processing tomatoes, a type that has been bred to be mechanical and to ripen uniformly, have intermediate accumulations with significant exceptions. Heinz 1370 is a widely grown cultivar for the production of paste; it was determined to be a mutation of a vacuolar cadmium transporter and is considered a cadmium excluder. Other heirloom beefsteak varieties on the other hand, are metal accumulators, and are found to contain two to three times as much cadmium in the fruit placental tissue compared to modern hybrids. A high heritable component to this genotypic variation. Linkage maps for the tomato lines with reduced Cd accumulation suggest that the regions of chromosomes 3, 6, and 10 contain a candidate gene for Cd accumulation, including both metal transporters and chelators. Breeding programs are underway to develop cultivars with low levels of metals, but these programs require that other essential nutrients (e.g. zinc and iron) also be kept at low levels. Also, cherry tomatoes are eaten whole with the skin on and may be more susceptible to the accumulation of surface-deposited metals from air borne fallout while beefsteak tomatoes are usually peeled prior to processing.

7.2 Growth Stage Sensitivity Flowering and Fruit Set as the Most Vulnerable Windows

Heavy metal exposure early in tomato development has a significant effect on the levels of metal build-up and on the effect on fruit quality. The flowering and fruit set stage is the most sensitive stage. Tomato plants are quickly using the carbohydrates and nutrients to developing flowers and young fruits during this time. Soil solution heavy metals present at this time are efficiently taken up and translocated due to high transpiration and active transport of photoassimilates to sink organs via the phloem. Exposure during the flowering stage causes a decrease in the viability of the pollen, abortion of ovules and poor fruit set. Fruits that do set may be far more contaminated with metals than fruits exposed to only during vegetative and/or late ripening stages resulted in a 60% decrease in marketable fruit yield and 40% increase in fruit cadmium content in comparison to continuous exposure. The early ripening phase (from the breaker to the red ripe stage) is also vulnerable because oxidative stress due to metal directly affects the synthesis of ethylene and accumulation of carotenoid. Unlike this, exposure to the early vegetative stage gives tomato plants a chance to produce phytochelatin and chelate the metals in the older leaves, thereby minimizing the fruit contamination[46]. In practice this means that the quality of irrigation water is most important when the plants are flowering and early fruit development is occurring (4-6 weeks after transplanting for determinate varieties).

7.3 Interactions Among Multiple Heavy Metals Synergistic versus Antagonistic Effects

Tomatoes are typically not contaminated with only one heavy metal in agricultural soils. Rather, a complex mixture of cadmium, lead, zinc, copper and other metals can either synergistically facilitate or inhibit uptake and toxicity in plants.

Interactions between metals sharing transport pathways are common, and typically antagonistic. In addition, the higher the level of a soil constituent (such as zinc) the less cadmium uptake occurs; this is due to competition for uptake between the two elements for the same cell membrane transporters (IRT1 and ZIP family transporters). Measuring soil zinc at 150-200 mg kg-1 reported in field studies has helped to limit fruit cadmium by 30-50% without causing zinc toxicity. In the same way, lead uptake decreases with high calcium availability, nickel and cobalt uptake decreases with high iron availability. The antagonisms indicate that an effective mitigation measure may be balanced fertilization with essential metals. There are, however, interactions that are synergistic. Two metals, cadmium and lead, when combined, can cause more oxidative stress than either would cause alone due to the fact that they target unique cellular components. Cadmium interferes with electron transport and lead inhibits antioxidant enzymes, leading to an overwhelming effect on cell defenses. Arsenic and cadmium synergize each other by increasing the influx of cadmium as a result of arsenic damage to the membrane. High zinc to cadmium ratio is protective and high cadmium to zinc ratio is dangerous. The future risk assessment needs to be advanced and should consider the most advanced mixture toxicity models, such as concentration addition or independent action models, to predict the tomato fruit quality outcomes under realistic field conditions.

7.4 The temperature, light intensity and water availability are called the environmental co factors.

Ambient environmental conditions have a strong effect on the expression of heavy metal toxicity in tomato. Metal induced oxidative stress is aggravated by high temperatures, which are the daytime temperature above 32 °C, as the production of ROS in chloroplasts and mitochondria is stimulated. Both the heat and cadmium stress contribute to an increase in reduction of photosynthetic efficiency and lycopene accumulation when compared to the effect of either stress individually. In contrast, the low temperatures below 15°C decrease transpiration rate and metal flow, decreasing fruit production, and also delaying the ripening process[47]. When the concentration of the metals in the soil does not change, the concentration of lead and cadmium in tomatoes will be greater under full sun than under partial shade conditions. But high light also stimulates the production of antioxidants and can help to some extent in reducing the damage effects of metals. The stomata respond to drought stress by closing, thereby decreasing transpiration and xylem metal transport, while concentrating metals in the soil solution that remains in the soil. Deficit irrigation can be used to increase tomato flavor by concentrating sugars, but can lead to the adverse effect of increased fruit metal concentrations due to decreases in fruit water content, whereas metal mass remains constant. The management of irrigation, therefore, has to take into account the enhancement of water quality and risk to food safety.

7.5 Soil Microbial Community Effects Arbuscular Mycorrhizal Fungi as Protective Agents

The tomato root soil microbiome has a crucial effect on the availability and uptake of heavy metals. Especially important are the arbuscular mycorrhizal fungi (AMF) that are symbiotic with tomato roots. These fungi grow within the root cortical cells and trunk into the soil, thus increasing the root volume by hundreds of square metres per gram of soil. Extraradical hyphae absorb water and nutrients, such as phosphorus, zinc and copper and transport them to the plant in return for carbohydrates[48]. AMF, for the case of heavy metals, offer a double benefit. Firstly, the cell wall of mycelium of fungi is rich in chitin and glomalin, which have high binding capacity for cadmium, lead and other cations and they bind the metals within the additional radical mycelium and stop them from entering the root symplast. Second, the genes involved in metal chelation are upregulated in plants by AMF colonization and the vacuolar sequestration process is strengthened. Meta analysis of tomato studies indicates that AMF inoculation can decrease the shoot cadmium level by 30-60 percent and fruit cadmium level by 20-40 percent, while increasing plant biomass and fruit yield. There are differences in the effectiveness of the AMF species and they will respond to soil pH and phosphorus status[49]. AMF colonization is inhibited when soil phosphorus is high, because plants decrease the allocation of carbohydrates to the fungi in the presence of high soil phosphorus. Intensification of tillage, fungicide application and monoculture result in the loss of these beneficial functions of soil microbial communities.

8. Mitigation strategies to minimize heavy metal accumulation and retain quality of tomato fruit.

8.1. In situ immobilization involves immobilizing contaminants in the soil in place.

The most basic approach to avoid excessive accumulation of heavy metals in tomato fruits is to reduce the bioavailability of the metals in the soil matrix. In situ immobilization – amendments that bind or precipitate metals to form non labile metal in the absence of soil removal from the field[50]. Pyrolytic organic residues under oxygen limitation are used to produce biochar, which has proved to be among the best amendments. It has a high surface area and has a porous structure with a lot of oxygen containing functional groups that make it strongly bind to cadmium, lead, nickel and copper. Biochar applied to contaminated soil at 5 to 20 t ha-1 has been demonstrated to decrease the level of Cd in tomato fruits by 40 to 70 percent and increase the soil organic matter and water holding. Compost quality, however, can be variable and certain composts may contain high levels of metals, depending on the quality of the composting feedstocks. The practice of liming (increasing the pH of the soil) is usually done by adding calcium carbonate or calcium hydroxide to the soil and is particularly effective at reducing the bioavailability of cadmium and lead. The soluble concentration of cadmium decreases by about half for each pH unit increase above 6.5. Phosphate amendments (e.g. rock phosphate and triple superphosphate) will bind lead by forming highly insoluble pyromorphite, a lead phosphate mineral. However, phosphate can also decrease the bioavailability of arsenic, but can also mobilize cadmium when used in soluble forms, so careful consideration should be given to which source of phosphate to use[51]. Despite this, however, the most inexpensive and readily applicable response to contaminated areas is in situ immobilization for smallholder farmers.

8.2 The management of water is at the top of the list for the development of a healthy pasture:

Heavy metal inputs to tomato cropping systems are directly related to the quality of the water used in irrigation; and one of the most direct controls is to replace contaminated water with clean water. If groundwater or surface water sources are contaminated, alternatives are rainwater harvesting, treated wastewater, or mixing up clean water with contaminated water to lower the concentration of metals to below the thresholds[51]. For situations where clean water is not available, shifting irrigation scheduling can lead to decreased uptake of metals. A saturated soil condition followed by an unsaturated soil condition (alternate flooding) causes a decrease in the bioavailability of cadmium[52]. Cadmium precipitates as cadmium sulphide under flooded anaerobic conditions and this compound is very insoluble. In Cd dominant contamination, keeping the water level low and keeping the crop under shallow flood during fruit development reduces the Cd level by 50-70%. The formation of arsenite can be reduced by irrigation of contaminated soil in an aerobic manner with shallow, frequent irrigation for arsenic dominant contamination[53]. Drip irrigation will lower the amount of water applied and the movement of metals up from deeper soil layers,.The dynamic of soil type and water management is very important and site-specific recommendations are essential for successful mitigation.

8.3 Microbial Interventions Plant Growth Promoting Rhizobacteria:

Bacteria in the tomato rhizosphere have the ability to decrease the uptake of heavy metals through various mechanisms. The production of siderophores by rhizobacteria, including plant growth promoting rhizobacteria (PGPR) like Pseudomonas putida, Bacillus subtilis and

Rhizobium species, in which they are small molecular weight chelators with high affinities to iron, but also bind cadmium, lead, and copper[54]. The activity of the free ions of toxic metals in the soil solution is lowered by siderophore complexation, which decreases their bioavailability[55]. Other PGPR strains excrete EPFs which interact with metal cations on bacterial cell surfaces that trap the metal cations prior to entry into root cells. In addition, some bacteria can use enzymes to convert arsenate to less toxic arsenite and/or volatilize arsenic as trimethylarsine. When tomato plants are inoculated with selected strains of PGPR, levels of Cd and Pb in the fruit have been seen to be reduced by 25–45 per cent during pot and field trials. The effectiveness of PGPR is strain dependent and requires strain compatibility with the tomato cultivar and the soil microbial community that is native to the soil[56]. The simultaneous inoculation with AMF and PGPR can give synergistic effect as AMF increase soil exploration and bacteria trigger the defense mechanisms of the plants.. Microbial interventions combined with composts (organic amendments) offer a source of carbon for bacteria growth and extra metal binding sites which is a good low-cost option for smallholder farmers.

8.4 The foliar application of selenium, silicon and salicylic acid are recommended under these conditions.

Heavy metal uptake from the roots to the fruits can be decreased by applying some of these compounds directly to the leaves of tomatoes. An element that is essential to humans but not plants, selenium has been of special interest. Low concentrations (5 to 10 micromolar) of selenium on foliar application lead to the activation of the phytochelatin synthase genes and stimulate the production of glutathione, thus improving the plant's ability to store cadmium and lead in the foliar vacuoles. Selenium is also an antioxidant, which helps to decrease oxidative damage caused by metals to fruit ripening machinery. Research indicates that foliar selenium application can cut down the level of cadmium in tomatoes by 30-50 percent, but without affecting yields. Silicic acid is absorbed and is deposited in the cell walls, where it chelates metals, thereby inhibiting their movement by apoplast and cell walls. Leafy applications have decreased the amount of fruit lead and nickel 20 to 35 percent[55]. However, foliar applied salicylic acid was able to reverse cadmium induced decreases in lycopene concentration without any significant reduction in fruit metal concentration. Foliar application is an option for farmers as it is easy and cost-effective, but may require multiple applications in highly contaminated environments.

8.5 Breeding and Genetic Engineering: Overexpression of Metal transporters:

Genetic solutions to reduce the uptake and/or improve sequestration of metals in tomato fruits without the use of external inputs are needed for long-term and sustainable mitigation of heavy metal accumulation. There are cultivars of low cad content identified in conventional breeding which are being utilized for crossing programs to incorporate the small cad content into high yielding commercial cultivars[57]. Now it will be possible to select those QTLs present on chromosomes 3, 6 and 10. Tomato plants with an over-expressed vacuolar cadmium and zinc transporter gene (HMA3) exhibit greater vacuolar sequestration of cadmium and lower xylem loading, which results in a decrease in cadmium uptake. Reducing the levels of cadmium in fruit has been demonstrated in transgenic tomatoes under a root specific promoter, in which cadmium levels were reduced by 50–70%. The plasma membrane efflux transporter, PCR2 (Plant Cadmium Resistance 2), appears to remove cadmium from the root symplast and return it to the soil solution[58, 59]. The genes responsible for the biosynthesis of the chelators, phytochelatin synthase (PCS) and metallothionein (MT), have been also introduced to the host plants, some of which led to unexpected effects on essential metal homeostasis. It is theoretically possible to use Genome Editing with CRISPR Cas9 to disrupt the uptake transporters (e.g., IRT1) but knockouts of IRT1 would result in Iron Deficiency, meaning that tissue specific and/or inducible knockout strategies will be required. Although regulation and public acceptance are both obstacles to commercialization of modified tomatoes, the technology is in place and could be used in the future.

8.6 Peeling, Washing and Cooking effects in Post Harvest Processing.

Consumer and food processors can limit the consumption of heavy metals in tomato products by simple post harvest treatments. Running tap water during washing helps wash off the dust and soil particles that have been deposited on the surface of the tomatoes which can contain high levels of lead and chromium. Research has indicated that the amount of cadmium lost per square meter of peelings is from 15 to 25 percent of the total cadmium, and the amount of lead lost is from 30 to 50 percent of the total lead, depending on the source of contamination. There are variable effects when cooked, such as cooking, boiling and sauce making. When boiling tomatoes in water, the water soluble metal complexes are removed into the cooking water and then thrown away, decreasing metal intake. During cooking, the inclusion of acidic foods (e.g. lemon juice, vinegar) may increase the solubility of metals and, perhaps, their absorption, although this is rarely quantified. Metal levels in industrially processed tomato products are generally found to be higher in paste tomato products compared to fresh tomatoes, on a dry weight basis, and higher in ketchup than in fresh tomatoes. As a result, fresh tomatoes are recommended to be washed well in contaminated areas, and peeling tomatoes is recommended. These are simple steps that are not a complete loss of risk but offer a readily available level of protection.

9: Knowledge Gaps and future Research Directions:

9.1 Lack of Long -Term Field Studies Under Mixed Heavy Metal:

Apart from a few exceptions, most of the research on heavy metal accumulation in tomato has been carried out in a controlled environment such as a greenhouse or hydroponics system, with only one metal salt. These experiments have given us some insight into the mechanisms, but have little relevance to the complexity of the real-world soils that tomatoes face in which they are cropped with mixtures of cadmium, lead, arsenic, zinc and copper for several growing seasons[60]. There is a need for long term (5-10 years) field trials to gain understanding of the long-term effects of repeated applications of contaminated irrigation water and/or continuous addition of metals from fertilizers to soil metal pools, their uptake patterns, and fruit quality over time[61]. Seasonal changes in temperature and rainfall that affect the bioavailability of metals should also be considered in such studies. Further, most field surveys are single-cut, and do not reflect variation between years[61]. This lack of multi-metal field information over a long period of time reduces the ability to use relevant soil quality guidelines for tomato production and reduce risk assessments.

9.2 Need for Transcriptomic and Metabolomic Integration to Link Heavy Metal Stress Signatures with Specific Quality Gene Networks:

While several studies have already reported metal stress-induced downregulation of genes encoding lycopene, e.g. PSY1, or sugar accumulation, e.g. LIN5, a systems level view of the changes in the tomato fruit gene expression and metabolism of this stress is still missing[62]. There is no published research that combined the information from time resolved transcriptomics, proteomics, and metabolomics from the same fruit samples subjected to a gradient of Cd and/or Pb exposur. Ten genes that are always expressed in association with the concentration of metals in fruits, for instance, could be used to create a diagnostic test that breeders could use. Other compounds such as volatile organic compounds, glycoalkaloids, which are generally bitter tasting, and phenolic conjugates should be included in metabolomic profiling beyond the well studied carotenoids and sugars[63].. Large scale systems biology projects of tomato as a model fruit crop should be prioritized by funding agencies.

9.3 Lack of information on effects of Heavy Metals on VOCs involved in Tomato Aroma

The tomato flavor is more complex than just sugars and acids, with the presence of terpenes, esters, alcohols, ketones and aldehydes. Over 400 volatiles have been found in tomato fruits, of which around 20 are important to the delicious aroma that is liked by consumers. They are cis 3 hexenal (grassy), 6 methyl 5 hepten 2 one (fruity) and 2 phenylethanol (floral)[64]. Only less than ten studies have focused on the effect of heavy metal accumulation on the volatile composition of tomato fruits, and there are no studies that have addressed this issue using comprehensive two-dimensional gas chromatography coupled with mass spectrometry (2D-GCxGC/MS) or electronic nose (e-Nose) technology[65]. Initial data indicate that cadmium stress decreases the concentration of important volatiles and amino acids derived from lipoxygenase, likely due to a decrease in the activity of lipoxygenase enzymes and/or the availability of essential fatty acid precursors. This lack of information has economic consequences since aroma is one of the most important factors that influences consumer preference and willingness to pay.

9.4 The development of a Rapid non Destructive sensor for On Farm screening of Heavy metals is ongoing:

Existing techniques to analyze heavy metal levels in tomato fruits involve destructive sampling, acid digestion, and analysis by atomic absorption or mass spectrometry in the lab. These methods are labor intensive, time consuming and only suitable for a few producers[66]. The need is for the development of fast, portable, and non-destructive sensors that are able to provide an estimate of the fruit content of metals in real time at the farm gate and/or during harvest. X ray fluorescence (XRF) spectrometry is one of the promising techniques that have been miniaturized into handheld devices[67]. Another method is near infrared (NIR) spectroscopy: the overtones of molecular vibration are detected. Development and validation of sensors for various tomato cultivars and environmental conditions are high priority research that has a direct translational impact to food safety.

9.5 Aligning laboratory mitigation strategies and solutions to cost-effective farmer level solutions:

Several mitigation strategies which lower heavy metal levels in tomato in controlled settings are not validated or feasible at a smallholder farm level. However, biochar is very useful in pot trials, and its production, transportation and soil incorporation costs are too high for some farmers in low income countries. Likewise, microbial inoculants are effective in sterile potting mix, and less effective in soils with competition. The task is to create low cost adaptations and make the application protocols easier[47]. For example, the biochar made with simple kilns using agricultural waste is almost as effective as the engineered biochar. Low cost locally isolated PGPR strains multiplied with molasses based fermentation have the potential of being applied as coatings or as soil drenches[68]. It is important to involve farmer cooperatives in participatory research in order to co develop realistic solutions that are economically viable, culturally acceptable and effective in real world conditions.

9.6 Establishing Crop Specific Quality Based Threshold Values Not Just Safety Based Limit:

One of the greatest knowledge gaps from market perspective is the lack of quality-based threshold values for tomato fruit pulp, regarding heavy metals[69]. Existing regulations include the Codex standard of 0.05 milligrams of cadmium per kilogram of fresh weight of food, which was based on human health risk assessment[70]. These limits are not based on the concentrations of the metals where fruit quality components like lycopene content, sugar to acid ratio, firmness or aroma start to fall. Large scale dose response studies conducted with several cultivars, under various growing conditions and quality parameters are needed to establish these thresholds.

Conclusion and future standing point:

The collective evidence synthesized in this review establishes a clear and scientifically robust pathway linking heavy metal contamination of tomato cropping systems to the degradation of fruit quality. Heavy metals enter tomato plants primarily through root uptake from contaminated soil or irrigation water, with cadmium, lead, arsenic, and nickel being the most significant threats. The fate of each metal within the plant is determined by its chemical speciation and transport properties. Cadmium and zinc, being phloem mobile, are efficiently redistributed from source leaves to developing fruits, leading to measurable fruit accumulation even when soil concentrations are moderately elevated.

Lead and chromium, by contrast, are largely retained in roots and leaves, posing less risk for fruit contamination under root uptake scenarios but remaining hazardous through direct atmospheric deposition. Once inside plant cells, heavy metals induce a cascade of oxidative stress responses characterized by reactive oxygen species overproduction, lipid peroxidation, and damage to photosynthetic machine. This stress, in turn, triggers the downregulation of key genes responsible for fruit quality attributes. Lycopene biosynthesis is consistently suppressed through reduced expression of phytoene synthase (PSY1) and phytoene desaturase (PDS), while sugar accumulation declines due to inhibition of invertase genes including LIN5 and LIN8. Fruit firmness is compromised by altered pectin methylesterase activity. The net result is a tomato fruit that is smaller, less sweet, less red, less firm, and nutritionally inferior to fruits grown on uncontaminated soils. Although some phenolic compounds may increase as a stress response, this elevation does not compensate for the substantial losses in carotenoids and soluble sugars. Critically, the metal concentrations at which these quality losses first become detectable are often lower than the health based safety limits established by Codex Alimentarius, meaning that consumers may purchase legally safe but organoleptically poor fruits without any regulatory warning.

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