

Comparative Analysis of Heavy Metal's Toxicity in Plants Physiology and its Remediation**Shiza Tariq**

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Corresponding author email: shiza.uow.edu.pk@gmail.com**Abstract**

Heavy metals (HMs) contaminate the environment worldwide, alter microflora structure, diversity, and function, degrade soils, and reduce plant growth and yield; that is why HMs treatment is essential to maintain soil health. Traditional approaches to heavy metals are expensive, not environmentally friendly, and adversely affect the soil. Microbial application is an ecologically friendly tactic for the removal of heavy metals. Microorganisms augment soil health improvement, which is the vital enhancement of plants' response to abiotic stress via various mechanisms, including phytohormones, biosurfactants, exopolymers, antioxidant enzyme production, and organic acids. Heavy metals show an active part in nature since they are essential for plant growth stages as these are correspondingly tangled in relocating electrons and redox reactions, indeed a vital portion of numerous enzymes as a straight contributor and elementary role in the metabolism of nucleic acid but additional concentration consequences in numerous lethal paraphernalia. Hence, the present article comprised the evidence for an improved understanding of heavy metal toxicity in various plants and its accumulation mechanism by plants. The root is an imperative gateway for water absorption and organic nourishment in which an advanced metal concentration element likewise accompanies numerous lands, whichever non-essential (e. g. Fe, Cu, and Mn) or essential metal elements or else heavy metals (e. g. Al, Hg, Pb, Ag, and Cd). The way to control metal essentials entry in the cell includes chelation and protection of compounds able to nullify the impairment once the metal component arrives via metallothionein or photoheating composites. Moreover, before their existence, antioxidant compounds were released into vacuoles and additionally rooted in a cellular structure, preventing the dangerous absorption of metal from avoiding cellular injury and toxic effects.

Keywords:

heavy metal, tolerant crop, phytohormones, microorganisms, micronutrients, nano-bioremediation, cellular level plant toxicity

Highlights:

- Heavy metal pollution impact on plant's growth
- Microbial application (MA) is an environment-friendly technique to mitigate heavy metals.
- MA technique has been practiced efficiently under natural conditions.

1. Introduction

Heavy metals showed a half-life of more than twenty centuries, insistent in landscapes having densities $>5 \text{ g/cm}^3$ (Ilyushin et al., 2019). These heavy metals are categorized into two categories:

1. Essential: Heavy metals beneficial living organisms in a limited concentration, such as chromium (Cr), cobalt (Co), nickel (Ni), zinc (Zn), manganese (Mn), and iron (Fe).
2. Non-essential: Heavy metals are highly toxic, even at a small concentration, such as lead (Pb), mercury (Hg), and cadmium (Cd) (Navarrete-Forero et al., 2019).

Depending on toxicity level, metals are divided into three categories:

1. Average toxic metals include Z, Ni, W, Cu, V, Co, Cr, and Co.
2. Low toxic metals, including Mn, Fe, and Mo.
3. Highly toxic metals including U, As, Ag, Sb, Hg, Cd, and Pb.

1.1 Ways of heavy metal accumulation

In rocks, heavy metals exist naturally, and anthropogenic activities (mineral extraction, fossil burning, landfilling, refining of metals, vehicle manufacturing, dyes, vehicular emissions, and agrochemicals) have increased their accumulation (Li et al., 2019) (Fig. 1). Few bacteria subsidize the Hg buildup (dimethyl mercury or monomethyl mercury), ultimately dropping water quality (Kumar et al., 2017).

1.2 Types of Heavy metal accumulation

Plants show heavy metals produce toxicity symptoms in four anticipated mechanisms.

- (i) HM directly interacts with the sulfhydryl group (-sSS) of functional proteins.
- (ii) HM displaced 120 essential cations from their precise positions, thus initiating a failure of the function.
- (iii) Reactive oxygen species (ROS) are generated under HM (Yaashikaa et al., 2022).

1.3 Mechanism of heavy metal toxicity

1.3.1 Heavy metals destroy plants in several steps

1. Destruction of chlorophyll later adversely affects photosynthesis and leads to nutrient deficiency and plant expiry.
2. Disruption of plants' normal biochemical and physiological processes occurs when heavy metals imprison the network site of vital component ions.
3. The destruction of plant cellular membrane, DNA, electron transport chain, and physiological activities begins permanent impairment of plants (He et al. 2022).

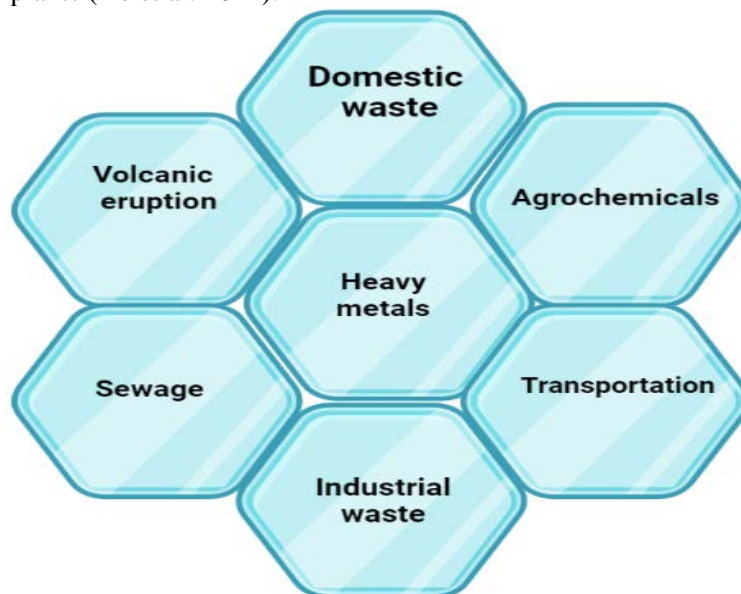


Fig. 1. Common HM sources accumulate in water and soil.

1.3.2 Harmful effects of heavy metal on the plant

Heavy metals above permissible limits disturb redox balance by creating reactive oxygen species, originating biomolecule modification and cell differentiation (Morris et al., 2022). Plants' acquaintance with Cd above the permissible limit (recommended safe range) causes a decline in water uptake, photosynthesis, nutrient absorption from the soil, chlorosis, and growth inhibition leading to root death (González-Velázquez et al., 2022). Zinc (Zn) concentrations in polluted soils often surpass those required as nutrients and inhibit many metabolic functions of plants, limit root and shoot development, and produce phytotoxicity (Natasha et al., 2022). Zn in excess also causes copper (Cu), phosphorus, and manganese (Mn) deficiencies in shoots (Schwalbert et al., 2022). Excess Cu, in addition to top soils from anthropogenic activities, including smelting and mining, plays a cytotoxic role that causes injury and brings stress to plants, leading to growth impedance and leaf chlorosis (Trentin et al., 2022; Bakshi et al., 2018).

The different concentrations of Hg^{2+} can bring physiological disorders, plant injuries, physical impediments to water transport in plants, and stomatal leaf closure (Saha et al., 2012; Wang et al., 2022). Municipal sewage sludge, mining, and smelting activities lead to Pb toxicity, adversely affecting plant morphology, photosynthetic processes, growth, variations in membrane permeability, enzyme activity drop, water imbalance, and disturbance in mineral sustenance (Halecki et al., 2022). Arsenate (As), as a correspondent of phosphate (P), strives for a similar uptake of root plasmalemma transporters (Tiwari et al., 2022; Marzban et al., 2016). The tolerance mechanism developed has been identified in a number of plant species; grasses show suppression of a high-affinity P/As uptake system that reduces As influx to a level at which the plant can efficiently detoxify it, presumably by constitutive mechanisms (Mondal & Singh, 2022) as a single-gene encoding achieves tolerance for the suppressed P/As transport (Afzal et al., 2022).

The excess of cadmium (Cd), lead (Pb), uranium (U), and copper (Cu) causes alterations in the cellular concentrations of essential micronutrients (MN) like calcium (Ca), iron (Fe), and manganese (Mn) that chlorotic leaves, decreasing net photosynthetic rate and changing ratios of chlorophyll a and b, while an excess of aluminum (Al) and cadmium (Cd) leads to lipid peroxidation of membranes, membrane leakage, change of lipid composition, inhibition of root elongation, increase in the volume of cortex cells and damages epidermis cells, all alterations due to the accumulation of HM may be related to the false signal generated similar to the oxidation state of essential nutrients like Mg and Ca (Azmat et al 2009; Pachorkar & Pawar, 2022; Priya et al., 2022; Zadel et al., 2022).

1.4 Heavy metal lenience on the cellular level

1.4.1 Extracellular metal speciation

Heavy metals availability in terrestrial ecosystems depends on soil characteristics, their physicochemical form, and plant species (Koptsik & Koptsik, 2022). A significant element of nutritional metal acquisition is the exudates released from roots into the rhizosphere through chelator assets. This complex creation intensifies the solubility of metals and offers a good uptake plant uptake. Flavins released from *Beta vulgaris* (Bhardwaj & Garg, 2022) and flavonoids from *A. thaliana* facilitate heavy metal tolerance (Shomali., 2022). These compounds can include an exhibition of reductive activity in the direction of redox-active metals, changing the metal's redox state when forming various complexes (Cesco et al., 2010).

1.4.2 Intracellular metal speciation

An appropriate cellular compound will ensure heavy metals' entrance into the cytoplasm instantly to inhibit the handling of possibly lethal, free cellular metal ions and provide involvement in metabolic pathways like detoxification.

1.5 Heavy metal tolerance mechanism in plants

Precipitation of heavy metals in plants was completed strappingly via the rhizosphere's pH variation or anions excreting like PO_4^{2-} . The plant root's surface showed the binding of numerous heavy metals in the adsorption procedure. Heavy metals (Ni, Cd, Sr & Pb) distillate quickly in the root tissues of plants (Castañeda-Espinoza, 2022). The generation of ROS in plants sustains balance, but under harsh stress conditions with heavy metals, the ROS damages various plants' physiological functions, such as photosynthetic pigments synthesis and enzyme activities (Azmat et al., 2009; Long et al., 2022). Moreover, ROS spells the plant biofilm system and induces the peroxidation of unsaturated fatty acids, leading to the disturbance of the membrane structure.

A plant's tolerance to toxic metals is associated with the plant's antioxidant system (Kiany, 2022). Cellular-level biochemical and physiological responses that result in ultrastructure fluctuations evade the adverse effect of metallic harmfulness. Root develops structural changes to protect the plant from the lethal effects of metals.

Plants are highly capable of solubilizing and absorbing various types of soil nutrients by helping to generate chelating agents and change pH and redox reactions oxidation (Pachokar & Pawar, 2022; Caregnato et al., 2008). *Brassica juncea* (Indian mustard) and *Eichhornia crassipes* (water hyacinth) have the highest tendency to absorb heavy metals from soil and water, respectively. Plants accumulate metals depending on several factors, such as metal species, metal concentration, substrate, and environmental conditions. None of the plant species has the potential to accumulate all types of metals from contaminated soil.

Plants may show metal-specific behaviours in natural and amendment-induced conditions. Nickel (Ni) hyperaccumulation is reported in most plant species compared to other metals. We can only say that any plant has the highest tendency to accumulate all heavy metals at once unless we know the metal species and its concentration. Induced accumulation depends upon the metal species plants with higher growth and transpiration rates, lower growth periods, higher biomass, and climate sensitivity are considered (Mishra et al., 2020). The roots of water hyacinths can absorb heavy metals. *Siam* weed has a high ability to absorb heavy metals like lead, iron, and Mn. In a study, ferns like *Pteris vitata* gathered heavy metals like cadmium and arsenic.

However, it is hard to cultivate ferns like other plants, which is why plants with tough seed coats will survive the first metal exposure. Plants develop effective mechanisms to manage heavy metal stress for survival, including excluding plasma membranes, restriction of absorption and transport, induction of stress proteins, and synthesis of specific heavy metal transporters (Gavrilescu, 2022). Hyper-accumulators exclude metals from their tissues to minimize metal accumulation, especially in their aboveground tissues. At the same time, hyperaccumulating plants are characterized by a shoot/root ratio of metal accumulation higher than achieved overexpression of transport systems required for enhanced sequestration, tissue-specific expression of proteins and high metal chelator concentrations (Wang et al., 2022).

One of the plant's leading approaches to tolerate high heavy metal concentrations is chelation (molecular chemical bonding of metal ions). Chelation happens when ligands (components that relate to the central metal ion's electronic orbitals and form secondary valence bonds that result in a complex molecule) bind through the donor atoms to a heavy metal ion.

1.6 Heavy metal tolerance and soil pH

Soils from the polluted area presented a high buffer capacity, and the control samples displayed a distinctly poorer resistance to pH changes in the soil environment. Particular focus was placed on cadmium due to its high mobility in soils, even with neutral and slightly alkaline pH. The analyses revealed that in areas heavily polluted by long-term industrial activity, it is essential to conduct extensive geochemical studies on the presence and circulation of particularly toxic elements. This is because every environmental factor, especially pH, may significantly affect their mobility, causing metal ions to become more or less active or increasing or decreasing ecological risk related to their presence (Huang et al., 2019).

1.7 Antioxidant enzymes accumulation

The term 'antioxidant' refers to a class of compounds that protect cells from damage caused by exposure to certain highly reactive species like ROS. The network and coordination of antioxidants are solely responsible for removing, neutralizing, and scavenging ROS (Hussain et al., 2019).

1.8 Bioaccumulation and biosorption

Bioaccumulation is defined as the phenomenon of living cells, whereas biosorption mechanisms are based on dead biomass. Biosorption possesses many advantages over bioaccumulation, including cost, temperature, selectivity, temperature, and degree of uptake. e.g., *Spirulina*, a phytoplanktonic alga, bacteria, and yeast. Fungi have a high potential to absorb heavy metals from wastewater, resulting in high-quality reusable water (Thakare et al., 2021).

1.9 Roles of micro and macronutrients in heavy metal tolerance

Selenium (Se) is required as a micronutrient to convert heavy metals into less hazardous and volatile forms. However, its higher concentrations lead to its toxicity and are considered a global pollutant. Plants take Se and convert them into volatile forms like dimethyl selenide (DMSe). The goal of biotechnological Se phytoremediation has been to improve Se tolerance, bioaccumulation, and volatilization. (Li & Liu, 2022). In *Brassica juncea*, the over-expression of the APS gene from *Arabidopsis* increased the reduction of selenate to organic Se forms, whereas plants accumulated mostly selenite (Schiavon & Santoro, 2022).

1.10 Roles of polyamines in heavy metal tolerance

To ensure the safety of living cells under unfavourable circumstances, polyamines (PAs) play an essential role in regulating the response under both abiotic and biotic stresses. The relative abundance of higher PAs (spermidine, Spd; spermine, Spm) vis-à-vis the diamine putrescine (Put) and PA catabolism determines the stress tolerance in plants. Climate changes and increasing demands for the production of maize have made it pressing to improve the stress tolerance strategies in this plant. It is imperative to understand the role of PAs in response to various environmental perturbations. In this review article, an effort has been made to summarize the recent literature on the role of PAs in conferring stress tolerance in the golden crop. The responses in terms of PA accumulation, their mechanism of action, and all the recent genetic manipulation studies carried out in the PA metabolism pathway, ameliorating the range of abiotic and biotic stresses have been discussed (Ramazan et al., 2022).

1.11 Hormonal regulation of heavy metal tolerance

Gibberellins, cytokinin, abscisic acid, ethylene, auxins, jasmonate, brassinosteroids, and salicylic acid regulate numerous physiological progressions and metal stress tolerance. Additionally, it is anticipated that 979 or more growth hormones have been discovered. Strategies including exogenous hormonal application and endogenous contents genetic manipulation play a significant role in plant yield and stress tolerance improvement in numerous species (Raza et al., 2022).

1.12 Peptides

Phytochelatins (PCs) are best-characterized plant chelators synthesized by the γ -Glu-Cys moiety of GSH transpeptidation and activated under heavy metal exposure, playing a pivotal role in metal ion regulation in plant cells (Guo et al., 2008b).

1.13 Trace elements

Studies revealed the defending role of trace elements in enhancing the antioxidant ability and shielding photosynthetic tissues (Natasha et al., 2022). Mn-arbitrated upgrading of Cd toxicity was linked with a reduced Cd uptake. Advantageous nutrients such as Silicon and Selenium make the main contribution under metal stress.

1.14 Fungi and heavy metal tolerance

Arbuscular mycorrhizal fungi (AMF) develop symbiotic systems with over 80% terrestrial floras. The AMF advances symbiotic relationships with many plants under Cd stress. An extensive mycelial network of AMF promotes the mineral nutrients absorption and utilization, augments the plant root morphology, originates changes in root exudates, promotes plant growth, and changes the Cd availability in soil. Organic acids in low-molecular-weight roots countersign complexation and chelation reactions, reducing heavy metal capacity and lessening the toxicity of Cd (Dhalaria et al., 2020; Rask et al., 2019). Under Cd stress, AMF improved the growth of maize and increased root surface area, length, branch number, and volume with increased succinic acid and malic acid secretions. *Phanerochaete chrysosporium* is a fungus proven effective for lead (Pb) (He et al., 2022; Begum et al., 2019).

1.15 Heavy metals affect the cellular redox environment

Heavy metals interrelate with the redox environment in the cell by persuading reactive oxygen species generation in redox-active metals can generate ROS through reactions like the Fenton and Haber-Weiss cycle (Han et al., 2022). Detoxification of heavy metals consumes the main component of cellular redox homeostasis.

Antioxidant defense systems retain the premeditated ROS at a low level, yet heavy metal stress dislocates the equilibrium between ROS generation and detoxification (Han et al., 2022). Depending on plant species, heavy metal accumulation alters enzyme capacities. *Targets erecta* is a Cd accumulator that shows reduced GR, CAT, and SOD in the

direction of cadmium contact. In contrast, *Avena strigosa*, a Cd-tolerant accumulator, shows augmented activities to the same Cd concentrations (Siddiqui et al., 2022). The antioxidant defense system is advantageous in heavy metal tolerant plants, and their meditation must be prudently explained when we investigate oxidative stress and redox imbalances brought by heavy metals.

1.16 Physiological and molecular biology of heavy metal tolerance

1.16.1 Genome

Plant *C. Sativa* genome sequence develops a diploid genome ($2n = 20$) containing a pair of sex chromosomes and nine autosomes. In contrast, its wild-type genome sequence offers the basis for the molecular pathway elucidation intricate in tolerance against HMs; thus, hemp plants upgraded polluted HMs, especially Cu-polluted soil (Khan et al., 2021).

1.16.2 Transgenic plants

The development of transgenic plants to enhance metal remediation capacity is a suspicious approach that comprises the desirable gene transfer and insertion from an external source into a plant of concern in plant DNA recombination, making foreign genes with improved traits and heritable (Mohanty et al., 2022). Metal stress generated the production of ROS, ensuing oxidative stress. Transgenic plants augment HMs tolerance through overexpression of genes and intricate antioxidants. To expand the ability to accumulate metals, overexpression of genes intricates in metal translocation, sequestration, and uptake. Limited metals are soluble soil components and are instantly available for absorption by plant roots, e.g., Zn and Cd. Bioengineering improves the characteristic ability of roots to release compounds that mobilize ions via soil pH reduction or forming metal complexes to target metals with negligible bioavailability. One more approach to rise metal translocation is the overexpression of chelators, facilitating undertaking from plant roots to the shoots and facilitating intracellular sequestration into the vacuoles (Podar et al., 2022).

1.16.3 Mutagenesis

Transgenic methods advance unintentional gene flow, beginning with external species to adjacent relatives, which is why such plants are analyzed by monitoring agencies and rejected by consumers. For plant mutagenesis, an alternative approach induces random mutations in the genome. Mutagens fast neutron and ethyl methane sulfonate have been used for crop improvement. Ethyl methane sulfonate is the least destructive, producing point mutations, minor DNA deletions in DNA, and base insertions. At the same time, fast neutrons cause chromosome loss and large deletions. Genetically modified microorganisms immobilize microbial cells to eradicate heavy metals (Maqsood et al., 2022).

1.16.4 Genome editing

Genome editing technology includes transcription-activators like zinc-finger nuclease, CRISPR/Cas-mediated gene mutation, and effector nucleases through innovative and specific tools for plant genetically engineered desired mutations. Deletions and insertions successfully modify genomes. A modification of the CRISPR/Cas assemblage, the base-editing system, can generate single-nucleotide polymorphisms or single-base change, allowing for a side-by-side precision not probable with a mutagenesis strategy resulting in many random mutations (Ranjbar & Malcata, 2022).

1.17 Plants' secondary metabolites and bioactive metabolites adjustment under heavy metal stress

Plants secondary metabolites reduced glutathione (GSH), ascorbate (AsA), jasmonic acid (JA), serotonin (5-HT), salicylic acid (SA), indole-3-acetic acid (IAA), gibberellic acid (GA3) moderation of manganese (Mn^{2+}) and aluminum (Al^{3+}) stress accompanying with acidic soils containing heavy metals in excess in maize, wheat, and rice (Arnao et al., 2021).

1.18 Methods of remediations and management of heavy metal tolerance

Various methods have been established for heavy metal remediation in contaminated soils, which are assembled into 3 types: chemical, biological and physical methods (Wu et al., 2022).

1.18.1 Chemical remediation

Chemical remediation is an efficient method to remove heavy metals from the soil, including practices like floatation, chemical reduction, ion exchange, flocculation, and coagulation that transform heavy metals into less toxic foam that cannot be effortlessly absorbed through plants (Hashmi et al., 2022). A developing technique acknowledged as nano-remediation eliminates heavy metals using nanoparticles that successfully eliminate Zn and Cr with various chemical methods (Tyagi & Pandey, 2016; Ahmad et al., 2022).

1.18.2 Agents' application for heavy metal removal

Chelating agents such as ethylenediamine tetra-acetic acid (EDTA) and chitosan were applied in assessment with the reaction of a locus compound like sodium citrate for nickel and copper removal from heavy metal contaminated sites (Chugh et al., 2022).

1.18.3 Biological method of heavy metal removal

Biological remediation is convened into two types:

- (1) Bioremediation (accomplished by microbial application).

(2) Phytoremediation (accomplished by utilization of higher plants).

1.19 Bioremediation

In bioremediation, heavy metals from the environment were removed using various types of biological representatives. Bioremediation is the most excellent active tool for soil recovery and management contaminated with toxic metals, and it utilizes numerous biotic mediators together with bacteria, fungi, algae, and higher plants. Microorganisms are the primary tools for the remediation of heavy metal-contaminated areas (Saravanan et al., 2022).

The HM tolerance by the microbes is an important mechanism that assists in HM reduction by up to 99.9 percent (Fig.2). Microorganisms express resistance in numerous ecological conditions together with water, soil, industrial and municipal waste. Heavy metal resistance in microorganisms is primarily ruled by the various transposon resistance systems mediated by plasmid and chromosomes. The plasmid arbitrates the all-out resistance apparatus with high effectiveness to a specific cation and anion. Microbial application to remediate metal-contaminated soils because of their ability to bioremediate through altering the reactivity and mobility of the metal contaminants. Microorganisms' application for remediation is a highly discriminating approach for the removal of solid metals at a low cost (Shomali et al., 2022).

Microorganisms can absorb Cd from a medium using the biosorption method in an aqueous solution. Bioremediation of metal using microbes is an effective and, cheap, environmentally-friendly strategy leading to the biotransformation of wastes. Heavy metal remediation is associated with biological oxygen demand, turbidity, total suspended solids, and chemical oxygen demand. The application of plant growth promotes rhizobacteria and acts as an efficient biofertilizer to alleviate worldwide dependency on unsafe agrichemicals. The microscopic inhabitants of the rhizosphere comprise fungi, bacteria, actinomycetes, algae, and protozoa. Nonsymbiotic bacteria accompanying the rhizosphere, advantageous for the plant, typically include the cyanobacteria (Allorhizobium, Mesorhizobium, Bradyrhizobium, Sinorhizobium and Rhizobium genera).

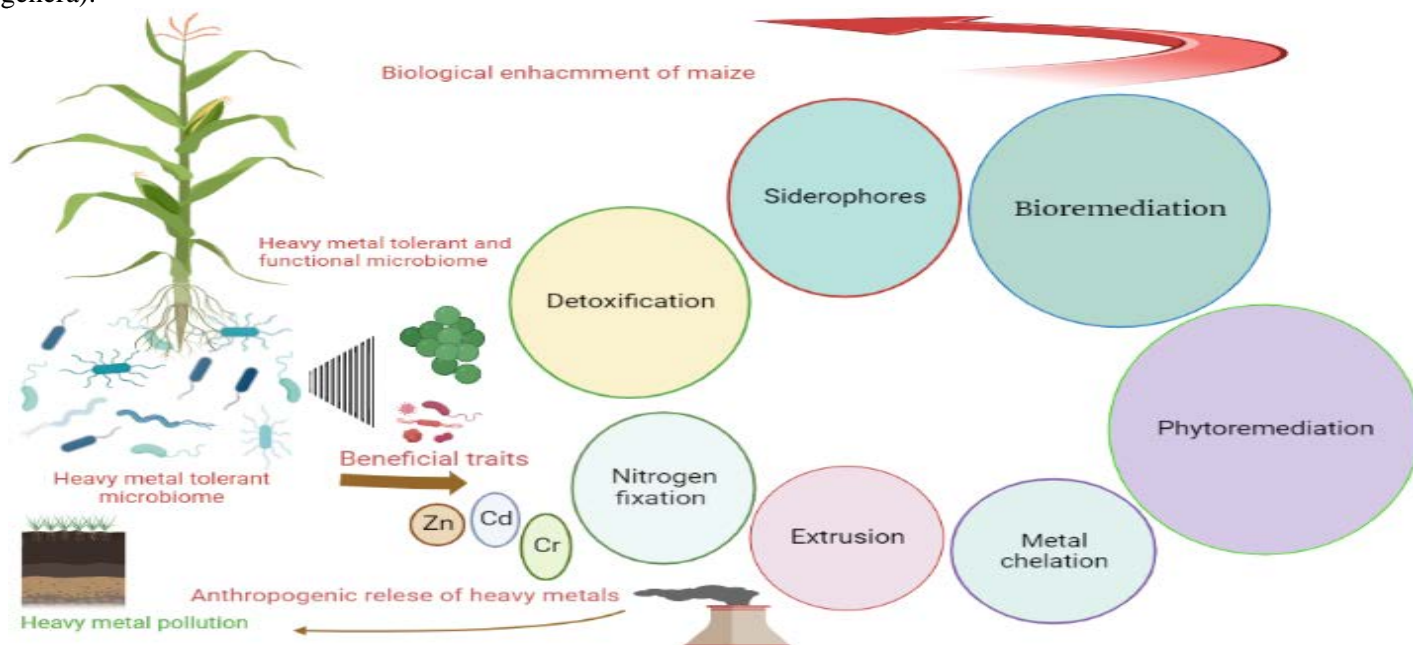


Fig.2. Different ways of Heavy Metals tolerance in plants and soil environment.

1.20 Phytoremediation

Phytoremediation is a viable, sustainable, cost-effective alternative for cleaning contaminated soil. The term phyto refers to plants and mediums to "eliminate a malicious". Germany, China, Taiwan, and Japan are well-known countries for phytoremediation applications. In phytoremediation, green plants are used to decompose, detoxify, and remove heavy metals. This progressive technique is also helpful in recording pollution levels, which was founded on applying hyper-accumulator plants that are tremendously tolerant to heavy metals (Oladoye et al., 2022). Phytoremediation is a plant-aided bioremediation practice that is extremely capable of remedying polluted soils involving plants. *Cannabis sativa*, commonly known as industrial hemp, is an auspicious contender for phytoremediation and is considered suitable for reclamation soils with slight to moderate heavy metal content and can be applied with numerous conventional remedial methods for the remediation process (Placido & Lee, 2022). Phyto mining eliminates heavy metals from polluted soil around ancient mines. Hemp has deep roots and is tolerant to the accumulation of different metals. Phytoextraction of metals is one more practical technique that includes applying metal-accumulator plants to remediate metal-contaminated soils (Igiri et al., 2018).

Phyto stabilization includes immobilization and transcription of heavy metals in roots, decrease in erosion and leaching, maintenance of rhizosphere aerobic environment, and organic matter alteration to stabilize or immobilize heavy metals. Phyto stabilization practices metal-tolerant plants for heavy metal immobilization in polluted soils by declining their bioavailability in the environment (Radziemska et al., 2022) to wander these toxins to groundwater or food cycle (Interior-Hallegado et al., 2022).

Rhizo filtration is a practice of eradicating pollutants from wastewater by absorption of heavy metals to alter rhizosphere pH. Bhat et al. (2022) discovered that *Zea mays* showed an advanced ability to bioaccumulate and uptake Hg. In South Africa, a sewage water treatment plant known as KwaZulu-Natal, a rhizofiltration expedient, was established with *Kyllinga nemoralis* and *Phragmites australis* to extract heavy metals from wastewater. Heavy metals were placed in the plant, and reference portions of the rhizo-filter were used in variable meditations (Hadi & Bano 2010). Results showed that Cd levels rose by 33 and 21 percent in the root system of *K nemoralis*, representing that the arrangement is operative for heavy metal extraction from wastewater (Odinga et al., 2019).

1.21 Nanotechnology in bioremediation

Nanotechnology addresses environmental complications by means of advanced solutions through effective physiochemical properties (extra added surface reaction site, excessive surface activity along with other exclusive magnetic, optical properties effective for toxic metal remediation and decent catalytic proficiency (Wang et al., 2019; Zhou et al., 2021). Nanoparticles are small units in 1-100 nm size range (Sachdev and Ahmad, 2021). These showed remarkable potential in green agriculture to regulate oxidative stress in numerous plants (Sachdev and Ahmad, 2021; Rai and Jajoo, 2021). Nano particles, titanium oxide (nTiO₂), zinc oxide (nZnO), aluminum oxide (nAl₂O₃), silicon dioxide (nSiO₂), cerium dioxide (nCeO₂), copper oxide (nCuO), carbon nanotubes (CNTs) and magnetite (nFe₃O₄) show incredible potential to scavenge ROS, improving the soil characteristics, enhance antioxidant enzymes activities, improving crop yield and mitigating the plant stress (Wang et al., 2021; Adrees et al., 2021). In plants, nanoparticles accumulate in the cell wall and bind heavy metals to form various complexes. In plant tissues, these complexes hinder the mitigation of heavy metals. In soil, nano-iron transforms arsenic and stabilizes it (Furgal et al. (2014). Cu-NPs reduce Cr translocation via effective Cr immobilization in soil, thereby supporting plant growth by dismissing severe cellular oxidative stress (Noman et al., 2020). Similarly, the amendment of meta-sodium silicate significantly reduced the bioavailability of lead by increasing the sorption of Pb onto ferrihydrite and adequate precipitation of PbSiO₃ in soil (Zhao et al., 2017). Nanoparticles (Table) enhance defense capacity regulation with transport genes responsible for the transport of heavy metals in plants; moreover, they improve soil strength and reduce permeability and compressibility (Taipodia et al., 2011).

Table: Heavy metal tolerance enhancement nanoparticles foliar application in Various Plant.

Nanoparticles	Plant treated	Concentration	Positive effect	Reference
ZnO-NPs	<i>Oryza sativa</i> L.	0, 50, 75, 100 mg/L	Reduction in the Cd accumulation in crop.	Ali et al. (2019).
	<i>Lactuca sativa</i> L.	100 mg/L	Reduce Pb Cd and Pb in the roots.	Sharifan et al. (2019)
	Tomato	50 mg/L	ZnO-NPs promoted photosynthetic capacity by enhancing antioxidant activities, which led to ROS scavenging.	Faizan et al. (2021)
Fe-NPs	Wheat	0, 5, 10, 15, 20ppm	Improved plant gas exchange and chlorophyll synthesis reduced the phytotoxicity by increasing the content of chelating agents (proline, GSH and PCs)	Hussain et al. (2019).
TiO ₂ -NPs	<i>Zea mays</i> L.	0, 100, 250 mg/L	Reduces the Cd accumulation in plants.	Lian et al. (2020)
	Glycine max L.	0, 10, 100, 1000 mg/L	Nano-TiO ₂ amendment alleviated oxidative stress caused by As exposure.	Xiao et al. (2021)
	<i>Coriandrum sativum</i> L.	0, 40, 80, 160 mg/L	Improved restricts bio-availability of Cu agronomic traits (seedlings, biomass and yield.	Sardar et al. (2021)
MgO-NPs	Glycine max L.	200 mg/ kg	Reduce the ROS	Ahmed et al. (2021)

FeO-NPs	Wheat	0, 25, 50, 100 mg/kg	ameliorate the cadmium	Manzoor et al. (2021)
SiO ₂ -NPs	Moso bamboo	100, 200 µM	Enhance seedling biomass under Chromium stress.	Emamverdian et al. (2021)
S-NPs	Brassica napus L.	300 mg/L	Sulfur (SNPs) alleviated Hg-induced oxidative stress	Yuan et al. (2021)
MgO-NPs	Daucus carota	5 mmol/L	Detoxified ROS to mitigate Pb stress mitigation and ROS production	Faiz et al. (2021)
Fe ₃ O ₄ -NPs	Oryza sativa L.	5, 10, 15 ppm	Alleviated the arsenic stress	Khan et al. (2021)

1.22 Physical remediation

The traditional reclamation practice follows soil capping, mixing, washing, excavation, and solidifying soil. Heavy metals, by physical methods, differ depending on the metal's physicochemical properties (Kumpiene et al., 2019). Incompetence for a low variety of pollutants causes permanent variations in the soil, leading to ecosystem disturbance and the production of secondary contaminants. (Gao et al., 2022).

1.23 Phytovolatilization

Plants alter various contaminants into the least dangerous volatiles, which are further unconstrained into the air via a foliar system, a successful approach to eradicating carbon-based pollutants and heavy metals (Liang et al., 2022). *Astragalus racemosus* converts Se into dimethyl diselenide, while *Arabidopsis thaliana* converts Hg²⁺ to Hg⁰, enhancing Hg volatility by phytovolatilization (Agarwal et al., 2019). In phytovolatilization, harvesting or disposing of HM pollutants are collected from the soil and spread as gaseous compounds (Saxena et al., 2019).

1.24 Heavy metals removal by composting

Composting is a widely accepted method for the removal of heavy metals from municipal solid waste, poultry manure, sewage sludge, tannery waste, and pig manure. The compost of waste material can be used as fertilizer to enrich the soil with numerous elements such as P and N, K, and many other nutrients. The higher heavy metal concentration of compost confines it from being applied as a soil conditioner; that is why numerous biological chemicals and chemical agents, including natural zeolite, sodium sulphide, bamboo charcoal, bamboo vinegar, lime, and red mud are added during the composting process. Fungus *Phanerochaete chrysosporium* effectively removes lead in the compost.

The advent of different genetic engineering methods has enabled us to manipulate the genetic materials of microbes and enhance their biodegrading efficiency. Several procedures involving recombinant DNA technology, gene cloning, and genetic modification have been done to improve the bioremediation ability of the microbes in the presence of different hydrocarbons and heavy metals (Kumar et al. 2020). However, very few studies have been conducted on the application of genetic engineering to create a better strain for the degradation of plastics.

1.25 Computational and genetic engineering for remediation

Gene editing assists in locating appropriate genes essential for the degradation and metabolism of pollutants and appropriate host organisms, e.g., *E. coli* (in which these genes are expressed). The key processes involved in a polymerase chain reaction are site-directed mutagenesis and antisense ribonucleic acid (RNA) technology. In antisense RNA technology, a novel tool for gene editing as artificially synthesized antisense RNA can efficiently be involved in the regulation of gene expression in host cells. Instead, site-directed mutagenesis is practiced to modify genetic activity linked with degradation. A similar enhanced biodegradation ability was observed in a consortium of marine microbes (Syranidou et al. 2019). However, despite their ability to perform better in laboratory conditions, most of these genetically modified organisms have displayed unsatisfactory results in field studies.

Genetic engineering is a practical approach to exploit potential genes in plants that are involved in As metabolism. Genotypic variation identification in *Oryza Sativa* L. is a crucial step towards safe rice varieties production under As contaminated soil. Significant genotypic variation has been discovered in rice diversities for As accretion that is attributable to radial oxygen loss and differential expression of transporters.

1.25.1 Gene editing tools

Gene editing tools have been applied to the genome engineering of microorganisms and plants to express specific genes (Jiang et al., 2022). Zinc finger proteins, more recently, transcription activator-like effector nucleases and the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 are tools for genome editing (Gaj et al. 2013). This assists in gene manipulation to achieve the gain and loss of function experiments and alter various gene expressions.

1.26 Bioinformatics

Bioinformatics an effective tool to enhance the biodegradation (Purohit et al. 2020) with numerous databases, including The Environmental Contaminant Bio-transformation Pathway Resource, The University of Minnesota Biodegradation Database, BioCyc database and MetaCyc database that are related to biodegradation pathways used evaluate the biodegradation by delivering data on the metabolic pathways, the microbial genes and enzymes associated with the process (Caspi et al. 2020). These computational methods assist in recognizing enzymes to locate metabolic pathways of interest to forecast the biodegradation routes of heavy metals, highlighting a platform to explore novel approaches for the biodegradation of heavy metals can be designed (Ali et al. 2021).

Bioinformatics resources are a precondition for obtaining data to initiate microbial bioremediation research on recalcitrant compounds (Ofaim et al., 2019). Oxygenase is a class of enzymes that transfer O₂ to oxidize the chemical compound. OxDBase provides data on reactions (oxygenases-catalyzed) and is a controlling tool appropriate for heavy metal removal research (Singh, 2018). The bionemo (Biodegradation Network Molecular Biology) database comprises entries for gene sequencing code data for biodegradation and links gene redulation and transcription (Libis et al., 2016b).

1.27 Genetically modified organisms

Various genetic engineering techniques, genetic tools, and synthetic biology has allowed the development of genetically modified microbial scavengers for the mitigation of diverse types of pollutants (Wang et al. 2019). However, several regulatory hurdles hamper the use of genetically modified microorganisms in an onsite experiment. Also, these genetically modified microbes have shown their efficiency in laboratory conditions, but onsite experiments are required to validate their effectiveness.

A diverse range of genetic tools has been developed to prevent the negative impacts of genetically modified organisms on the field, e.g., suicide genetic systems (Honjo et al. 2019) and antibiotic gene-free genetic engineering tools (Ji et al. 2019). Moreover, CRISPR/Cas 9 is a modern tool that makes manipulation precise (French et al. 2020). Apart from inducing synthesis of amino acids (proline and histidine), amines, organic acids, and plant antioxidant α -tocopherol and glutathione, some nitrogen-containing metabolites like some peptides (phytochelatins, metallothioneins, and ferritins) have been reported to play an essential role under heavy metal stress. In the following section, we will discuss the roles of peptides in heavy metal tolerance.

2. Conclusion

The detailed literature search reveals that heavy metals are harmful, but some can be advantageous at limited/optimum concentrations. Metal toxicity causes numerous diseases that ultimately lead to the mortality of Plants. Increasing organic nutrients in the soil as remediation agents can be attained with compost and manure. Bioremediation can be improved by establishing methods of microbial isolates resistant to heavy metal and understanding and advancement of the remediation mechanisms to remove the pollutants at a low cost. Different experimental approaches are needed at molecular and genetic levels to explore plant cell death under heavy metal stress. The recent advances in metagenomics analysis, nanotechnology, genomics, and bioinformatics have assisted in the advancement of metal-tolerant plants in a limited time. Culture-independent techniques can open up new avenues for the discovery of novel metabolic pathways and enzymes. In the metabolic engineering field, researchers can engineer microorganisms as self-eliminate bacterial scavengers to remediate heavy metals at a low cost.

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