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## Elicitors fortifies the plant resilience against metal and metalloids stress

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### ABSTRACT

This review addresses plant interactions with HMs, emphasizing defence mechanisms and the role of chelating agents, antioxidants and various elicitor molecules in mitigating metal toxicity in plants. To combat soil contamination with HMs, chelate assisted phytoextraction using application of natural or synthetic aminopolycarboxylic acids is an effective strategy. Plants also employ diverse signaling pathways, including hormones, calcium, reactive oxygen species, nitric oxide, and Mitogen-Activated Protein Kinases influencing gene expression and defence mechanisms to counter HM stress. Phytohormones enhance the enzymatic and non-enzymatic antioxidant defence mechanism and the level of secondary metabolites in plants when exposed to HM stress. Also it activates genes responsible for DNA repair mechanism. In addition, the plant hormones can also regulate the activity of several transporters of HMs, thereby preventing their entry into the cell. Elicitor molecules regulate metal and metalloid absorption, sequestration and transport in plants. Combining of different elicitors like jasmonic acid, calcium, salicylic acid etc. effectively mitigates metal and metalloid stress in plants. Moreover, microbes including bacteria and fungi, offer eco-friendly and efficient solution for HM remediation. Understanding these elicitors, microbes and various signaling pathways is crucial for developing strategies to enhance plant resilience to metal and metalloid stress.

### NOVELTY STATEMENT

This review provides recent advancements in understanding how plants mitigate heavy metal (HM) and metalloid stress, highlighting the synergistic potential of different elicitors and microbial interactions for enhanced remediation and plant resilience. By elucidating the complex interplay between chelating agents, antioxidants, and signaling molecules like jasmonic acid, calcium, and salicylic acid, it offers a comprehensive overview of how these elements collectively enhance plant defence mechanisms. Additionally, the review underscores the eco-friendly potential of microbial elicitors, which through diverse mechanisms such as metallothionein synthesis, contribute to effective detoxification and biotransformation of HMs. This integrative approach not only deepens our understanding of plant responses to HM stress but also paves the way for innovative and sustainable agricultural practices.

**Abbreviations:** ABA: abscisic acid; APCA: aminopolycarboxylic acid; BR: brassinosteroids; CK: cytokinin; EDDS: ethylene diamine disuccinate; EDTA: ethylene diamine tetraacetic acid; GA: gibberellic acid; HM: heavy metal; JA: jasmonic acid; MAPK: mitogen-activated protein kinase; NLMWOA: natural low molecular weight organic acids; NO: nitric oxide; NTA: nitrilotriacetic acid; ROS: reactive oxygen species; SA: salicylic acid

### KEYWORDS

Aminopolycarboxylic acids; metalloid stress; metal toxicity; microbial elicitors; Mitogen-Activated protein kinase; phytohormone signaling

## Introduction

Rapid industrial growth has led to an increase in the production and environmental discharge of heavy metals (HMs) and metalloids. Over the past decade, industrialization has accelerated, contributing to the rise in HM and metalloid pollution. Both natural processes and human activities, such as the weathering of soil minerals, the use of treated wastewater, sewage sludge, fertilizers, and various industrial practices, release large amounts of toxic compounds into the

atmosphere (Zhang et al. 2024a). The adaptation of these plants to abiotic challenges can be better understood by looking at the hyperaccumulation of metals and metalloids in plant tissues. Although trace metals and metalloids were always present in the crust of the Earth, human activities like mining, irrigation, and the use of fertilizers and pesticides have increased their concentrations in soil (Tang et al. 2023). Plants absorbed both required and non-essential elements from this range of metals and metalloids. While essential trace metals such as zinc (Zn), copper (Cu), iron (Fe) and

cobalt (Co) play an active role in plant metabolism, high quantities of these metals can be harmful to plants because they surpass the threshold for tolerance (Arif et al. 2016). In optimal concentrations, these metals participate in redox processes and are crucial to plant metabolism (Ghori et al. 2019). However, through transporters intended for necessary elements, non-essential metals like lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), mercury (Hg), and chromium (Cr) can enter plants. HMs are dangerous to plants even in low concentrations because they cause reactive oxygen species (ROS), which can cause oxidative damage to proteins, lipids, carbohydrates, and DNA, among other plant components (Russo et al. 2022). Crop plant growth has also been observed to be hampered by HMs such as Hg, Co, Cu, Cr, Cd and Zn, which have long been deposited in the soil through the dumping of sewage and industrial waste and numerous metalloids and HMs, are used in the industrial and commercial sectors, such as in semi-conductor devices, electronics, alloying, and the medical field. These materials leak into the soil system and have an impact on plant growth and production (Caparrós et al. 2022).

Phytotoxicity can occur when non-essential metal concentrations build up in plants and essential metal concentrations surpass thresholds, resulting in growth retardation and suppression of leaf and root growth. These pollutants subsequently accumulate in soils, leach into groundwater, and contaminate water sources (Okerefor et al. 2020; Alengebawy et al. 2021). This contamination poses serious environmental, agricultural, and public health risks while also threatening biotic communities within ecosystems (Noor et al. 2022; Xie et al. 2022). Hazardous metals and metalloids have been widely dispersed across the Earth's surface, with their presence in soil and water increasing due to human activities over millennia. A critical concern for global agriculture is the rising concentration of HMs in fertile soil, which now exceeds safe thresholds (Angon et al. 2024).

To withstand HM and metalloid stress, plants either avoid absorbing these elements from the soil or activate detoxification mechanisms to manage those they do absorb. Over time, plants have developed sophisticated defence systems to survive in environments containing HMs and metalloids. One strategy involves modifying their root systems or releasing compounds into the soil that bind to HMs, preventing their uptake. However, if HMs are absorbed, plants initiate detoxification processes to minimize their harmful effects. HM-induced stress generates ROS, which cause oxidative damage to plant cells (Zhao et al. 2023). To mitigate this, plants produce metal-binding proteins like phytochelatins and metallothioneins, which bind to HMs in plant tissues, forming less toxic complexes. This chelation process prevents HMs from interfering with essential cellular functions. Moreover, plants utilize phytohormones and their signaling pathways that accompany them to combat the stress that HMs induce. Hormones such as salicylic acid (SA), ethylene (ET), and abscisic acid (ABA) are crucial in regulating plant responses to environmental stressors. These hormones improve the plant's resistance to HM toxicity by controlling metabolic processes, gene expression, and physiological reactions (Zhang et al. 2022).

The production of crops and its quality can be negatively impacted by plant morpho-physiological, biochemical, and molecular changes caused by high levels of HMs and other metalloids in the soil (Tripathi et al. 2023). Toxic HMs reduce biomass and limit growth by interfering with the plant's ability to absorb nutrients and its metabolic processes. Furthermore, the level of methylglyoxal has increased, as has the inhibition of photosynthesis. Several physiological and metabolic processes in plants are regulated by some metalloids at extremely low concentrations, such as Si, B (~1–100 ppm), and Se (>5 ppm). Also, metalloids play a crucial function in promoting various cellular enzymes that participate in diverse redox processes (Dhiman et al. 2024). In addition, grains and fruits may cease to mature, and the pigmentation of leaves may alter in response to excessive concentrations of HMs. Moreover, it has been shown to impact plant nutrient balance, production, and fruit quality (Angulo-Bejarano et al. 2021; Singhal et al. 2023). Plant breeders and biotechnologists have expressed their concern about the decline in agricultural production, which is a threat to food security. Elicitors are molecules capable of inducing the production of compounds stimulating any type of plant defence which lead to enhanced resistance toward biotic or abiotic stress conditions. Elicitors mitigate metal or metalloid stress effects by enhancing antioxidant molecules and accumulation of bioactive secondary metabolites in plants. Depending on their nature or origin, elicitors may be biotic or abiotic (Kaushal et al. 2023; Lone et al. 2023). In light of the aforementioned, this review article discusses HM and metalloid interactions with plants, as well as the role of various elicitors such as chelating agents, phytohormones,  $\text{Ca}^{2+}$ , ROS, NO, and microbial signaling molecules in plant defence against metal and metalloid stress and [Figure 1](#) represents the various mechanisms and strategies for HM stress amelioration in plants.

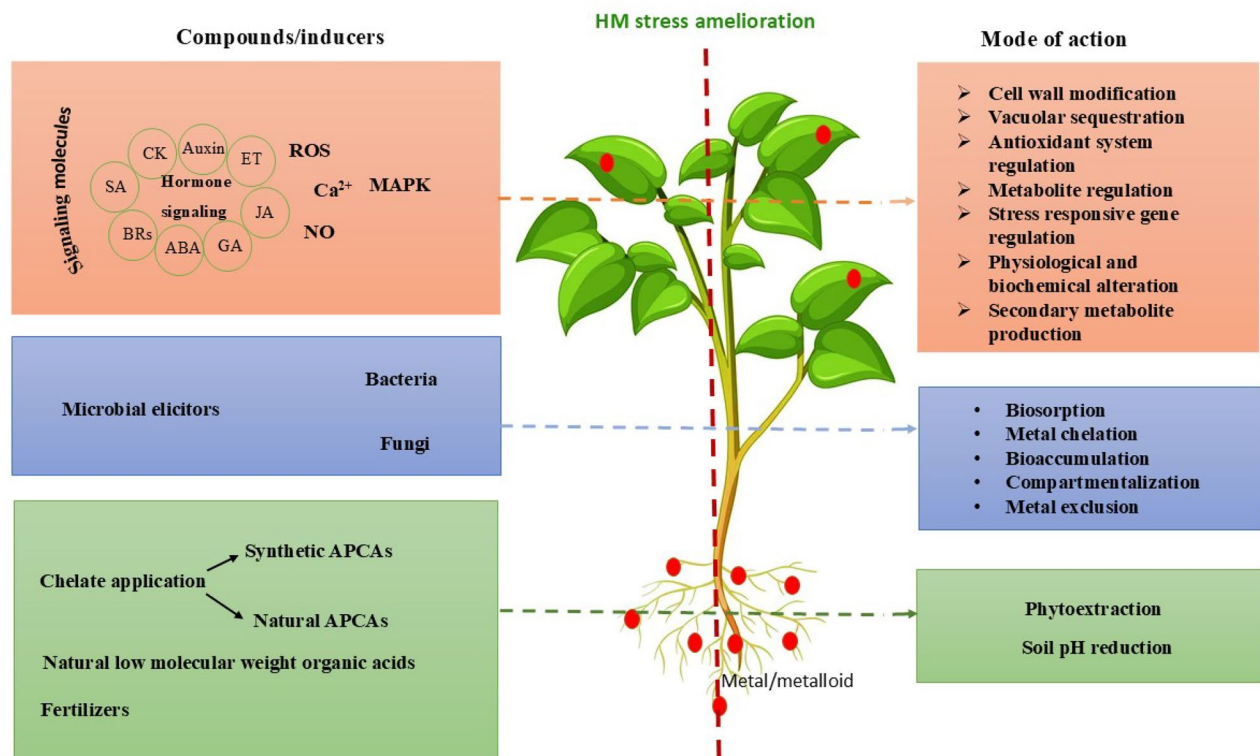
## **Chemical compounds as allies in combating metal toxicity**

HM contamination in the soil is a prevalent environmental challenge resulting from global industrialization. The decontamination of HM polluted soil is imperative to reduce associated risks and ensure environmental health and ecological restoration. Two widely employed methods to increase the availability of HM content in plants are chelate application and soil pH reduction (Sharma et al. 2023). Synthetic chelates, like ethylene diamine tetraacetic acid (EDTA), infiltrate plant roots and form soluble complexes with metals, thereby increasing HM availability (Deng et al. 2024). Soil pH can be lowered using the application of acids or acid-producing fertilizers (Murtaza et al. 2015). [Figure 2](#) illustrates the different methods of chemical compound application for combatting metal toxicity in plants.

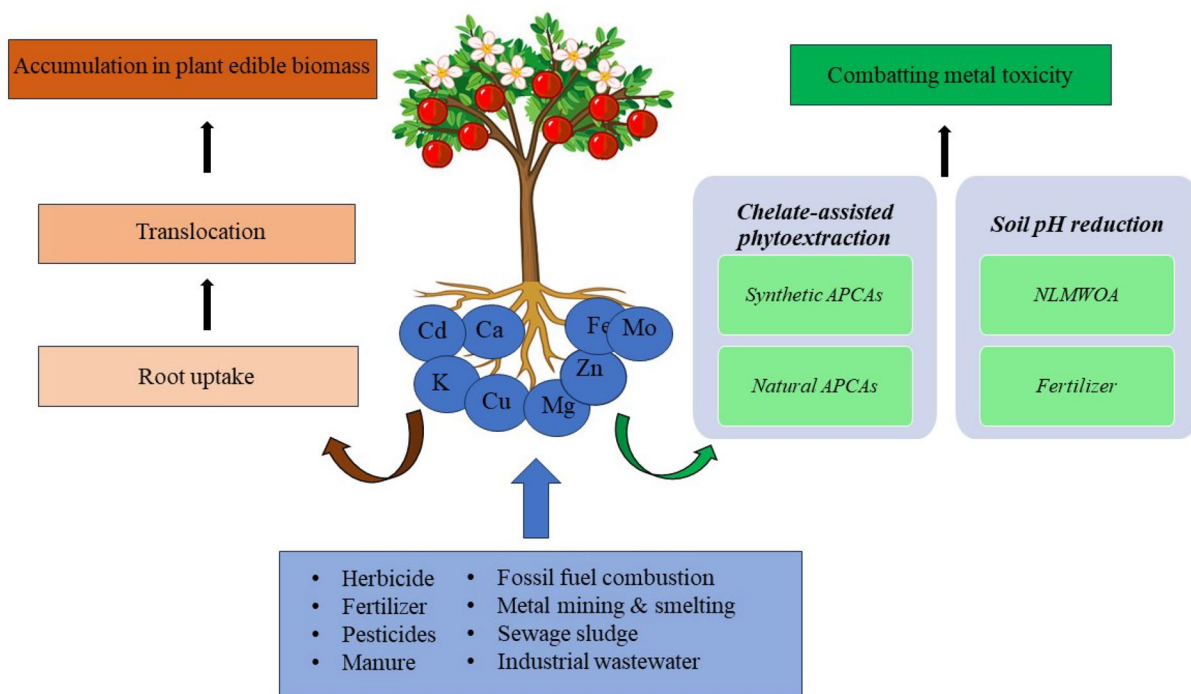
### **Chelate-assisted phytoextraction**

#### **Application of synthetic APCAs (Aminopolycarboxylic acids)**

Synthetic chelates, like EDTA, has been employed for over 50 years to provide micronutrients to plants in both soil and hydroponic environments. Since the late 1980s and early



**Figure 1.** Mechanisms and strategies for heavy metal (HM) stress amelioration in plants. The figure outlines various compounds and inducers such as signaling molecules, hormones (SA, CK, Auxin, ET, JA, ABA, etc.), microbes (bacteria and fungi), and chemical compounds (chelates, APCAs, fertilizers) that aid in alleviating HM stress. The modes of action include cell wall modification, vacuolar sequestration, antioxidant regulation, stress-responsive gene regulation, and others. Additionally, mechanisms like biosorption, metal chelation, bio uptake, and phytoremediation strategies such as phytoextraction and soil pH reduction are shown to contribute to mitigating metal/metalloid toxicity in plants. APCAs - Aminopolycarboxylic acids; MAPK - mitogen-activated protein kinase.



**Figure 2.** Plants response to metal stress and different methods of chemical compound application to combatting metal toxicity in plants. APCAs - Aminopolycarboxylic acids; NLMWOA - natural low molecular weight organic acids.

1990s, EDTA has been proposed as a potential chelating agent for phytoextraction operations. The effectiveness of these implementations varies based on factors like metal

species, soil metal concentration, soil composition, and the amount of EDTA applied in shoots and roots (Evangelou et al. 2007). The application of EDTA in *Helianthus annuus*



resulted in a noticeable rise in the transportation of Ni and Cd from the roots to the shoots. However, no movement of Cr was observed in the same study (Chen and Cutright 2001). Hydroponic studies with *Indian mustard* proposed that EDTA acts as a chelating agent for Pb, adhering it externally to the plant. The resulting soluble Pb-EDTA compound is subsequently absorbed and deposited in the leaves (Kumar and Prasad 2018). It was hypothesized that in soil, protonated EDTA enters the root and produces metal complexes, and thus increases metal transport to the shoots of *Brassica napus* (Wenzel et al. 2003). The stability of the concentrations of metal-EDTA complexes in soil solutions can vary depending on the metal. These complexes can either completely dissolve (in the case of Zn) or partially dissociate (in the case of Pb) upon plant absorption. This was demonstrated using extended X-ray absorption fine structure analysis of bean (*P. vulgaris*) (Sarret et al. 2001). These findings suggest that while EDTA is effective for phytoextraction, the behavior of metal-EDTA complexes is highly metal-specific, and their effectiveness depends on the interaction between the chelate, plant species and environmental conditions.

Various studies have investigated synthetic APCAs, including diethylene triamino pentaacetic acid (DTPA), trans-1,2-hydroxyethylene diamine tetraacetic acid (HEDTA), N-(2-hydroxyethyl) iminodiacetic acid (HEIDA), cyclohexylene dinitrilo tetraacetic acid (CDTA), N,N'-di(2-hydroxybenzyl) ethylene diamine N,N'-diacetic acid (HBED) ethylenediamine-N,N'-bis(2-hydroxyphenyl)acetic acid (EDDHA) and ethylene bis (oxyethylenetrinitrilo) tetraacetic acid (EGTA). The success of different treatments with APCAs in promoting HM phytoextraction and causing metal desorption from the soil depends on the HM and the type of plant (Evangelou et al. 2007). Zuluaga et al. (2023) reported that supplying various chelates in a series DTPA > EGTA > EDDHA > EDTA > HEDTA > DTPA was most effective in raising Pb accumulation in maize and peas. In cabbage shoots (*B. rapa*), Shen et al. (2002) suggested that the order EDTA > HEDTA > DTPA was most effective for increasing Pb accumulation. These findings emphasize that the choice of APCA and the sequence of its application are crucial for maximizing the effectiveness of HM phytoextraction. Therefore, the APCAs to be applied and the order of application is very important for effective action of the same.

#### Application of natural APCAs

Ethylene diamine disuccinate (EDDS) is biosynthesized by specific bacterial strains. Alternatively, it can be synthesized using maleic anhydride and ethylene diamine, as demonstrated by Asemave (2018). Several studies using EDDS have been conducted in the past ten years, showing that it is effective in boosting the absorption of certain metals. Like EDTA, EDDS increases the uptake of HMs, as substantially higher levels of the metals are being phytoavailable, which are efficiently absorbed by the plants and later translocated to the shoot (Evangelou et al. 2007). According to the reports, EDDS has greater efficacy in improving the absorption of Ni, Cu, and Zn compared to EDTA. While, EDTA was found to be more successful in mobilizing Cd and Pb (Meers et al. 2005). Moreover, the addition of 5 mmol kg<sup>-1</sup>

of EDDS and EDTA to soil greatly enhanced the absorption of Cd, Cu, Zn and Pb (Luo et al. 2005). Hauser et al. (2005) used 20 mmol kg<sup>-1</sup> EDDS in column studies, showing comparatively high effectiveness in mobilizing Zn, Cu, and Pb. These findings underscore the importance of applying APCAs like EDDS and EDTA in specific combinations and sequences to optimize the phytoremediation process for different HMs.

Nitrilotriacetic acid (NTA) has been primarily employed as a biodegradable chelating agent in detergents for the past 50 years. Despite its anticipated benefits, not much research has used NTA functions as a ligand to facilitate metal phytoextraction. Previously it was reported that NTA (1.8 mmol kg<sup>-1</sup>) had no discernible effect on Zn, Cu, or Cd absorption in sunflower compared to the control (Meers et al. 2005). NTA (500 µM) enhanced Cu uptake by *Nicotiana tabacum* in hydroponic experiments (Wenger et al. 2003). Chiu et al. (2005) found NTA to be more efficient in *Vetiveria zizanioides* and *Zea mays* than synthetic APCAs such as HEIDA, EDTA, HEDTA, EGTA, DTPA, and CDTA in extracting Zn and As from soil. Quartacci et al. (2005) proved that phytoextraction potential of *B. juncea* was improved with application of NTA. Also, the Cd concentration of the shoots enhanced by 2-fold and 3.3-fold when 10 and 20 mmol kg<sup>-1</sup> NTA were added to sandy soil, respectively, compared to non-treated samples. These results show that NTA can enhance the extraction of metals from soil, including Zn, As and Cd, and shows potential as a useful tool in phytoextraction tactics. To fully realize its potential in metal remediation efforts, more research into its use and efficacy across various plant species and soil types is necessary.

#### Soil pH reduction

##### Application of natural low molecular weight organic acids (NLMWOA)

Natural low molecular weight organic acids (NLMWOA) are organic acids with a small molecular size. Root-mediated release of organic compounds can have both indirect and direct effects on the solubility of essential and toxic ions. Indirectly, these compounds can impact physical properties of the rhizosphere, microbial activity and the growth dynamics of roots. Directly, they can cause chelation, acidification, oxidation-reduction reactions and precipitation in the rhizosphere (Evangelou et al. 2007). Yu et al. (2020) explored the use of tartaric, malic, oxalic, and citric acid to enhance the phytoextraction efficiency of *Celosia argentea*. The application of malic acid was also found to significantly boost the plant's ability to extract Cd, providing a potential strategy for improving phytoextraction efficiency in real-world environmental remediation efforts. This research contributes valuable insights into the selection of effective plant species and the role of LMWOAs in enhancing phytoremediation outcomes. Citrate is widely used as a complexing agent for mobilizing sorbed and precipitated uranium (U) in soil and in the extraction of nuclear reactor components, both *in situ* or *ex situ*. Additionally, plants such as *B. Chinensis*, *B. Juncea* and *B. narinosa* exhibited a 1000-fold increase in uptake of U compared to control on application of citric acid. However,

the lower concentration (5 mmol kg<sup>-1</sup>) of citric acid did not result in a prominent rise in the concentration of Cu, Pb Cd and Zn in shoots (Luo et al. 2005). Similarly, in another study, Meers et al. (2005) reported that the inclusion of citrate at a concentration of 220 mmol kg<sup>-1</sup> did not result in an elevated absorption of Zn, Ni, Cd, and Cu in *H. annuus* compared to the control sets. It is important to note that the impact of citrate additions on the uptake of HMs varies significantly among various plant species and the metal absorbed.

Leng et al. (2021) conducted research on the phytoremediation capacities of three garden plants *Liriope platyphylla*, *Iris tectorum*, and *Photinia × fraseri* for remediating trace elements (TEs) from artificial soils derived from municipal sludge. Among the tested plants, *L. platyphylla* was found to have the largest biomass per unit soil area, making it the most effective in absorbing TEs. The addition of citric acid further improved its uptake by boosting the biological enrichment factors. It also highlights the role of citric acid in enhancing the phytoremediation process. The phytoremediation indexes for *L. platyphylla* ranged from 1.16 to 29.7, indicating its strong potential for environmental cleanup. This makes *L. platyphylla* a promising candidate for large-scale phytoremediation, particularly when combined with citric acid treatments.

Saffari and Saffari (2020) recommend citric acid as the most effective chelating agent for enhancing Cd phytoremediation in *Calendula officinalis*. This is due to its ability to significantly increase Cd translocation to the shoots while simultaneously minimizing oxidative stress in the plant. In contrast, while EDTA proved highly efficient in increasing Cd mobility in the soil, its application led to increased oxidative stress and reduced plant growth, making it less favorable for long-term phytoremediation efforts. The findings underscore the potential of *Calendula officinalis* as a Cd-hyperaccumulator, capable of extracting substantial amounts of Cd from contaminated soils when paired with an appropriate chelator like citric acid. It was demonstrated that *Zea mays*, with the aid of chelating agents, can effectively enhance the phytoextraction of Cu and the dissipation of organic pollutant pyrene in the rhizosphere when soil is co-contaminated with both HM and organic pollutants. Even though both EDTA and citric acid individually had negative effects on plant growth, when used in combination, synergistically promoted plant growth in HM and pyrene co-contaminated soils. This synergism not only reduced the detrimental impacts of each chelate when used separately but also improved the remediation efficiency of both contaminants more than their individual applications. Moreover, Sharma et al. (2023) reported that Cd toxicity resulted in reduction of plant growth and photosynthetic activity, whereas ethylene glycol tetraacetic acid, EDDS, NTA and citric acid supplementation alleviated the toxic effect of Cd in *Solanum nigrum*. Recently, addition of natural or synthetic low-molecular-weight organic substances in soil with high contents of Cd, Pb, Zn, and Cu to facilitate phytoextraction by *Portulaca oleracea* was reported by Thalassinos et al. (2024). These finding highlights the potential of

chelate mixtures as a more effective approach for managing soils contaminated by multiple pollutants.

### Fertilizer application

The mobility of non-essential elements in soil is also influenced by various factors, such as soil composition, soil redox, metal concentration and speciation, pH conditions, permeability, precipitation and the application of phosphate fertilizers. Research has demonstrated that the addition of phosphate fertilizer greatly enhances the movement of As. This can be attributed to either the substitution of solution-phase orthophosphate for solid-phase As or the competition for adsorption sites with phosphorus in the soil (Gao et al. 2023). Cao et al. (2003) conducted a study on the Chinese brake fern (*Pteris vittata* L.), which is known for its ability to accumulate high levels of As. Their study proved that the supplementation of phosphate in soil greatly boosted plant As uptake by 265%. The enhanced mobility of As and its subsequent higher absorption by plants resulted from the substitution of phosphorus (P) by As at the soil binding sites (Cao et al. 2003). In another study conducted by Huang et al. (2012a) proved the positive effects of applying di-hydrogen phosphates salt at three different levels (22, 88, and 352 mg P kg<sup>-1</sup> soil) on the growth of *Sedum alfredii* and its uptake of metals in paddy soil contaminated with a combination of Zn and Cd. The inclusion of phosphates resulted in a substantial enhancement in the absorption of Zn by *S. alfredii*, primarily attributed to the amplified concentration of Zn in the shoots and the production of dry matter. The greatest uptake of Zn and Cd through plants was reported in the KH<sub>2</sub>PO<sub>4</sub> and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> treatments. These studies highlight the potential of phosphate fertilizers in increasing plant uptake of As and other metals, providing insights into phytoremediation and agricultural management in contaminated situations.

### Exploring elicitors: Signaling molecules in plant defence against metal and metalloid stress

Plants detect and recognize the presence of HMs through a variety of receptors. The signals from these receptors are further transmitted to the inside of the cell with the aid of different secondary messengers. They regulate the cell's activity and react to the HM stress signal by influencing the stress-responsive genes expression and modifying the activity of different proteins within the cell. Signal transduction mechanisms involving hormones, calcium (Ca<sup>2+</sup>), ROS, nitric oxide (NO), and mitogen-activated protein kinase (MAPK), are important in HM stress signaling (Table 1). The second messengers produced or activated by the elicitor/signal-receptor complex can amplify the original signal and integrate many down regulators of the signaling cascade thus can effectively mitigate HM stress. In response to HM stress, plants employ many mechanisms to protect themselves, including chelation, vacuolar sequestration, modulation of HM transport and storage, formation of antioxidants, and alteration of the cell wall composition (Kaur et al. 2021).

**Table 1.** Various heavy metal induced signaling pathways in plants and their responses.

Heavy metal	Signal transduction pathway	Plant	Protein alteration	Gene alteration	Plant response	References
As, Cd	Auxin	<i>Oryza sativa</i>		ASA2, YUCCA2, AUX1 and PIN5	Root growth inhibited	Ronzan et al. 2018
As, Cd	Auxin	<i>Arabidopsis thaliana</i>		YUC6, LAX3 and PIN1	Root growth inhibited	Fattorini et al. 2017
As	NO	<i>Brassica juncea</i>		<i>BjPIN5</i> and <i>BjPIN3</i>	Growth inhibited	Praveen et al. 2019
As	Ca <sup>2+</sup> , ROS, MAPKs	<i>Oryza sativa</i>	MAPKs	Involved in secondary wall synthesis, phytohormone signaling and synthesis, protein kinases and phosphatases	Transcriptional regulation in growth inhibition, detoxification and protection in rice roots	Huang et al. 2012b
As	SA	<i>Artemisia annua</i>		<i>ADS</i> , <i>CYP71AV1</i> , <i>DBR2</i> , and <i>ALDH1</i>	Enhancement of the secondary metabolite artemisinin and increased size of trichomes	Kumari et al. 2018
As	Hormonal	<i>Oryza sativa</i>		<i>WRKY4</i> , <i>bHLH</i> , <i>AP2-EREBP</i> , <i>NAC</i> , <i>ATAF</i> , <i>CUC</i> , <i>OZG2 Prx</i> , <i>GST</i> , <i>oxidoreductase</i> , <i>cytochrome P450 genes OsABCC9</i> and <i>ZIP3</i>	Stress responsive pathways regulated	Di et al. 2021
Cd	Auxin	<i>Oryza sativa</i>		<i>OsPIN1b</i> , <i>OsPIN1c</i> and <i>OsPIN9</i>	Root growth inhibited	Wang et al. 2021b
Cd	Auxin, NO	<i>Arabidopsis thaliana</i>	PIN1, PIN3, and PIN7, TIR1, IAA17		Auxin level decreased and root meristem growth inhibited	Yuan and Huang 2016
Cd	ET, JA	<i>Arabidopsis thaliana</i>	EIN3/EIL1, ERFs, COI1	<i>NRT1.8</i> , <i>NRT1.5</i>	Stress tolerance	Zhang et al. 2014
Cd	MAPKs, Auxin	<i>Oryza sativa</i>	MAPKs	<i>OsYUCCA</i> , <i>OsPIN</i> , <i>OsARF</i> , and <i>OsIAA</i> , <i>OsMAPK2,12</i> , <i>14</i> , <i>44</i> , <i>3</i> , and <i>OsMSURPK2</i>	Hindering auxin biosynthesis and resulted in limited root growth and elongation	Zhao et al. 2013
Cd	Auxin	Poplar		GH3	Increased degradation of auxin activity, increased peroxidase activities, and increased lignification and decreased stem growth	Elobeid et al. 2012
Cd	ET	<i>Arabidopsis thaliana</i>		ACS, ACS2 and ACS6	Increased ET biosynthesis	Schellingen et al. 2015
Cd	MAPKs, ET	<i>Lycium chinense</i> , tobacco		<i>LchERF</i> , <i>LcGSH1</i> , <i>LcMCK</i>	GSH accumulation due to <i>LcMCK</i> gene expression and the ET signal transduction pathways	Guan et al. 2016
Cd	CK, Auxin	<i>Arabidopsis thaliana</i>		<i>WOX5</i> , <i>SCR</i> , <i>PIN 1</i> , <i>2</i> , <i>3</i> , <i>7</i>	Root growth inhibited	Bruno et al. 2017
Cr	ABA, ET, JA, GA	<i>Oryza sativa</i>	CDPK and NADPH oxidase	Protein kinases, receptor-like cytoplasmic kinase, LRK10-like kinase type 2, and protein phosphatase 2C, <i>WRKY</i> and <i>apetala2/ ET</i> response factor	Various stress responsive pathways activated	Trinh et al. 2014
Cd+Zn	Auxin	<i>Oryza sativa</i>		<i>7 OsYUCCA family genes</i> , <i>9 OsPIN genes</i> , <i>25 OsARF genes</i> , <i>26 OsIAA genes</i> and cell cycle genes	decreases Cd content and increased auxin content and root growth	Zhao et al. 2019
Cr	BRs	<i>Oryza sativa</i>		SOD, catalase, POD, APX, GPX	Stimulates antioxidant system and mitigate HM stress by integrating with phytohormones	Sharma et al. 2016
Hg	ET	<i>Medicago sativa</i>		<i>ERF1</i> and <i>AP2</i>	Increased ET and stress response	Montero-Palmero et al. 2014

### Hormone signaling

Auxin plays a vital role in recognizing and responding to HM stress. It is also particularly significant in promoting root development. The root is the primary organ that experiences the HM stress. Therefore, comprehending auxin signaling and response in HM stress is of significant importance in studies related to HM stress. HMs can influence and change the balance of endogenous auxin by modifying its production, movement, interaction, conjunction, and breakdown. The HM will impede the movement of auxin from the shoot to the root primarily by reducing and restraining the activity of the auxin efflux protein, PIN protein, and AUX1 (LAX3) (the transporter responsible for auxin influx). Moreover, inhibition of the *YUCCA* gene, which encodes flavin monooxygenase proteins, leads to a decrease in auxin

biosynthesis in root cells (Dai et al. 2023). The above-described processes disrupt the balance and distribution of auxin by HM stress. The Cd treatment resulted in the overexpression of the *Gretchen Hagen 3 (GH3)* gene in poplar. The protein encoded by this gene is accountable for the process of conjugation and degradation of auxin, resulting in a reduction of auxin levels within cells (Saini et al. 2021). HMs such as Cd, Al and As have the ability to alter the level of miRNA, thereby impacting auxin homeostasis in cells. MiR160 specifically targets the AtARF10 and AtARF16 proteins that regulate root cap formation. The MiR390 controls lateral root growth by controlling tasiRNA production. TasiRNA targets transcription factors, such as AtARF2, AtARF3, and AtARF4 (Marzi et al. 2024; Zhang et al. 2024b). Overall, the growth of roots is diminished when exposed to HM stressors, resulting in a decrease in the surface area of roots in

contact with the HMs. This is a precautionary action taken to minimize the exposure of metals to the roots.

Exposure to HM stress leads to an upregulation in the production of ET, which subsequently causes changes in plant metabolism and cell wall composition (Dai et al. 2023). The rise in ET levels can be attributed to the upregulation of ET biosynthetic genes, such as 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and ACC synthase (ACS) enzymes, and elevated expression of EIN2 and the transcription factors ERF1 and AP2 (Saini et al. 2021; Ur Rahman et al. 2023). When exposed to Cu, the ACS transcript level was higher in potato and tobacco (Zhang et al. 2024b). et al. alleviates HM stress in plants by interacting with ROS, by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, while simultaneously promoting the development of antioxidative enzymes. The exogenous application of ET resulted in an augmentation of the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione S-transferase (GST) in *Brassica juncea* when subjected to Zn stress (Saini et al. 2021).

HMs degrade cytokinins (CKs) in cells, and thus reduces its level in plants. HM stress causes downregulation of genes involved in CK production and genes that respond to CK (Dai et al. 2023). The impact of naturally occurring CK in reducing HM stress is unexplored. However, the use of exogenous cytokinin to alleviate HM stress has been extensively studied and documented. Nevertheless, there are studies that suggest an increase in CK levels through the upregulation of the biosynthetic gene *IPT* (isopentyl transferase) and the downregulation of the catabolic gene *CKX* (cytokinin oxidase) (Ur Rahman et al. 2023). CK can enhance the plant growth under HM stress by improving cell division as well as increasing the amount of antioxidants and sugars, which helps in reestablishing ROS homeostasis in plants (Zhang et al. 2024b).

HM stress leads to a decrease in the production of gibberellic acid (GA) (Dai et al. 2023). Moreover, using exogenous GA under HM stress can mitigate the negative consequences of HM stress (Ur Rahman et al. 2023; Saini et al. 2021). GA enhanced tolerance as well as growth and development in *Chlorella vulgaris* under low levels of Cd and Pb stress. Germination parameters was improved in maize under HM stress, by the application of GA due to increased nutrient level and stability of membrane. GA can also modulate the phloem mobility, by altering the source-sink formation and can improve photosynthesis in plants under HM stress (Emamverdian et al. 2020). The DELLA protein is a key regulatory protein in signaling cascade of GA and plays essential role in enhancing cell cycle inhibitors such as SIAMESE (SIM) and Kip-related protein 2 (KRP2), that inhibit cell cycle which is inevitable for cell survival during HM stress and thus modulate stress tolerance (Achard et al. 2009; Braat and Havaux 2024).

ABA primarily mitigates HM-induced stress by regulating the aperture of stomata in the distressed plants. As a result, the movement of HM from root to shoot is restricted as the movement of water through transpiration is also reduced. ABA regulates the activity of stress-responsive genes,

including those responsible for synthesizing phytochelatin, metallothioneins, and phenylalanine ammonia-lyase, as well as antioxidant enzymes and genes synthesizing osmolytes, through a signal transduction pathway (Kumar et al. 2022). ABA suppresses *IRON-REGULATED TRANSPORTER1* (IRT1) transporter genes expression, which are essential for absorption of HM, particularly Cd. Furthermore, the transcription factor abscisic acid-insensitive5 (ABI5) decreases the absorption of Cd by interacting with R2R3-type MYB transcription factor, MYB49. MYB49 enhances the accumulation of Cd in plants by upregulating TFs bHLH38 and bHLH101 that upregulate the expression of IRT1 transporter genes, involved in Cd uptake. MYB49 also upregulate the expression of HM-associated isoprenylated plant proteins (HIPP22 and HIPP44) that enhance Cd accumulation. The ABI5 binds with MYB49 and inhibit its activity, thus the above mentioned upregulation of proteins needed for Cd accumulation cannot be done, thus ABA effectively decrease the Cd accumulation in plants (Zhang et al. 2022). During HM exposure, ABA level significantly increases and effectively regulates several metabolic, physiological, and gene expression pathways to counteract the HM stress. HM stimulates, production of ABA, by upregulating 9-cis-epoxy carotenoid dioxygenase (*NCED2, 3, 4*) and abscisic acid-deficient 4 (*ABA4*) genes. Additionally, it enhances the levels of signaling molecules involved in the ABA signal transduction pathway, such as protein phosphatase 2C (PP2C4, 5) and basic leucine zipper (bZIP10, 12) (Dai et al. 2023).

Although ABA is reported to restrict the entry of HMs into the plant, it has equal potential for enhancing the HM absorption into the plants. The exogenous administration of ABA was found to enhance the expression and functionality of HM ATPase (HMA4) that transport HMs from the roots to the shoots. Additionally, it stimulated the production of zinc/iron-regulated transporter (ZRT/IRT)-related protein (ZIP) family, essential for the transportation of HMs, including Zn and Cd, across the plasma membrane. Also, it can stimulate the activation of additional transporters that transport Mn, Fe, Zn, Cd, Cu, Co and Ni which include yellow stripe-like (YSL), natural resistance-associated macrophage protein 3 (NRAMP3), and plant cadmium resistance 2 (PCR2), ATP-binding cassette transporter G (ABCG) and ATP-binding cassette transporter C (ABCC). The activation of the antioxidant enzyme system by ABA can decrease the production of ROS during HM stress, leading to an increase in root density and photosynthesis in plants under HM stress (Saini et al. 2021). Moreover, when plants are stressed by As, B, Cd, or Zn, polyamines help them deal with it by crosstalking to other phytohormones and increasing the production of phytochelatin and metallothionein and thus reduce the harmful effects of metals and metalloids in plants (Gupta et al. 2024).

Brassinosteroids (BRs) employ a defence strategy to counter HM stress by activating antioxidant enzyme system (Dai et al. 2023). Additionally, it can increase the levels of phytochelatin and proline. It increases the amount of proline in plants that are under HM stress by increasing the synthesis of a key enzyme delta-1-pyrroline-5-carboxylate



synthase (P5CS), necessary for proline synthesis. Additionally, it regulates hormone balance by promoting the production of IAA, putrescine, and cadaverine (Ur Rahman et al. 2023). The putrescine and cadaverine are important polyamines that helps to reduce cellular damage and have essential role in alleviating stress in plants (Ozmen et al. 2023). The BES1/BZR1 transcription factor has a role in maintaining homeostasis under the control of BRs signaling pathway. These TFs controls the expression of many genes, including the stress responsive genes that are under the signaling cascade of BRs (Shi et al. 2022). Researchers most often use bioactive BRs such as brassinolide (BL), 28-homobrassinolide (28-HomoBL), and 24-epibrassinolide (24-EpiBL) to improve HM tolerance through the involvement of BRs in signaling pathways. The external application of 24-epiBL and 28-homoBL to *Brassica*, *Raphanus*, *Vigna* and wheat reduced Ni stress by enhanced activation of antioxidant enzymes. Similarly, foliar application of BRs in *Brassica juncea*, *Phaseolus vulgaris*, *Cicer arietinum*, and tomato plants was most effective in alleviating Cd stress, particularly by enhancing the antioxidant machinery (Zhang et al. 2024b).

The primary mechanism by which the SA mitigates HM stress is through the promotion of cell wall production. This process enhances the wall's thickness, which can aid in attaching the HMs to the cell wall, preventing their presence in metabolically active regions of the cell. This process is often accomplished through the action of pectin methyl-esterase, which generates unbound carboxyl groups capable of binding with HM by demethylating the pectin. SA can modify the composition of the cell wall by increasing the levels of ferulic and p-coumaric acids, hence altering the hemicellulose and lignin content. Moreover, SA can influence the synthesis of glutathione a significant non-enzymatic antioxidant and a constituent of phytochelatins. The SA can increase the production of glutathione by upregulating the serine acetyltransferase enzyme, which facilitates the creation of cysteine, a necessary component for glutathione production (Saini et al. 2021). Additionally, it enhances the expression of glutathione synthetase and glutathione reductase, actively preserving this antioxidant system. Moreover, ABC transporters are activated which sequesters the HM-PC complex into the vacuoles on application of SA. Furthermore, it can improve the absorption of minerals by enhancing the function of H<sup>+</sup>-ATPase (Dai et al. 2023).

Additionally, SA can improve the levels of secondary metabolites such as artemisinin, spermidine, and putrescine under HMs stress. The process by which it increases the accumulation of the latter two has to be deciphered. The production of artemisinin is facilitated by the elevated activity of specific genes, namely aldehyde dehydrogenase 1 (*ALDH1*), cytochrome P450 monooxygenase (*CYP71AV1*), artemisinic aldehyde  $\Delta$ 11 (13) double-bond reductase (*DBR2*) and amorpha-4, and 11-diene synthase (*ADS*). The interaction between SA and auxin, mediated by the MYB1 transcription factor (TF), promotes the growth of lateral roots. This interaction also leads to the upregulation of osmotins, WRKY proteins, heat shock proteins and peroxidases. It is hypothesized that SA reduces DNA damage caused by HM stress by activating RAD51D and RAD51, responsible for

DNA repair. The precise signaling mechanism by which SA improves HM stress is not yet fully understood, but it is hypothesized to occur through the MAPK signaling cascade (Saini et al. 2021). The foliar application of SA not only protected Ni and Pb-stressed *Phaseolus vulgaris* plants from the negative consequences of HM stress, but also enhanced nitrate reductase, enzymatic antioxidants and enhanced metabolites, while reducing free radicals, ROS and electrolyte leakage. Under Cr stress, *Zea mays* also showed similar results on application of SA (Zhang et al. 2024b).

Jasmonates or jasmonic acid (JA) enhance the antioxidant defence mechanism in plants when exposed to HM stress. It boosts the synthesis of enzymatic antioxidants and increases the glutathione levels. Glutathione synthetase (GS) and gamma-glutamylcysteine synthetase (gamma-GCS) genes involved in glutathione production are upregulated by the action of JA (Saini et al. 2021). Additionally, it exerts a beneficial influence on the activity of ATP-sulfurylase (ATP-S). ATP-S produce adenosine-5'-phosphosulfate (APS), which is reduced to sulfide (S<sup>2-</sup>) and gets integrated into cysteine (Cys). Thus, Cys participates in synthesizing glutathione and thus it was inferred that JA play role in preserving the GSH pool for antioxidant defence (Anjum et al. 2015). Studies have also demonstrated the beneficial effects of JA on the photosynthetic apparatus. The reduction in photosynthesis due to various stressors could be partially alleviated by the positive influence of JA on photosynthetic apparatus. However, the specific mechanism by which it protects photosynthesis of plants under stress remains unclear. In addition, the JA can also regulate the activity of several transporters of HMs, thereby preventing their entry into the cell and their accumulation in the shoot or promoting their movement through the vascular system. The transporters affected by JA activity are ABCC1, PCS1, PCS2, and HMAs. Furthermore, it has been observed that LOX, 12-oxophytodienoic acid reductase 3 (OPR), needed for JA synthesis and the key signaling molecule, jasmonate ZIM-domain (JAZ), that function in JA signaling is augmented under HM stress (Saini et al. 2021). By lowering lipid peroxidation and ROS, JA improved the ability of faba and rapeseed plants to handle Cd stress. In Cd stressed pea plants, the application of MeJA enhanced the PSII efficiency, antioxidant potential, and overall photosynthesis. Applying MeJA in *Brassica* under As stress reduced ROS production by enhancing the transcription of lipoygenase genes needed for JA production (Zhang et al. 2024b).

### Calcium (Ca<sup>2+</sup>) signaling

Ca<sup>2+</sup> serves as a ubiquitous secondary messenger in multiple cellular signaling pathways and plays a crucial role as signaling molecules under HM stress. The suppression of root growth under HM stress can be attributed to the disruption of Ca influx, as certain HMs like Cd can interfere with Ca due to their comparable ionic radii. Additionally, it can inhibit the movement of HMs from the roots to the shoots. When exposed to Cr stress, it specifically hinders the transfer of Cr from the roots to the shoots in rice (Mukta et al. 2019). Ca-dependent protein kinase (CDPK), calmodulin protein (CaM), calmodulin-like protein (CML), calcineurin

B-like protein (CBL) and CBL-interacting protein kinases (CIPKs) are the most common  $\text{Ca}^{2+}$  sensors in a cell (Huang et al. 2017). HMs can enhance the intracellular  $\text{Ca}^{2+}$  levels in plants and the various  $\text{Ca}^{2+}$  sensors binds with  $\text{Ca}^{2+}$  and modulate the activity of various HM stress responsive genes by activating various downstream signaling pathways in plants (Shabbir et al. 2022). The CDPKs can phosphorylate NADPH oxidase, membrane channels, transcription factors, and MAPKs, thus can modulate their activity to enhance HM stress tolerance (Mansoor et al. 2023).  $\text{Ca}^{2+}$  signaling can enhance ROS production in apoplast by activating NADPH oxidase. This event further increases  $\text{Ca}^{2+}$  level and activate antioxidant as well as ROS scavenging machineries to decrease ROS. Thus, the  $\text{Ca}^{2+}$  and ROS crosstalk and signaling reduce HM toxicity in plants (Ravi et al. 2023). In maize, the ZmCDPKs can phosphorylate ion channels such as SLAC1 in guard cells that regulate stomatal closure (Du et al. 2023). The CaM sense and transmits the message to transcription factors, specifically the Calmodulin-Binding Proteins (CBPs) such as CAMTAs, bZIPs, WRKY IIDs, CBP60s, MYBs, MADs box, and NAC proteins. Calmodulin-binding transcription activators (CAMTA) activates several stress-sensitive genes, either through direct or indirect mechanisms (Baek et al. 2023). Using a transgenic method, in a recent research, researchers observed that the expression of *Populus euphratica* CPK21 in *Arabidopsis* improved their Cd tolerance by interacting with the cation and HM transporters in the plants. This interaction effectively boosted the antioxidant system, thereby lowering the production of Cd-induced ROS. They also improved the water status of the stressed plants by interacting with plasma membrane intrinsic proteins (Yin et al. 2024).

### Reactive oxygen species (ROS) mediated signaling

HMs stress induce the generation of numerous ROS, they in turn activate various signaling pathways and activation of antioxidant enzymes to alleviate the HM toxicity. Additionally, HM also pave way for the enhancement in the synthesis of suberin and lignin content, leading to cell wall remodeling. Hyperaccumulator plants, like *Thlaspi caerulescens*, exhibit U-shaped lignification and suberization of the root endodermis. Moreover, the phenylpropanoid pathway is controlled and amplified to produce lignin, particularly monolignols, under HM stress. In addition, HM stress triggers the creation of several secondary metabolites through the phenylpropanoid pathway. Application of Cd in soybean resulted in an increase in both phenylalanine ammonia-lyase activity and lignin concentration. Apart from this elevated level of ROS can also stimulate phenolic compounds synthesis, hence improving the plant's ability to counter HM stress (Berni et al. 2019). The ROS produced by Cd toxicity reduced root growth in wheat by arresting cell division at G1 phase. Furthermore, the  $\text{H}_2\text{O}_2$  causes the oxidation of BZR1, which in turn activates BR signaling and its interaction with phytochrome interacting factor 4 (PIF4) and auxin response factor 6 (ARF6) facilitate adaptation to environmental stresses (Tian et al. 2018).

### Nitric oxide (NO) signaling

NO affects cellular activity by posttranslational modifications (PTMs) to regulate the formation of ROS and cellular metabolisms. NO is a potent chemical that exhibits high reactivity and serves as a signaling molecule within cells. It is produced from arginine by the enzymatic action of nitric oxide synthase (NOS) (Terrón-Camero et al. 2019). NO signals are typically transmitted through specific PTMs, such as the addition of a nitrosyl group to the reactive thiol group of cysteine, resulting in the formation of S-nitrosothiol (SNO). This process is termed as S-nitrosylation, where the signal is sent to a neighboring protein molecule through transnitrosylation. This redox signaling mechanism enables plants to cope with oxidative stressors caused by HMs. Therefore, when exposed to oxidative stress caused by HMs, the process of S nitrosylation occurs in proteins, particularly in the mitochondrial proteins responsible for the electron transport chain and citric acid cycle. This process aids in protecting the plant from experiencing an oxidative burst (Sharma et al. 2020).

Additionally, this phenomenon conceals the death signals, safeguarding the plant cells against apoptosis (Fernando et al. 2019). NO can improve the concentration of phytochemicals in plant cells, leading to the chelation and storage of HMs in vacuoles. It can boost the levels of GSH, which is a precursor to PCs. In addition, it can modify the structure of metallothioneins, and thereby enhancing resistance to HM stress. Further, it improves the functionality of both the enzymatic and non-enzymatic antioxidant systems (Sharma et al. 2020). In a recent study in *Hibiscus cannabinus* L., the exogenous application of NO helped in Cd induced stress alleviation by maintaining the enzymatic antioxidant system and increasing photosynthesis (Cao et al. 2024).

### The mitogen-activated protein kinase (MAPK) pathway

The HM can modify the expression of MAPK genes, which in turn can modify the hormonal balance, ultimately influencing the plant's response. The presence of Cd decreases the activity of specific MAPK genes in rice (*OsMAPK2*, *12*, *14*, *44*, *OsMSRMK3*, and *OsMSURPK2*). Consequently, these genes downregulate the genes responsible for the biosynthesis and transport of auxin (*OsPIN*, *OsARF*, *OsYUCCA*), as well as cyclin-dependent kinases (*OsCDK*, *OsCKL*, and *OsCYC*) necessary for cell cycle progression (Ur Rahman et al. 2023).

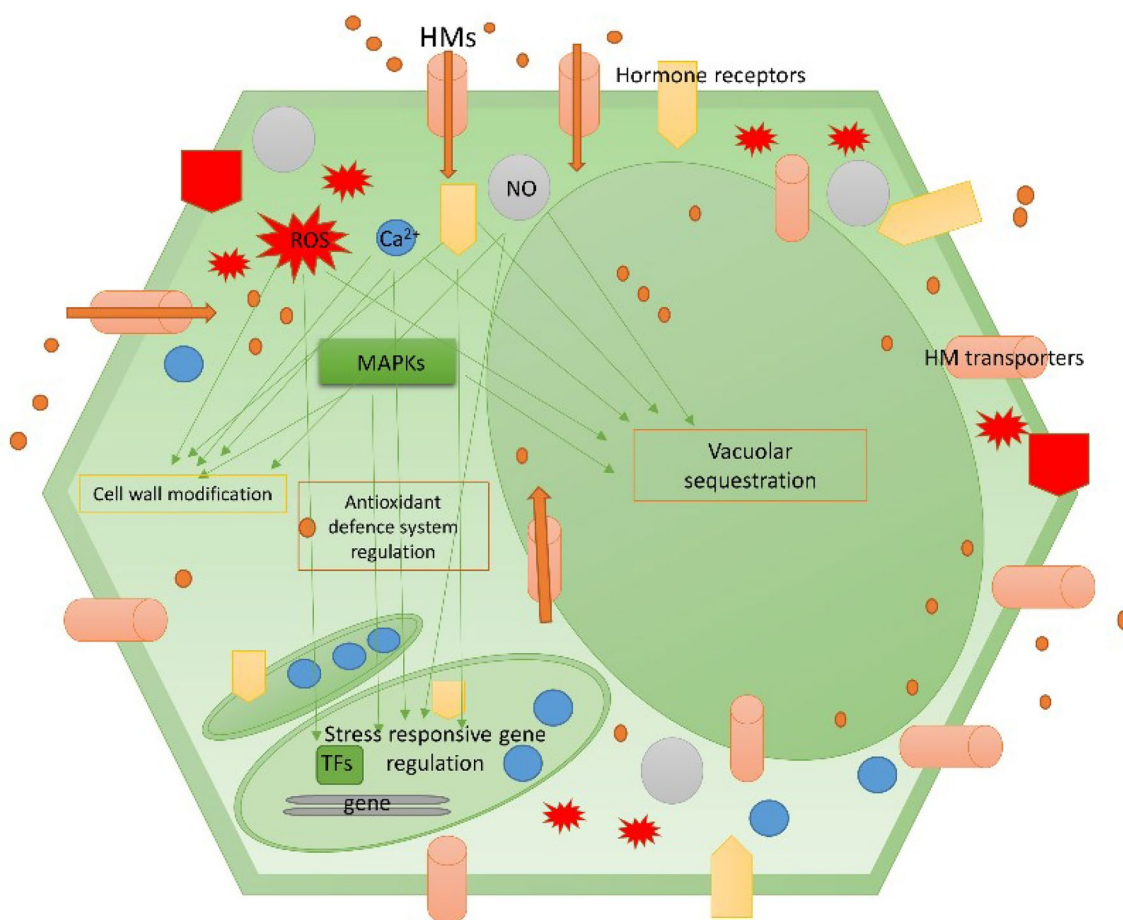
The MAPKs serve as the primary signaling molecules that mediate HM toxicity by controlling the processes of HM absorption, sequestration, and transport. The main contributors in this context are MAPK3 and MAPK6. The increased expression of these MAPKs controls the activity of several transporters (ZIP, CPx- and P1B-ATPase, NRAMP, cation diffusion facilitator, and ABC transporters) and chelators (IRT1, FRO2, and FIT) (Jalmi et al. 2018). Exposure to HMs activates various signaling pathways, including those mediated by NO, ROS, and phytohormones. These signals engage in mutual interaction and initiate the MAPK cascade. MAPK signaling cascades result in the phosphorylation of

many substrate proteins, including MKP1 (Mitogen-Activated Protein Kinase Phosphatase), esterase, and MAPKAPK (MAP kinase-activated protein kinase). Recently, Niekerk et al. (2024) reported that MAPK signaling cascades activate downstream transcription factors (WRKY, bZIP, MYB and HSF) to enhance particular stress responses (detoxification, HM-uptake, HM-transport, and growth and development) under various HM stresses. In addition, it stimulates the activation of certain transcription factors, such as WRKY42, WRKY22, and MYB, which have a role in plant defence mechanisms (Kaur et al. 2021). These transcription factors subsequently stimulate defence genes expression such as metal transporter genes, PCs, MTs, antioxidant-related genes, and so on. Thus, the plants exhibit an increased capacity for HM tolerance or accumulation (Li et al. 2022).

Signaling pathways are inevitable for plant defence mechanisms against HM stress. While SA and JA are widely known as signaling molecules involved in plant defence, plants are also regulated by hormonal, calcium, NO, ROS and MAPK signaling pathways. Detailed study of these mechanisms can be helpful in the development of strategies to protect plants from HM toxicity. Figure 3. illustrates the various signaling pathways (hormonal, ROS,  $Ca^{2+}$ , MAPKs and NO) which integrate and respond to HM stress by modulating the stress responsive gene regulation, cell wall modification and sequestering the HMs into the vacuoles in plant cells.

### Synergistic strategies: Integrating compounds and elicitors against metal and metalloid stress

In elicitation studies against metal and metalloid stress, a range of chemical agents such as phytohormones, metals, signaling molecules and their combinations are widely used. Various studies have found that elicitors like ET, JA, SA, and NO are effective for HM tolerance of plants both individually and in combination (Giri and Zaheer 2016; Kamali et al. 2024). Treatment of *Cajanus cajan* seeds with methyl jasmonate was found to be effective to alleviate  $CdCl_2$  (5 mM) toxicity (Kaushik et al. 2024). Similar kind of metal toxicity relieving power of JA was noted in *Lycopersicon esculentum*, wherein 100 nM of JA seed treatment mitigate lead stress through maintaining the enhanced level of secondary metabolites, organic acids and metal ligation compounds (Bali et al. 2020). Through potential mechanisms such as  $Ca^{2+}$  signaling, improved ROS scavenging activity, and chelation ability, toxicity of HMs and metalloids in plants can be reduced through elevating the JA levels; suggesting that the generation of JAs during stress may be influenced by  $Ca^{2+}$  signaling (Chen et al. 2021).  $Ca^{2+}$  is found to alleviate HM toxicity in a variety of plants viz. *Brassica juncea*, *Sesamum indicum* against Cd stress (Ahmad et al. 2015; Abd-Allah et al. 2017). Earlier studies have reported that the combined effect is more prominent than



**Figure 3.** The various signaling pathways (hormonal, ROS,  $Ca^{2+}$ , MAPKs and NO) integrate and respond to HM stress by modulating the stress responsive gene regulation, cell wall modification and sequestering the HMs into the vacuoles.



the individual application of elicitors/chemical compounds in seeds or plants to alleviate metal and metalloid toxicity.

Combination of 40mM Ca<sup>2+</sup> and 100µM JA is effective against As stress in tomato seedlings *via* suppressing chlorophyllase activity and increasing the activity of δ-aminolevulinic acid dehydratase (δ-ALAD), also the activity of glyoxalase I (Gly I) and Gly II was enhanced by the combined application of Ca<sup>2+</sup> and JA, drastically increasing the biosynthesis of chlorophyll (Siddiqui et al. 2022). Likewise, synergistic application of calcium chloride and kinetin in *Phaseolus vulgaris* was found to be effective in reducing the toxicity induced by Ni and/or Pb. The treatment lowered proline, malondialdehyde content, total phenols, POX, SOD, Ni and Pb contents (Khalil et al. 2017). The deleterious effects of Cd<sup>2+</sup> were mitigated by exogenously supplied Ca<sup>2+</sup> (2mM) and 200µM Si, which led to the restoration of seedling growth and suppression of Cd<sup>2+</sup> absorption in rice (Srivastava et al. 2015). Likewise, nitric oxide and ascorbic acid contribute to the development of tolerance against Cd, which is one of the most cytotoxic pollutants. By altering the DNA methylation profile, transcriptionally upregulating genes involved in terpenoid metabolism, boosting protein concentration, increasing proline level, augmenting flavonoids, and elevating the activity of antioxidant enzymes, these two chemicals mitigated the cytotoxicity of Cd. Elevation in the expression of two cytochrome P450 monooxygenase genes (*CYP71D178* and *CYP71D180*) contribute in mitigating the toxicity. Indeed, it has been confirmed that CYPs have a role in the metabolism of terpenoids and phenylpropanoids (Farahani et al. 2024).

SA along with kinetin and/or calcium reduced the toxicity of HMs in *Phaseolus vulgaris*. Before seedlings were

subjected to hazardous concentrations of Ni (2.5mM) and Pb (0.5mM), seeds were soaked in 0.1mM SA, either alone or in conjunction with 30ppm kinetin or 40mM calcium chloride. Here, under Ni and/or Pb stress, plants emerged from SA treated seeds in conjunction with kinetin or Ca<sup>2+</sup> exhibited increased antioxidant enzyme activity and proline buildup (Khalil et al. 2021). Various studies also revealed the metal and metalloid stress mitigation properties of hydrogen sulfide and its synergistic action with signaling molecules such as Ca<sup>2+</sup>, NO, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), ABA, SA, JA, melatonin, proline, etc. (Wang et al. 2021a).

## Role of microbes in metal stress mitigation

Many biological and physicochemical strategies have been used for removing HMs from the environment. Although these physicochemical procedures are quick, their expense and technical complexity make them difficult. Additionally, they end up in secondary pollution and have a negative impact on the chemical, physical, and biological characteristics of soil (Glick 2010; Ullah et al. 2015). Biological remediation is thought to be the most successful approach for removing harmful metals from the environment. In light of global climate change and overuse of fertilizers in agricultural fields, the employment of plant growth-promoting microbes for the bioremediation of HM-polluted soil is one of the feasible strategies. Many microorganisms, including bacteria, algae, and fungi have been used for cleaning the HM-contaminated environments (Table 2). They can function as elicitors helping the plants to grow and improve their capacity to withstand and collect HMs. Their ability to

**Table 2.** Some of the key studies related to the microbial remediation of heavy metal contamination.

Microorganism used	Heavy metal	Tested concentration	Observation	References
<i>Bacillus</i> sp.	Pb and Cu	1000 mg L <sup>-1</sup>	<i>Bacillus</i> sp. (ATS-1) is an easily cultivable, cost-effective and efficient biosorbent for removing Cu and Pb ions from the aqueous solutions.	Tunali et al. 2006
<i>Pseudomonas putida</i> SP1	Hg	280 µM	<i>P. putida</i> SP1 can be potentially applied in the bioremediation of Hg. It reduces Hg <sup>2+</sup> to Hg with the help of reductase enzyme.	Zhang et al. 2012
<i>Arthrobacter viscosus</i>	Cr	100 mg L <sup>-1</sup>	Dead and living biomass of <i>A. viscosus</i> are promising biosorbents for reducing Cr(VI) to Cr(III) from aqueous solution at highly acidic conditions with 100% efficiency for 100 mg L <sup>-1</sup> Cr.	Hlihor et al. 2017
<i>Bacillus cereus</i> RC-1	Cd	5–60 mg L <sup>-1</sup>	Dead cells of <i>B. cereus</i> RC-1 are superior biosorbents for Cd(II) than live cells. Cd(II) accumulated mainly on the cell wall followed by intracellular uptake.	Huang et al. 2013
<i>Acinetobacter</i> sp. B9	Cr	35 to 425 mg L <sup>-1</sup>	<i>Acinetobacter</i> sp. B9 can tolerate high Cr concentrations and reduce the concentration of Cr from the media. Simultaneous removal of increased concentrations of Cr, Cr(VI), and Ni was found when the strain B9 was used for bioremediation of industrial wastewater.	Bhattacharya and Gupta 2013
<i>Microbacterium oxydans</i> CM3 and CM7	Cu and Ni	10–15 mg L <sup>-1</sup>	The maximum Cu and Ni removal percentages of CM3 and CM7 were found to be 94.15%, 91.11%, 86.32% and 83.24%, respectively.	Heidari et al. 2020
<i>Aspergillus</i> sp.	Cu and Pb	200–1400 ppm	Biosorption capacity of <i>A. flavus</i> was found to be 20.75–93.65 mg g <sup>-1</sup> for Cu(II) with initial concentration 200–1400 ppm, while that of <i>A. niger</i> for Pb(II) was 3.25–172.25 mg g <sup>-1</sup> .	Iram et al. 2015
<i>Purpureocillium lilacinum</i>	Cr	0.1 g L <sup>-1</sup>	Cr(VI) was reduced to less toxic Cr(III) and this reduction was pivotal for the bioremediation of Cr(VI). <i>P. lilacinum</i> isolate survive in high Cr concentrations, indicating its potential for effective bioremediation of Cr-contaminated sites.	Kerga et al. 2023
<i>Termitomyces clypeatus</i>	Cr	10–100 mg L <sup>-1</sup>	Cr(VI) biosorption involved more than one mechanism such as physical adsorption, ion exchange, complexation and electrostatic attraction and followed in two subsequent steps – Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup> biosorption at the protonated active sites (amino, carboxyl and phosphate groups) and reduction of Cr(VI) to Cr(III) by reductive groups (hydroxyl and carbonyl groups) on the biomass surface.	Ramrakhiani et al. 2011
<i>Bacillus thuringiensis</i> KUNi1	Ni	0.5–7.5 mM	This strain removed 82% of Ni from the medium. <i>Bacillus</i> sp. have high potential for Ni sequestration both <i>in situ</i> and <i>ex situ</i> detoxification of Ni-contaminated sites.	Das et al. 2014



synthesize the diverse array of bioactive substances like enzymes, secondary metabolites, and phytohormones render them indispensable in enhancing tolerance to HMs and facilitating their removal (Thakur et al. 2019).

Microbes can consume waste and transform them into simple, nontoxic byproducts or molecules, which can improve plant growth and survival under HM toxicity. Applying metal-resistant strains in single, consortium or immobilized forms has produced successful results for HM remediation (Tiwari and Lata 2018). Through chelation, acidification, and precipitation, microbes also increase the bioavailability of metals from soil. For instance, organic acids generated by plant roots and bacteria decrease the pH of the soil and aid in metal ions sequestration (Mishra et al. 2017). The potential of microbes for extracting and/or recovering metals has led to a significant surge in interest on methods involving HM uptake by microbes in recent years. Particular holistic processes underpinning the adaptive strategies of microbes will provide light on the mechanisms underlying stress adaptation and metal tolerance. Detoxification and definitive biotransformation capabilities can be achieved through enzyme engineering in microbes for increased rates of degradation.

### Heavy metal remediation by bacteria

Bacteria have proven to be a reliable tool for removing HMs from environments that are hazardous to them because of their adaptability to quick mutation and development. The benefits of bacterial potentiation have been specifically linked to high specific growth rates; their superiority as bioremediation agents over other microbial equivalents is attributed to their doubling time and bigger surface area-volume ratio. The survival of bacteria in a metal-toxic environment is facilitated by their stress-induced conversion into nontoxic forms (Mathivanan et al. 2021). Moreover, by mobilizing and/or immobilizing, absorbing, and transforming, bacteria reduce the toxicity of HM ions (Hassan et al. 2017). A variety of both free-living and symbiotic plant growth promoting rhizobacteria (PGPR) are found in the soil surrounding the plant roots, and they have the ability to positively impact plant development and productivity by producing growth regulators through the provision and facilitation of nutrient uptake from the soil. PGPR can function as possible elicitors for abiotic stress tolerance, including HM resistance (Chamkhi et al. 2021; Selwal et al. 2024). By building complexes with certain metabolites, siderophores, and bacterial transporters, they restrict the bioavailability of HMs (Ahemad 2012; Nadeem et al. 2014). According to Pandey et al. (2013), Cd resistant *Ochrobactrum* sp. and As and Pb-resistant *Bacillus* spp. aid in bioremediation and the growth promotion of rice cultivars in HM contaminated soil.

The biotic and abiotic elements influencing the efficacy of detoxification determine the efficacy of bacterial bioremediation, which includes biosorption, bioaccumulation, biotransformation, biomineralization, and biodegradation techniques (Zhang et al. 2020). In bacterial bioremediation, stress management refers to the collective mechanisms that include morphological modifications, metallothionein

synthesis occurring within the cell, and siderophore production occurring outside the cell to support the bacteria's defence mechanisms against metal toxicity and environmental adaptation. Enzymatic detoxification, efflux pump system, and biotransformation of metal ions are examples of additional and exemplary processes that support the natural stress tolerance mechanism and enable efficient pollution management and subsequent detoxification (Bourles et al. 2020). Kim et al. (2015) have examined the possibility of using zeolite-supported sulfate reducing bacteria (SRB) to improve the removal of  $\text{Cr}^{6+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Cu}^{2+}$  from contaminated saltwater. It was found that zeolite carriers immobilized with SRB can improve HM removal. The removal of  $\text{Cu}^{2+}$ ,  $\text{Cr}^{6+}$ , and  $\text{Ni}^{2+}$  was effective by 98.2, 99.8 and 90.1% at a concentration of 100 parts per million of the HMs, respectively.

A crucial first line of defence against metal toxicity is constituted by morphological changes, which efficiently provide the mechanisms of adaptation. *Pseudomonas aeruginosa* strain 4EA on exposure to lead nitrate resulted in decreased cell size and shrunken appearance (Naik and Dubey 2011). Similarly, in *Lysinibacillus fusiformis*,  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ion accumulation resulted in reduced cell size with bulged irregularity (Mathivanan et al. 2016). These encouraging data highlight the distinct patterns of stress management and bioremediation, which call for in-depth research on differential expression profiling in order to draw boundaries for resistance tactics and morphological profiles. The efficient adsorption of Ni and Co in *Klebsiella variicola* is determined by the porosity of its cell wall (Afzal et al. 2017). This morphometric porosity evaluation provided further information about the targeted delivery and biosorption of particular HMs. *Pantoea agglomerans* showed morphological changes for different metal ion concentrations. These variations showed patterns of variability in cell morphology, including elongation, curved ends, irregular giant cells possessing pleomorphic aggregation, dumbbell appearance, and cell membrane distortions (Mohite et al. 2018).

The efflux pump systems, such as the CBA efflux transporter, ABC transporter, P-type ATPases and cation diffusion facilitator are responsible for metal expelling in cellular architecture. Effective translocation is emphasized by the role that ABC transporter play a role in importing and exporting substrates like ions, sugars, and complex chemical compounds in bacteria. They are also involved in the secretion of many compounds, including peptides, lipids, proteins, hydrophobic medications, and polysaccharides, as well as the removal of harmful substances (antibiotics) from cells. A membrane transporter called ArsK (encoded by the *arsK* gene) facilitates the removal of  $\text{As}^{3+}$  from the intracellular region of the *Escherichia coli* cells (Shi et al. 2018). P-type ATPases efflux systems, expressed by *ZntA* and *CadA* located in the chromosome were responsible for eliminating  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  from the cells of *Ralstonia metallidurans* (Legatzki et al. 2003).

Enshaei et al. (2010) documented the role of intracellular sequestration and thus maintaining homeostasis and providing the cellular foundation for bacterial defence that emancipates detoxification through the synthesis of metallothionein.

Cysteine residues give metallothioneins their mechanistic activity, and thiol groups take care of necessary metals like Zn, Fe and Cu as well as non-essential metals such as Ag, Hg, Cd, As and Pb (Yin et al. 2019). It has been observed that overexpressing metallothionein production enhances metal sequestration at higher concentrations of Pb, boosting the effectiveness of bacterial biotransformation (Murthy et al. 2011; Dar et al. 2013). Increased metallothionein production was shown to occur under conditions of Cd and Cu stress in *Pseudomonas putida* and *Pseudomonas aeruginosa* (Enshaei et al. 2010). Permina et al. (2006), found that chromosomal inheritance pattern of eubacteria has higher prevalence of HM tolerance.

Utilizing metal-resistant bacteria can effectively extract metal from polluted surroundings. Gaining insight into the regulation of HM resistance is valuable for the purposes of biological waste treatment and assessing the potential consequences of industrial activity on natural ecosystems (Permina et al. 2006). In order to produce viable and new strains for large-scale bioremediation, the specific action of bioremediation and its interrelationship could therefore offer deeper insights for targeted mutagenesis experiments and genome engineering, confirming the safety and specificity of bacterial bioremediation.

### Heavy metal remediation by fungi

Fungi have developed both extracellular and intracellular defence mechanisms against the harmful effects of HMs. Their exceptional capacity for metal uptake and recovery makes them valuable as biosorbents for removing hazardous metals from both aquatic and terrestrial habitats. Both living and dead fungal cells play crucial roles in inorganic chemical adhesion (Tiwari et al. 2013). For example, hazardous  $\text{Cr}^{6+}$  was transformed into less toxic  $\text{Cr}^{3+}$  by the dead fungal biomass of *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Aspergillus niger*, and *Penicillium chrysogenum* (Park et al. 2005). Extensive research on the tolerance of fungi to HMs revealed the role of the extracellular mechanisms in metal chelation and cellular binding, which prevent metal ions from entering the cell. In addition to this, intracellular strategies involve conjugation of the metal ion with organic ligands and proteins (Priyadarshini et al. 2021). Thus, we can summarize that fungi employ a combination of biosorption, metal chelation, bioaccumulation, compartmentalization and efflux transport to manage metal exclusion. For example, *Trichoderma viride* exhibited significant Cu tolerance through bioaccumulation, where  $3.4\text{g L}^{-1}$  of the biomass eliminated 81% of Cu(II) in 72h (Anand et al. 2006).

The functional groups present in fungal cell wall polymers including carbonyl, hydroxyl, carboxyl, ester, amino, phosphate, sulfhydryl, and imidazole establish binding interactions with metal ions. When *Acremonium pinkertoniae* was cultivated in the presence of copper sulfate, it accumulated Cu ions, resulting in bluish-green colored mycelium. These Cu ions were incorporated into the glucan-chitin complex by forming coordination bonds with hydroxyl groups of the polysaccharides and oxygen and nitrogen atoms of the amide

group (Zapotoczny et al. 2007; Jamir et al. 2024). Additionally, biosurfactants produced by *Candida sphaerica* were reported to have high removal efficiencies of 95, 79 and 90% for Fe, Pb, and Zn, respectively (Luna et al. 2016). Understanding these mechanisms can provide valuable insights for developing effective strategies for environmental metal removal and detoxification.

Fungi possesses immense potential for mitigating the toxicity of HMs in natural environments and regulating the damages caused by HM pollutants. For instance, *Aspergillus* sp. was found to remove 85% of the Cr from the synthetic medium in a bioreactor system, against 65% from the tannery effluent (Srivastava et al. 2007). Furthermore, Gola et al. (2016) investigated the growth kinetics and HM removal capacity of *Beauveria bassiana*, aiming to develop effective remediation technique for multiple HMs in contaminated water. Continued exploration of microbes-mediated metal remediation could further enhance our ability to address metal and metalloid pollution and advance sustainable environmental management strategies.

### Conclusions

In conclusion, the review underscores the urgent need to address the escalating threat of HM and metalloid pollution resulting from rapid industrialization, which adversely impacts plant health, agriculture, and ecosystems. Elevated metal concentrations disrupt crucial physiological processes, jeopardizing crop yield and quality. The study provides a comprehensive examination of plant interactions with HMs, highlighting the pivotal role of chelating agents, antioxidants, and elicitor molecules in mitigating metal toxicity. Strategies such as chelates, soil acidification and the activation of diverse signaling pathways are discussed, emphasizing their contributions to gene expression and defence mechanisms. Elicitor molecules including JA,  $\text{Ca}^{2+}$  and SA, exhibit synergistic benefits in mitigating metal stress, while microbial elicitors present eco-friendly solutions through detoxification and biotransformation mechanisms. Research has shown that elicitors, either alone or in combination, can increase plant HM tolerance. Combined application of elicitors/chemical compounds to seeds or plants, have a greater cumulative impact than when used alone to mitigate metal and metalloid toxicity. Moreover, many microbes, including bacteria and fungi have been used for cleaning the HM-contaminated environments. Overall, understanding these various elicitors, microbes and signaling pathways is deemed crucial for developing effective and sustainable strategies to enhance plant resilience against the pervasive challenges of metal and metalloid stress.

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No potential conflict of interest was reported by the authors. The authors hereby confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. All the authors have seen and approved the manuscript for submission.

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