Heavy metal induced regulation of plant biology: Recent insights

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Abstract

The presence of different forms of heavy metals in the earth crust is very primitive and probably associated with the origin of plant life. However, since the beginning of human civilisation, heavy metal use and its contamination to all living systems on earth have significantly increased due to human anthropogenic activities. Heavy metals are non-biodegradable, which directly or indirectly impact photosynthesis, antioxidant system, mineral nutrition status, phytohormones and amino acid-derived molecules. Due to the toxic behaviour of some heavy metals, the endogenous status of chemical messengers like phytohormones may get significantly influenced, leading to harmful impacts on plant growth, development and overall yield of the plants. It has been noticed that exogenous application of phytohormones, i.e., abscisic acid, salicylic acid, auxins, brassinosteroids, cytokinins, ethylene and gibberellins can positively regulate the heavy metal toxicity in plants through the regulation of the ascorbate–glutathione cycle, nitrogen metabolism, proline metabolisms, transpiration rate, and cell division. Furthermore, it may also restrict the entry of heavy metals into the plant cells, which aids in the recovery of plant growth and productivity. Besides these, some defense molecules also assist the plant in dealing with heavy metal toxicity. Therefore, the present review aims to bridge the knowledge gap in this context and present outstanding discoveries related to plant life supportive processes during stressful

conditions including phytohormones and heavy metal crosstalk along with suggestions for future research in this field.

Keywords: Plant hormones, metal stress, redox homeostasis, hormone crosstalk.

1. Introduction

Environmental contamination is one of the most crucial challenges in today's world (Ali and Khan 2017). In particular, contamination caused by heavy metals (HMs) is a major concern and a serious environmental menace (Hashem et al. 2017). The rapid expansion of industries and urban areas has led to HM pollution to the environment, with an increased movement or translocation rate in recent years (Khan et al. 2004). The main natural sources of HMs are the breakdown of rocks and volcanoes, whereas human activities that introduce HMs to the environment include agricultural practices such as pesticide/insecticide treatments as well as chemical fertilisers and effluents from industries such as mining/smelting. The burning of fossil fuels is another source by which HMs enter the environment (Spiegel 2002). Because HMs cannot be degraded, they persevere in the environment for a long time, entering the food chain or food web, and consequently lead to health hazards for all living beings.

Amongst HMs, cadmium (Cd) is one of the most hazardous for different organisms. Cd moves rapidly through the soil, so it is easily absorbed by plants and translocated to different organs (Rahim et al. 2016). Based on the concentration of Cd, plants can be categorised as Cd accumulators and avoiders. By contrast, lead (Pb), a nonessential metal, causes toxicity in plants for a long time, with adverse changes in morphology, growth, seed germination, photosynthetic activities, water/nutrient content, and enzymatic activities in various plant species (Nas et al. 2018). These effects were found to be more prominent with increasing concentration and longer exposure. After reacting with the sulfhydryl group (thiol,

R-SH), Pb inhibits the activities of enzymes by producing reactive oxygen species (ROS) that cause oxidative bursts (Zulfiqar et al. 2019).Another nonessential metal, arsenic (As), also causes toxicity in plants at high concentrations by inhibiting root length and proliferation, reducing biomass generation, and interfering with various metabolic activities that drastically affect the growth and reproductive potential of plants (Sharma et al. 2021). However, at low As concentrations, in hyper-accumulator plants, growth may not get negatively impacted due to the fact that these plants have better As tolerance level (Wang et al. 2015). Exposure to As causes the generation of free radicals, which ultimately results in oxidative stress. As binding to plant cells may lead to protein folding or conformational changes. The proteins that bind to As are transcription factors, signalling proteins, metabolic enzymes, and certain structural proteins (Hussain et al. 2018).

Innumerable biochemical and physiological activities of plants are affected by HMs, and the toxicity changes with the type and concentration of the metal, as well as the plant species exposed. HMs also play important roles in plant growth, along with the pH and composition of the soil. Some HMs, including zinc (Zn) and copper (Cu), activate enzymes and act as cofactors, whereas certain HMs, including mercury (Hg), Cd, and As are lethal and cause reduced growth and, thus, cell death at high doses. Plant roots are the primary site for the uptake of HMs; however, these metals can also be absorbed by the surface of the leaves (Shahid et al. 2017). Various reports indicate that HMs influence various plants by altering the activities of antioxidative enzymes (Gjorgieva Ackova 2018). Cd metal interference changes the uptake, accumulation, and transport of various elements, including calcium (Ca), potassium (K), and manganese (Mn), in the upper part of the plants, thus affecting the water status. With increasing concentrations of HMs, plants are adversely affected in terms of reduced growth, chlorosis, discolouration of plants, and ultimately death.

HM stress leads to changes in various physiological activities at the cellular or molecular levels, such as inactivation of enzymes, blockage of functional groups of molecules that are metabolically significant, displacement of vital elements, and disturbance of membrane integrity. Phytotoxicity is primarily caused by the addition of ROS or reactive nitrogen species, which are released through natural physiological processes, including photosynthesis, the Krebs cycle, and the Calvin cycle (Rascio and Navari-Izzo 2011). In addition, the Fenton reaction leads to the interaction of antioxidants and metals, resulting in the generation of ROS. Toxicity symptoms generally confirm the oxidative stress produced by the HMs (Sharma and Dietz 2009). Nontoxic metabolites are synthesised by the complex formation of a metal-binding peptide, metallothionein, and phytochelatin that provides protection to cells and the organisms (Singh et al. 2003; Muller et al. 2015).

Oxidative homeostasis can be measured by determining certain things like whether homeostasis has been disturbed or oxidative stress has been caused. Consequently, the products generated by the reaction of free radicals and oxidised molecules can act as markers. Malondialdehyde and protein carbonyls are the by products of lipid peroxidation, and even 8-oxo-deoxyguanosine may be recognised as a distinctive marker of the oxidative burst (Fryzova et al. 2017). Malondialdehyde and protein carbonyls are measured by simple analysis of biological samples using spectrophotometric techniques (Morales and Munné-Bosch 2019). The expression of enzymes acting as antioxidants can lead to the detoxification of ROS. These enzymes act as markers and can be triggered in various plant tissues to reduce oxidative stress. Superoxide dismutase (SOD) is a key enzyme with potential antioxidant properties and acts as the first line of defence against oxidative stress produced by HMs in plants (Rady and Hemida 2015). In addition, other enzymes, including catalase and peroxidase, are expressed against stress (Naz et al. 2015). Figure 1 provides the overview of heavy metal generated oxidative stress and its regulation by plant's internal defense system.

2. Uptake, translocation and sensing of heavy metals in plants

Metal mobility depends on various factors and soil properties, such as organic material content, oxides, soil structure, and the creation of different soil layers (Mehes-Smith et al. 2013a). A wide range of factors, including the combination of metals with moving colloidal dimensions and the development of organic and inorganic metal complexes (which do not adsorb easily to the solid surfaces like soil particles), can boost the transport of materials. In metal distribution and horizontal mobility, soil topography also plays a key role. The strength of the plasma membrane, which is negative on the cytosol side, helps to regulate metal cations by means of secondary carriers such as channel or proton conveyor proteins (Mishra and Dubey 2006; Tangahu et al. 2011). Furthermore, depending on the form and function of the plant, a wide range of mechanisms and dynamics help to absorb HMs.

The two key pathways, the apoplastic- and the symplastic, used in the reuptake of metals by plants have been well researched (Rabêlo et al. 2017; Dalir and Khoshgoftarmanesh 2014). In apoplasmic movement, only non-cationic metal chelates can freely spread due to the greater potential for exchanging apoplast cations (Mishra and Dubey 2006). In addition, the bulk of the metal ions absorbed in the plant's vascular system are not soluble. In symplastic movement, the bulk of highly concentrated metals absorbed are transferred through the xylem using the stele after passing the plasma membrane (Thakur et al. 2016; Saxena and Misra 2010). The plasma membrane has a negative potential that promotes the development of cationic metals. Metals can enter through the xylem via three main steps: (1) root cell sequestration of metal ion; (2) symplastic movement to the stele; and (3) metal entrance into the xylem tissue. Once in the xylem, metals are transported by membrane proteins via the Casparian strip; energy is required for the use of active transport systems. The cation channels in the membrane of the cell allow the metals to be translocated across the

concentration gradient. **Uptake and translocation mechanisms of heavy metals have been shown in figure 2.**

Metals are mobilised, transported, and catalysed by H^+ extrusion across the membrane through ATP-dependent proton pumps (Singh et al. 2003; Brunner et al. 2008). Pectin binding studies showed that binding preferences depend on pectin source (e.g., $Al^{3+} > Cu^{2+} > Pb^{2+} > Zn^{2+} = Ca^{2+}$ or $Cu^{2+} = Pb^{2+} > Cd^{2+} = Zn^2 > Ca^{2+}$, $Pb^{2+} >> Cu^{2+} > Co^{2+} > Ni^{2+} >> Zn^{2+} > Cd^{2+}$ or $Pb^{2+} = Cd^{2+}$) (Debbaudt et al. 2004). Pectin is a heterogeneous polysaccharide family present in the primary cell wall and middle lamella (Guo et al. 2015). Several plants have the ability to reduce root cell wall pectin, and enhance the pectin methylation, resulting in avoidance of heavy metals like Cu due to low metal binding potential of less pectin containing root cells (Colzi et al. 2011). The major functional groups of pectin are hydroxyl-, carboxyl-, amide-, and methoxyl-groups, and these have traditionally been linked to HM binding (in particular carboxyl groups, which allow divalent and trivalent HM ions to bind), high biometal absorption, and the potential elimination of HMs (Mata et al. 2009). The quantifiable evidence concerning the adsorption potential of pectins in the form of food additives indicates their applicability to successfully eliminated Co^{2+}, Pb^{2+}, Cu^{2+} and Ni^{2+} ions in animals and humans. Plant transporters also act as shuttles through plasma membrane for cationic nutrients as well as toxic cations (Singh et al. 2003). The structures of crucial metals are recognised by special membrane proteins, which bind to the metals and then translocated to the cellular compartments. The sequestration of metal ions in the vacuolar space prevents them from reaching cell sites where the key activities of cell division and respiration occur. This is the key mechanism of tolerance in plant species and, thus, an important defensive mechanism against metal toxicity. Some plants have a significant number of protein transporters (Hawkesford 2003). Specific chelators (e.g., organic acid chelators such as malate, citrate, histidine, or nicotianamine) may be involved in the xylem

translocation of metal cations. As the metal is associated in a chelate, it moves in the xylem without being adsorbed by the xylem's high potential for cation exchange.

Arsenic is one of the pollutants that is notoriously toxic to living organisms, including humans and can be found in the environment. Arsenate (As^{5+}) transport is regulated through the phosphate (PO_4^{3-}) transporter in plant species like *Brassica juncea* and *Oryza sativa*. The activity of high-affinity phosphate transporter systems determines the accumulation of arsenate in concentrations following the Michaelis-Menton kinetics. Similar to As^{5+}, As^{3+} use multifunctional aquaglyceroporin, a subset of the aquaporin family, as a transport channel. Of note, these aquaglyceroporins have been identified in various plant species (Mishra and Dubey 2006; Meharg and Jardine 2003). In addition, the absorption of Cd in plants is promoted by various pathways utilising transport proteins. The variable histidyl regions of the ZNT protein allow for Cd^{2+} binding and subsequent transport through a mechanism by which metals are collected and transported (Mishra and Dubey 2006). Cd may also be transported through natural resistance-associated macrophages proteins, a special metal transport mechanism that reportedly absorbs high Cd concentrations in *Arabidopsis* (Mishra and Dubey 2006; Tangahu et al. 2011).

Ni can exist as crystalline inorganic minerals or as precipitation, depending on the soil pH. Higher Ni content is largely based on at least two factors: sequestration and/or translocation in the roots of a metal-accumulator plant (van der Ent et al. 2018). Ni can be transported as a Ni citrate complex or as a Ni-peptide complex to provide high Ni mobility in plants. Ni can also be transmitted as a Ni-histidine complex. In higher plants, an H^+/ATPase-produced proton-electrochemical gradient drives the main active mechanism by which plasma and tonoplasts from plant cells absorb and transport water. Although no specific Ni-uptake systems have been identified in higher plants, two major Ni-uptake strategies exist in prokaryotes: Ni^{2+} permeases regulated by the Ni-cobalt transporter and NiK systems

belonging to the ATP-dependent binding cassette (ABC) transporter family. ABC carriers are an important group of membrane proteins that promote the active transport of ligands through biological membranes.

Pb deposition in the soil from car exhaust dust and other various industrial gasses can lead to the contamination of plants, as well as animals that feed on plants (Tangahu et al. 2011). Plants primarily absorb Pb through passive absorption by the roots. In addition, various studies of plant proteins have shown that Pb transport can be promoted across the membrane. The ability of tobacco and Arabidopsis to absorb Pb have been shown to be regulated via NtCBP 4 and AtCNGC1 protein expression (Mishra and Dubey 2006).

The biological response in plants, animals, and humans to metals is extremely variable and depends on the concentration in the tissue and whether its function is necessary or not. Because plants can absorb HMs, accurate tests for the ecotoxicology in risk assessment should include knowledge of the transport of metals from soil and/or air to plants.

3. Heavy metal stress impact life supporting processes in plants

3.1. Impact of heavy metals on photosynthesis

Heavy metals influence photosynthetic functions directly or indirectly, though the impairment of different physiological and biochemical processes related to the photosynthetic process, a topic which has been explored since '80s (Clijsters and Van Assche, 1985; Sharma et al., 2020).

For example, the functioning of photosystems is the main target of Cr (VI) in isolated chloroplast of pea (Bishnoi et al. 1993). The impairment of PSII is in some instances related to inhibition at various sites of PSII reaction centers (RCs) as observed in case of Cu toxicity (Baron et al., 1995) or due to impairment of plastocyanin functioning as observed in plants

subject to Hg toxicity (Kimimura and Katoh, 1972). In other cases, for example in case of Pb toxicity, it has been demonstrated that the metal affects photosynthesis by inhibiting activity of carboxylating enzymes (Stiborova et al., 1987). The inhibition is thought to be connected to the reaction of Pb with protein sulfhydril groups and, in turn, the enhancement of oxidative stress by increasing the production of ROS is observed (Reddy et al., 2005). The enhancement of oxidative stress is a common feature in plants under stress, in particular when the capability of light utilization is constrained by environmental cues, as HM (for reviews see Fryzova et al., 2017; Ghori et al. 2019). Notably, a study in which seedlings of spring barley (*Hordeum vulgare* L.) were exposed to a wide range of copper, zinc, chromium, nickel, lead and cadmium concentrations pointed outdose-dependent and ion-specific responses in terms of oxidative stress. As a consequence of oxidative stress, cellular membranes (including chloroplast membranes) become altered, causing electrolyte leakage accompanied by an enhanced lipid peroxidation (Belkadhi et al. 2014).

Another mechanism leading to inhibition of photosynthesis by HM is their substitution for Mg^{2+} in the chlorophyll molecules (Küpper et al. 1998). The authors described the consequences of this substitution and depicted the effect connected to the loss of functionality in most cases in chlorophyll molecules. The loss of functionality can also be associated to the reduction of biosynthesis of chlorophyll and accessory pigments, as observed in *Vigna radiata*, *Helianthus annuus*, *Brassica napus*, and *Thalaspi caerulescens*, exhibit photosynthesis inhibition (Küpper et al. 2007; Tran and Popova 2013; Wahid and Javed 2008, respectively) subjected to cd stress. Differently, in *Phaseoulus vulgaris*, Calvin cycle reactions are more likely than PSII and pigment biosynthesis inhibition to be the primary target of the toxic influence of Cd. The reduced demand for ATP and NADPH upon Calvin cycle inhibition causes a down-regulation of photosystem II photochemistry and of the yield of linear electron transport (Krupa et al. 1993). Accordingly, Siedlecka et al. (1997)

observed that the inhibition of Rubisco carboxylase activity may be considered as the primary plant response to Cd-stress and, consequently Cd-induced increase in ATP content, and in the ATP/ADP ratio was observed. Fe supply resulted in a revitalisation of the adenylate pool and Calvin cycle metabolites thereby ameliorating Cd toxicity.

3.2. Impact of heavy metals on oxidative stress

Oxidative stress arises when there is imbalance between accumulation and scavenging of oxidising biomolecules(disruption in the equilibrium between oxidising materials and antioxidative machinery) Redox metals including Fe, Cu, and chromium undergo redox cycling, whereas redox metals including Pb, Cd, Hg, and others deplete the cell of major antioxidants, particularly antioxidants and enzymes that include a thiol group. The development of ROS, such as hydroxyl radicals and superoxide ions, can be increased by either redox-active or redox-inactive metals. This increased ROS generation can overpower the intrinsic defences of antioxidant cells and lead to a condition that is known as oxidative stress. In general, it is possible to consider HMs' mutagenic potential and oxidative stress reactions by Fenton and Haber-Weiss (Jomova and Valko 2011) reactions. High levels of HMs lead to oxidative stress and, consequently, growth inhibition. There are numerous processes for the managing of ROS involving HMs (Juknys et al. 2012). Phytotoxicity caused by HMs can result from changes in various physiological processes induced by the inactivation of enzymes in cells/molecules, blocking functional groups of metabolically active molecules, or extracting essential elements and disrupting the integrity of the membranes (Singh et al. 2020). Fenton's reaction may be another way of achieving the requisite interaction between antioxidants and metals. In this case, the interaction effects are the ROS.

Redox metals (Cu and chromium) can create the most destructive ROS and hydroxyl radicals ($OH^\bullet$). Singled oxygen may be used to form certain kinds of superoxide ROS (O_2) in

metals that do not have a redox potential, such as Cd or plumes, Zn, or Ni. ROS can cause nonspecific protein and membrane lipid oxidation, DNA damage, and enzyme inhibition by triggering programmed cell death (Sharma et al. 2012). However, ROS plays an important role in the plant defence process and cannot be removed entirely from plants (Schutzendubel and Polle 2002).The detrimental influences of are ROS are concentration dependent. If the ROS concentration reaches the defence mechanisms threshold level, then oxidative stress may occur (Sharma et al. 2012).

3.3. Impact of heavy metals on antioxidant system

Plants have different antioxidant protection mechanisms to scavenge toxic radicals to defend themselves from oxidative stress (Sarma 2011; Zaimoglu et al. 2011; Khan et al., 2022; Ilyas et al., 2022). These defensive mechanisms can be classified into two main classes: low-molecular weight antioxidants (e.g., α-tocopherol), which are lipid-soluble membranes-associated antioxidants and water-soluble reductor agents (glutathione); and antioxidant enzymes, including SOD, POD (Adrees et al. 2015; Lou et al. 2015).

Within the antioxidant network, metal ions play crucial roles as these cofactors are necessary for the majority of antioxidant enzymes. All SOD isoforms, for example, produce bonded HM ions (Gupta and Ahmad 2013; Mehes-Smith et al. 2013b). Cu and Zn are the Cu/Zn–SOD associated cofactor of chloroplasts, and Mn-SOD is present in glyoxisomes. In the chloroplasts of some plants, Fe-SOD has been found. Metals are involved in free radical and direct or indirect ROS production in four ways: (1) direct electron transfer in reduction of one electron; (2) metabolic disturbance leading to a rise in ROS and free radical formation; (3) inactivation and decline of the antioxidant defence mechanism enzyme control; and (4) high molecular antioxidant weight depletion. ROS produced in the leaf cells can be extracted from

an antioxidant system with complex enzymes, such as catalase, ascorbate peroxidase, and glutathione peroxidase.

The key consequence of metal stress is higher ROS production due to impairment of the photosynthesis cycle by HMs (Rascio and Navari-Izzo 2011). In photosynthesis and respiration, ROS such as O_2^- and H_2O_2 are formed as the by products of electron transports (Kadukova and Kavuličova 2011). Nonetheless, complex regulatory mechanisms are integrated in plants such as ROS, plant hormones, ethylene, and abscisic acid (ABA), signalling molecules such as salicylic acid and jasmonic acid, and secondary messengers like Ca (Cotrozzi et al. 2017; Landi et al. 2019; Aftab and Roychoudhury 2021). However, when ROS are greater than physiologically normal levels, their accumulation causes oxidative stress in the cells, resulting in lipid peroxidation, macromolecular deprivation, membrane intrusion, DNA breakage, and the leakage of plant ions (Rascio and Navari-Izzo 2011; Cotrozzi et al. 2017; Landi et al. 2019).

4. Oxidative stress is a localised chloroplastic phenomenon found mainly in chloroplast ascorbate-peroxidases. Along with glutathione reductase and dehydroascorbate reductase, a process called Halliwell-Asada pathway is expected to eliminate H_2O_2. In addition, plant cells have relatively high levels of ascorbate, glutathione, and α-tocopherol, all of which are effective scavengers of oxygen. Lipophilic α-tocopherol is available in large amounts in thylakoid membranes wherein it inhibits lipid peroxidation chain propagation reactions. Proline is used to detoxify active oxygen in heavy metal–stressed *Brassica Juncea* and *Cajanus cajan*. In a number of plant species subject to HM stress, the accumulation of proline has been observed. Early toxicity was closely linked to metal-induced oxidative stress (Sharma and Dietz 2009). The production of nontoxic metabolites to protect cells and the entire organism is facilitated by complex metal-binding peptides, metallothioneins

(gene-encoded polypeptides), and phytochelatins (Singh et al. 2003). **Status of hormonal signalling and crosstalk under heavy metal stress**

Plant hormones are signalling molecules that play a significant role in the growth and development of plants. Different hormones have different modes of action. It has been reported that an individual hormone may possess different cellular and developmental activities; however, a single activity can be regulated by various plant hormones (Gray 2004). Hormones such as cytokinins, ABA, brassinosteroids, salicylic and jasmonic acid play significant roles in the overall development of plants and contribute to the signalling process.

Plant hormones are synthesised inside the plant body and are essentially needed by plants as they trigger modifications in physiological and molecular aspects of plants. These hormones play crucial roles during exposure to HM stress conditions (Fahad et al. 2015). They can control growth, reproduction, enzymatic activities, cell membrane permeability, and secondary metabolite synthesis (Wani et al. 2016). Effects of HMs can be estimated by reduced growth and productivity, consequences of HM uptake and translocation to various plant parts. Toxicity of HMs causes reduced root and shoot biomass, which is overcome by plant hormones.

Treatment of HMs stimulated the internal concentration of ABA. Upregulation of *NCED2/3*genes, the precursor genes of ABA, is caused by As metal stress. Similarly, vanadium metal also leads to the stimulation of signalling and synthesis of ABA triggered by the activation of certain genes. In the signalling of ABA, other genes including *PYL*, *PP2C* and *SnRK2* are also actively involved and are expressed in plants during exposure to HMs such as Zn and Cu (Wang et al. 2014). During the starvation of boron, *PIN1* triggers the alterations in the dispersal of the auxin hormone and, thus, retards root growth. Nitric oxide accumulation is induced by Cd toxicity, which causes suppressed auxin transport and

decreased root meristem size. In addition, nitric oxide plays a significant role in the signalling process of auxin during Cu stress (Petó et al. 2011). During HM stress conditions, the activity of antioxidative enzymes including SOD, catalase, and ascorbate peroxidase are stimulated, thus inhibiting the H_2O_2 content. Furthermore, brassinosteroids stimulates the activities of antioxidative enzymes and provides protection to plants against HM toxicity.

The production of ethylene also increases under HM toxicity due to *ACS* and *ACO* gene expression. *ACS2* and *ACS6* gene expression is regulated by MPK3 and MPK6 at the transcriptional level via the phosphorylation of the transcription factor WRKY33. In this process, WRKY33 attaches to *ACS2* and *ACS6* promoters (Bücker-Neto et al. 2017). In ethylene signalling, EIN2 acts as a vital component by playing a role of stress transducer. Exposure to Pb enhanced the transcript levels of this transducer in *Arabidopsis,* controlling AtPDR12 (an ABC membrane transporter) and leading to the elimination of Pb from the cytoplasm (Schellingen et al. 2014).

4.1. Heavy metals and abscisic acid

ABA is considered to be an important plant hormone with a significant role against abiotic stresses. Against different abiotic stress conditions, the internal level of ABA in plants was found to enhance signalling and activate gene expression (Sah et al. 2016). The modulation of biosynthetic genes of ABA occurs due to the exposure of plants to HMs such as As, Pb, Hg, and Cd, consequently stimulating the concentration of ABA inside the plants (Bücker-Neto et al. 2017). Protein-encoding genes are transcriptionally controlled by ABA (Nemhauser et al. 2006; Wani et al. 2017). By the use of ABA-deficient and insensitive mutants against Cd toxicity, promising findings have been reported that indicate that slowed growth due to Cd is not due to the consequence of ABA signalling. By contrast, the opening and closing of the stomata is controlled by ABA signalling, which triggers water balance

during HM toxicity (Sharma and Kumar 2002). However, there is a scarcity of information indicating the exogenous role of ABA in regulating HM stress. ABA contributes to maintaining the balance between the growth and subsistence of plants against the oxidative stress produced by HM toxicity.

4.2. Heavy metals and gibberellic acid

Gibberellic acid positively influences seed germination, stem and leaf growth, and fruit, flowering, and trichome formation (Yamaguchi 2008). In addition, it protects plants against toxicity of HMs and triggers adaptation under stress conditions, ultimately helping the plant's resistance (Maggio et al. 2010). Furthermore, gibberellic acid contributes to the signalling mechanism, but the meticulous mechanism of gibberellic acid translocation in different parts of plants is still a mystery (Gupta and Chakrabarty 2013). In *Zea mays* plants, expression of the *TaMYB73* gene was stimulated due to presence of gibberellic acid (He et al. 2012). DELLA proteins were also found to be involved in helping plants avoid stress, as they repress gibberellic acid activity (Wild and Achard 2013). Another report suggested that small doses of Zn can enhance the level of GA3 while high doses reduce GA3 (Atici et al. 2005).

4.3. Heavy metals and salicylic acid

Salicylic acid is a naturally existing phenolic compound that provides defence to plants against HM toxicity. This hormone plays a significant role in plant growth and development, fruit ripening, and protection against abiotic stress conditions (Rivas-San and Plasencia 2011). Along with ABA, it was applied to plants to regulate the drought conditions (Miura and Tada 2014). Salicylic acid regulates temperature, salt, HM stress, and ultimately cell death and provides resistance in conditions caused due to hypersensitive responses (Fahad and Bano 2012; Khanna et al. 2016). In *Hordeum vulgare* roots, salicylic acid content was found to increase during exposure toCd and ameliorate Cd stress by activating the

pathways that can detoxify Cd, apart from modulating the antioxidant defence system (Metwally et al. 2003). Salicylic acid was also beneficial in combination with other plant growth regulators such as jasmonic acid and ethylene (Jia et al. 2013). Against HM stress, array of signal transduction is produced as a consequence of plant activities in response to stress with the synthesis, accumulation, and translocation of phytohormones to the different plant parts (Matilla-Vazquez and Matilla 2014). Ethylene biosynthetic enzyme activities were enhanced upon HM stress by phosphorylation of *ACS2* and *ACS6* due to activation of MAPKs (Bucker-Neto et al. 2017). Salicylic acid may reduce HM toxicity by regulating various plant metabolic processes (Safari et al. 2019; Wei et al. 2018; Kohli et al. 2018; Guo et al. 2018), the scavenging, and/or reduced accumulations of ROS and/or an enhancement of antioxidant defence systems (Malik et al. 2019; Mohamed et al. 2019; Mostofa et al. 2019; Wang et al. 2019), the safeguarding and security of the membrane stability (Belkadhi et al. 2015), the interaction with plant hormones (Tamás et al. 2015), the increase of hemoxygenase (Cui et al. 2012), and the enhancement of efficiency.

4.4. Heavy metals and auxins

Indole-3-acetic acid (IAA) is a type of auxin that has multiple roles in plants. Apart from playing an essential role in the progression of plants in optimum conditions, it also protects them against stress and regulates growth (Bielach et al. 2017; Singh et al. 2021). In salinity stress conditions, this hormone helps the plants to adapt and it increases during the HM stress. However, reports suggest that stimulated doses of IAA can sometimes cause retardation in growth, possibly due to the disturbances in the hormone balance in plants exposed to stress (Fahad et al. 2015). Exogenous application of IAA triggers improvement in plant growth and reduces As accumulation which regulates As stress in plants (He et al. 2021). Reports indicate that the crosstalk of IAA, ethylene, and ROS plays a vital role in the modification of root systems via signal transduction (Jogawat et al. 2021).

4.5. Heavy metals and brassinosteroids

Among various plant steroids, 70 brassinosteroids have been recognised and have important roles in seed germination, phytomorphogenesis, cell expansion, flowering, vascular differentiation, stomatal formation, and plant senescence (Bajguz and Tretyn 2003; Hayat and Ahmad 2010). Different parts of plants including the roots, shoots, buds, pollen grains, fruit, and leaves possess endogenous brassinosteroids (Bajguz and Hayat 2009) and trigger plant growth even against HM/metalloid stress (Kour et al., 2021; Sharma et al., 2022). 24-Epibrassinolide is one of the most important brassinosteroids and stimulates growth by ameliorating HM stress (Shahzad et al. 2018). When plants are exposed to HM stress, the antioxidative defence system of antioxidative enzymes and antioxidants is modulated. Enzymes such as SOD, catalyse, and peroxidase (POD) and nonenzymatic compounds such as vitamin C and glutathione are involved in the regulation of different types of stresses including HM toxicity (Arif et al. 2016; Singh et al. 2020).

4.6. Heavy metals and cytokinins

Cytokinins play a crucial role in the regulation of plant development. During stress, the endogenous level of this hormone is changed, indicating its significant function in tolerating stress (Brien and Benkova 2013). HM stress leads to inhibition of cytokinin synthesis and its translocation from the roots to different parts of plants. During HM stress conditions, cytokinin involvement and interactions with other plant hormones is extensively enhanced (Ha et al. 2012). The role of cytokinins is the opposite of ABA: when plants are exposed to HM toxicity, the changes in the hormonal level are interdependent (Pospíšilová 2003).

4.7. Heavy metals and ethylene

Reports indicate that the biosynthesis of ethylene is stimulated during HM stress (Gora and Clijsters 1989). Physiological processes including photosynthesis are controlled by ethylene depending on the sensitivity of plants to the hormone (Iqbal et al. 2012). It was observed that HMs such as Cu or Cd stimulates the activity of 1-aminocyclo-propane carboxylic acid synthase (ACS) and, ultimately, the production of ethylene (Pell et al. 1997). At low HM concentrations, ethylene biosynthesis is primarily increased; however, high doses of metal lead to antagonistic effects in many plants (Gora and Clijsters 1989). S-Adenosylmethioninesynthetase is also involved in the synthesis of ethylene from methionine to S-adenosylmethionine. S-Adenosylmethionine has the potential to act as a substrate for the production of 1-aminocyclopropane-1-carboxylic acid by 1-aminocyclopropane-1-carboxylic acid synthase. Activation of 1-aminocyclo-propane carboxylic acid synthase (ACS) gene involved in production of ethylene has been reported to get stimulated under Cu stress in potatoes (Schlagnhaufer et al. 1997). However, Trinh et al. (2014) analysed the effects of chromium toxicity in rice seedlings and found the expression of ethylene biosynthetic genes such as ACS1, ACS2, ACO4, and ACO5 to be strongly induced during chromium signalling.

5. Impact of some defense molecules in heavy metal stress adaptation in plants

Plants subjected to HM stress display various responses and produce several kinds metabolites including proline, phytochelatins, histidine, glutathione, metallothioneins, oligopeptides, glutathione, phytochelatins, betaine, nicotianamine, putrescine, spermidine and spermine, some are at high mM levels. Other than the amino acid-derived molecules, many sugars including sucrose, fructose and trehalose, and few sulphurs and nitrogen-based metabolites have been identified to play essential roles in alleviation of free radicals, for membrane and enzyme defense, in addition to maintaining osmotic stress stability (Handa et al. 2018). Out of many metabolites, for the amino acid proline, a wide array of data implies crucial responsibilities under HM stress (Sharma and Dietz 2006; Szabados and Savoure

2010; Ghori et al. 2019). Proline has been demonstrated to serve as a molecular chaperone capable to secure protein integrity and strengthen the operations of diverse enzymes, for example its role includes the inhibition of protein aggregation and protection of nitrate reductase activity under HM stress (Sharma and Dubey 2005; Szabados and Savoure 2010). Pretreatment of *O. sativa* seedlings with proline decreased the Hg toxicity upon scavenging ROS, i.e. H_2O_2 (Wang et al. 2009). Roots cell wall is rich in metabolites namely carbohydrates, amino acids, phenolics and proteins with the functional groups –OH, –SH and –COOH that binds to di- and trivalent metal ions. Binding of HM ions to the root tissues prior to metal influx throughout the plasma membrane. The metal influx at high levels is concerned by metal ions outflow into the apoplast followed by chelation in cell cytoplasm by amino acids, phytochelatins, metallothionines and organic acids. The harmful effects of $^1O_2$ and $OH^•$ on photosystem-II can be limited by proline in individual thylakoid membranes (Saradhi and Mohanty 1997). The transgenic *N. tobacum* plants engineered for hyperaccumulation of proline with *P5CS* gene overexpression and acceleration of the proline biosynthetic pathway significantly reduces the accumulation of free radicals in cells (Hong et al. 2000).

Hg stress upregulated many genes that involved in aromatic amino acids viz., phenylalanine and tryptophan and enhanced the amount of free phenylalanine and tryptophan ratio in *O. sativa* seedlings (Chen et al. 2014). According to Chen et al. (2014) exogenous utilisation of amino acids to *O. sativa* roots improved the tolerance to HM stress and it was successfully decreased the HM ion induced ROS production, which confirmed that the genes involved in amino acid signaling pathways responsible for HM stress response. Similarly, in Cu stressed *O. sativa* leaves the higher activities of ornithine-δ-amino-transferase and P5CS was correlated with higher quantities of proline accumulation (Chen et al. 2001). In *Silene vulgaris* plants, metal induced proline synthesis was higher in a non-tolerant variety; despite constitutive proline quantity was higher in metal tolerant varieties (Schat et al. 1997).

Histidine is another amino acid reported in relation to Ni stress in *Alyssum lesbiacum* showed that the hyper accumulators that correlated with production of free histidine, and increased histidine ratio was stated in the xylem sap of *A. lesbiacum* under Ni stress treatments (Krämer et al. 1996). In addition to these, glutathione and phytochelatin amino acids also involve in metal binding and the accumulation of these amino acids is needs to be considered as vital response. All these studies established that the role of amino acids or its derived metabolites in hyperaccumulator is crucial because of the formation of consistent complexes with bivalent cations, thereby assisting largely in degrading HMs.

Conclusion:

HMs cause major environmental problems today due to the establishment of industries, development of urban areas, and other various anthropogenic activities. These HMs remain persistent in nature, which is the foremost cause of their hazardous effects on environment. Though most consideration concerning this matter has been engrossed on their toxicity in context with the humans, animals, and plants, which are even drastically affected due to their harmful effects. However, plants have the potential to protect themselves from HMs as they can uptake and translocate them to different upper aerial parts and their accumulation may occur for a long duration. HMs influences the oxidative homeostasis by the generation of ROS. When contaminating soil, they cause reduced growth and altered biochemical and physiological functioning in growing plants. Thus, more studies are required to better understand the effect of HM stress on plants and associated fields to preserve the ecological concord of our planet. The interactions of HMs with plants either show negative/adverse effects or certain resistance or activate tolerance strategies in the plants to combat their toxic effects. This review presented current evidence showing that plant growth and development are affected by HMs. The toxicity of these metals is caused mainly by their accumulation in the soil but can be quenched by hyper accumulator plants through

phytoremediation processes. Plants also use various strategies to enhance their ability to combat HMs including hormonal regulation, stress genes synthesis, and antioxidative defence system activation. However, additional work on the metal stress in plants must be conducted before coming to any definitive inferences.

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