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A Comprehensive Study on Heavy Metal Phytotoxicity and Bioremediation Strategies for Ecological Sustainability

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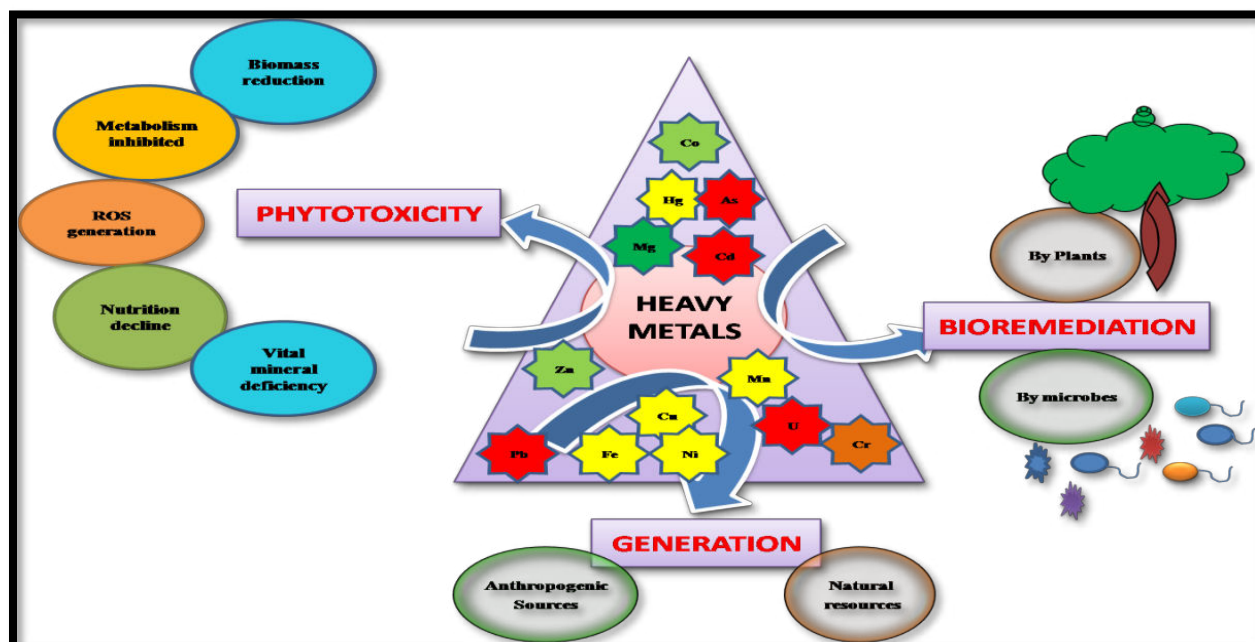
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Abstract: Heavy metals are environmental contaminants that threaten our ecosystems and pose health concerns to living beings through their continuous persistence and magnification in the environment as a result of natural as well as anthropogenic sources. The bulk of these contaminants are exceedingly potent for all life forms, due to which their thorough elimination from the environment is necessary, but existing treatment methods are unable to offer a practical and sustainable approach. Researchers now consider using microbes and plants for heavy metal mitigation as one of the most viable approaches. This review is focused on the general account of heavy metals and their toxicity symptoms in plants. It also states the different bioremediation approaches of plants and microbial cells for a sustainable ecosystem.

Keywords: bioremediation, environment, heavy metal, mitigation, pollutant, toxicity

Graphical abstract



1. Introduction

Heavy metals (HMs) are naturally occurring elements or metals possessing a high atomic mass of more than 23, a high atomic number larger than 20, a high atomic weight ranged within 63.5–200.6 g/mol and a high atomic density of at least 5 g/cm³. HMs are characterized by the phenomenon of recalcitration, which shows resistance against biodegradation (Figlioli et al.2019). Bishop, P. L. (2000) categorized heavy metals into three classes: precious metals (Ag, Au), radionuclides (U, Th, Ra, Ce), and toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, and Sn). HMs can also be further classified into essential and non-essential. Essential HMs serve a significant role in several oxidation-reduction reactions and influence the biochemistry and physiology of living organisms for proper growth and nutrition, but their higher amounts can be toxic. On the other hand, non-essential HMs are often associated with different serious concerns in both ecology and human health. Based on their toxicity, HMs can further be classified into extremely toxic and moderately toxic (Fig 1).

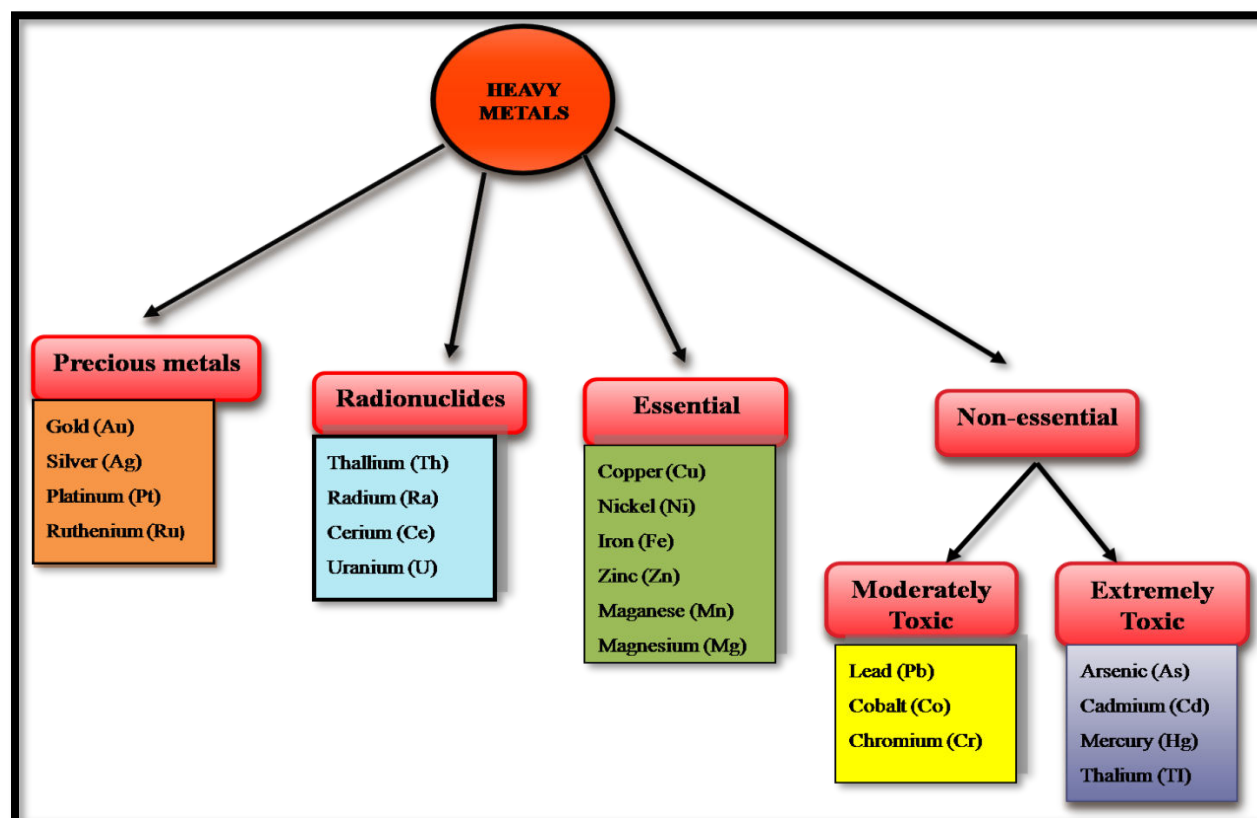


Fig. 1: Classification of heavy metals.

2. Origin of Heavy Metals

Heavy metals are naturally distributed in nature, but some anthropogenic activities are also responsible for their persistence in the surroundings. Natural sources are recognized as weathering of metal-bearing rocks, soil erosion, and volcanic

eruptions (Seaward and Richardson, 1989), while anthropogenic sources include mining, tailings (ore residues) activities, and wastes generated from geogenic operations and pharmaceutical industries. Repeated use of agrochemicals during agricultural practice also causes heavy metal magnification in the soil; from there, they get into the water during leaching. Emissions from fossil fuels (coal products, crude oil, and petroleum products) cause fluctuations in the natural level of HMs, making them persistent in the atmosphere (Tchounwouet al. 2012). Major sources of heavy metal pollution are given in Fig 2.

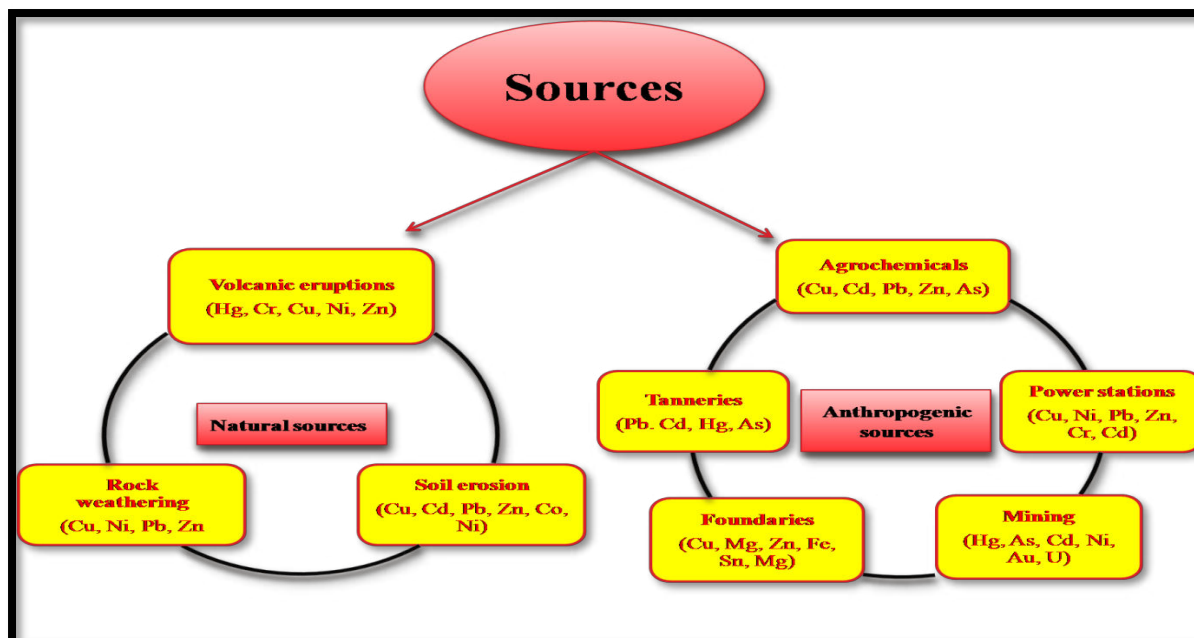


Fig. 2: Emerging heavy metals (HMs) concentration in the environment from major sources.

3. Heavy metals phytotoxicity

Potentially toxic levels of HMs can cause a variety of deleterious impacts on plant growth, photosynthetic & respiratory rates, mineral nutrition, and antioxidant activity. The details of the toxic effects of some important HMs on plants are given below.

Zinc (Zn) is an essential heavy metal that serves as an enzyme catalyst in various metabolic pathways such as chlorophyll production, auxin formation, and conversion of starch to sugar. It also helps the plant survive in the winter (Ghori et al. 2019). However, it is phytotoxic at higher concentrations and interferes with the other minerals uptake by plants (P, Fe, Mn, Cu), causing mineral deficiencies in plant tissues (White and Pongrac, 2017).

Chromium (Cr) alone makes up about 0.1 to 0.3 mg kg⁻¹ of the rocks and is considered the seventh most abundant element on earth (Cervantes et al. 2001). Cr toxicity in plants include reduced seed germination, slow growth, low yields, suppression of enzyme activity, photosynthesis impairment, imbalances in nutritional availability & oxidative mechanisms, and gene alteration (Kumar et al. 2016).

Copper is naturally distributed in the earth's crust at an average concentration of 60 mg/kg (Alloway, B. J. 2013). It is needed in a trace amount to maintain proper functioning. However, an excess of Cu may have negative consequences on overall productivity and survival of plants (Printz et al. 2016). Mir et al. (2021) outlined the detrimental consequences of excessive copper in plants, such as delayed germination, photosynthesis inhibition, ROS (reactive oxygen species) generation, and poor nutritional availability.

Cadmium (Cd) is listed 7th among the 20 most pernicious toxic metals due to its carcinogenic nature (Jaishankar et al. 2014). Plants exposed to cadmium toxicity suffer from reduced germination to decreased productivity. It also impairs the physiological processes (water exchanges, other vital minerals deficiency, and photosynthetic activity) by altering plant metabolism (enzymes/metabolites inhibition, ROS generation, oxidative stress) (Zulfiqar et al. 2022).

Nickel (Ni) is the 5th most abundant (0.08% of the earth's crust) metal used in manufacturing of utensils, coins, and electronics items (batteries) (Genchi et al. 2020). In plants, it interrupts mineral ion uptake and causes oxidative stress. Moreover, Ni poisoning also results in chlorosis, necrosis, and inhibition of many physiological processes (photosynthesis, transpiration) (Hassan et al. 2019).

Arsenic (As) is the 20th most abundant element on earth (Garbinski et al. 2019), with extreme toxicity and carcinogenicity (WHO, 2018). Arsenic phytotoxicity is not only limited to reducing plant biomass and nutritional quality (Hu et al. 2013), but also causes physiological changes in plants by disrupting a number of metabolic processes (Tripathi et al. 2015).

WHO/FAO (1988) listed iron (Fe) as the 2nd most abundant metal on the earth's crust. In plants, Fe is essential for a number of vital biological processes, including photosynthesis, mitochondrial respiration, metabolism (nitrogen & sulphur), and biomolecule synthesis (hormones & DNA) (Ibanez et al. 2020). However, high levels of Fe can generate oxidative stress by upsetting the cell's redox equilibrium and leading to alterations in the plants' morphology, metabolism, and physiological characteristics (de Oliveira Jucoski et al. 2013).

Manganese (Mn) is considered as crucial cofactor for photosystem II's (PSII) water-splitting activity (Andresen et al. 2018) in plants. However, prolonged exposure can lead to oxidative stress and photosynthetic disruption. As a result, young leaves suffer from interveinal chlorosis, while mature leaves have necrosis with black patches and wrinkles. Browning of roots also occurs to hinder the absorption and transfer of other mineral elements, causing their deficiency in the respective plant (Li et al. 2019).

Magnesium (Mg) regulates numerous enzyme-linked metabolic functions such as photosynthesis, glycolysis, genome regulation, protein formation and enzyme activation for CO₂ fixation & assimilation, etc. ((Chen et al. 2018; Lu et al. 2021) in plants and has been known to enhance biomass along with nutritional quality and fruit yield (Yan and Hou, 2018) but an excess amount can lead to bacterial spots on tomatoes and peppers due to interference with calcium ion regulation (Huber and Jones, 2013).

Cobalt (Co) occurs in a combined form with other HMs (Fe, Ni, Ag, Pb, Cu, and Mn) and in rock carbonates and minerals (Mahey et al. 2020). In plants, Co is a crucial part of cobalamin (vitamin B₁₂), which is necessary for the functioning of various N₂ fixation-related enzymes. Conversely, elevated levels of cobalt show detrimental effects on plant physiology and metabolism, resulting in pale leaves, discoloured veins, and leaf loss due to iron deficiency (Asatiet et al. 2016; Hu et al. 2021).

Lead (Pb) is extremely poisonous as it can disrupt cellular membranes, enzymatic activities, and DNA synthesis (Marzan et al. 2017). Plants accumulate Pb within their roots, where it causes inhibition of different physiological and biochemical processes specifically germination period, CO₂ and nitrate assimilation, photosynthesis, and plant growth & advancement (Naveed et al. 2020; Aqeel et al. 2021).

Mercury (Hg) is a persistent and volatile contaminant due to which it shows vast mobility in nature (Pacyna, J. M. 2020). Mercury interacts with plants either by direct application as an antifungal agent (seed disinfectant) or foliar spraying as herbicide (Patra and Sharma, 2000). Mercury phytotoxicity is associated with inhibition of photosynthetic rate, impermeability of cell membranes, disrupting protein structure, causing oxidative damage, and replacing other vital mineral elements such as Mg (Patra et al. 2004; Israr et al. 2006).

Uranium (atomic number: 92; atomic density: 19.1 g/cm³) is one of the most toxic pollutants, posing mutation, carcinogenicity, and DNA alteration due to its

radioactive properties (Qi et al. 2019; Gudkov et al. 2016). Rapid advancement in nuclear technology generates enormous amounts of uranium waste (Iltonet al.2008), causing significant danger to our ecosystem (Chen et al. 2021). In plants, nitrogen and protein metabolisms are hampered by uranium activity (Chen et al. 2023). Expression of photosynthetic genes is also hampered, which alters the photosynthetic metabolism and inhibits ETC, resulting in unusual energy supply and a slowed respiration rate (Zhang et al. 2020).

4. Acceptable level of heavy metals (HMs) (Table 1).

The acceptable limit for each HM in the environment varies according to its toxicity, affected medium, exposure time, etc. Hence, the World Health Organization (WHO) has mandated permissible concentrations of heavy metals in plants, drinking water, and soil to minimize their noxious effects on ecosystems (Table 1).

Table 1: Acceptable levels (ppm) of heavy Metals in plants, water and soil.			
Heavy metal	Plants(per kg)	Drinking water (per litre)	References
Cd	0.02	0.01	Hasan et al. 2012
Pb	2	0.05	Hasan et al. 2012
Hg	-	0.001	WHO, 2004
Cr	1.30	0.1	Hasan et al. 2012
Ni	10	0.2	Hasan et al. 2012
Cu	10	2	Hasan et al. 2012
Fe	20	1.0	Shah et al. 2013 Patil and Ahmed, 2011
Zn	50	5	Shah et al. 2013
As	-	0.05	WHO, 2004
Co	-	0.01	WHO, 2004
Agricultural soil (per kg)			
Cd	0.003		Chiromaet al. 2014, Aneyoet al. 2016
Pb	0.1		Chiromaet al. 2014
Hg	0.05		World Bank, 1998
Cr	0.1		Chiromaet al. 2014, Aneyoet al. (2016)
Ni	0.05		Chiromaet al. 2014, Aneyoet al. (2016)
Cu	36		WHO, 1996

5. Heavy metal remediation: plants and microorganisms

Cleaning up of environment by removing toxic chemicals/pollutants by living organisms is termed bioremediation. When microorganisms are employed to treat the contaminants, it is termed microbial remediation, but in the case of plants, it is called phytoremediation.

5.1 Plants defense mechanisms (Fig 3).

Plants remediate HMs-polluted environments by utilizing two general approaches (a) Site stabilization: in-situ techniques that reducing HM's acute toxicity through decreasing their mobility & bioavailability such as phytoimmobilization, which is also known as phyto-stabilization or phyto-sequestration and (b) Direct removal of heavy metals by "clean-up" strategies involving phyto-degradation, phyto-extraction, phyto-volatilization, and phyto-accumulation to combat heavy metal stress (Table 3).

a. Site Stabilization: In-situ technique

Phytostabilization/Phytoimmobilization/Phytosequestration

Phytoimmobilization entails plants to immobilize pollutants (heavy metals) in the soil or root portion rather than in aerial or above-ground parts preventing their entry into food chain (Gil-Loaiza et al. 2016). Such plants include *Festuca rubra* (red fescue) and *Dactylis glomerata* (orchard grass) (Kacprzak, 2014). Some studies also conclude that *F. rubra* is efficient enough to immobilize Cu, Pb, Mn, and Zn from metal-contaminated sites emerging via industrial activities such as mining, energy, and fuel emissions (Yin et al. 2014).

b. Clean-up Strategies

Phytodegradation

Enzymes secreted from plant roots are employed in phytodegradation to break down pollutants. These enzymes (nitroreductases and dehalogenases) are uniquely able to break down hazardous pollutants such as pesticides and chlorinated solvents. For phytodegradation, poplar trees are frequently utilized to degrade copper (Cu), cadmium (Cd), and zinc (Zn) (Greipsson, S. 2011).

Phytoextraction

This tactic depends on a plant's capacity to absorb and store large amounts of hazardous contaminants (heavy metals) that get detoxified in their above-ground parts or aerial sections (leaves), which can be later harvested and processed further (Rascio and Navari-Izz, 2011). Some examples of plants with efficient phytoextraction capacity include *Elsholtzia splendens* for copper (Cu), *Thlaspi caerulescens* (alpine

pennygrass), *Pteris vittata* (Chinese brake) for arsenic (As), *Brassica carinata* and *Helianthus annuus* for lead (Pb), *Brassica juncea* for mercury (Hg), *Astragalus bisulcatus* (the two-grooved milkvetch), and *Stanleya pinnata* for selenium (Se) (Favas et al. 2014; Freeman et al. 2006; Tangahuet al. 2011; Furiniet al. 2015).

Phytovolatilization

Some plants can absorb toxic substances from the soil, and then release their volatile forms into the atmosphere by transforming them into less harmful forms. Many inorganic chemicals (Se, As, and Hg) can be volatilized by different plant species, such as selenium (Se) by *Brassica juncea* (Banuelos et al. 1993) and mercury (Hg) by *Nicotiana tabacum* (Rayuet al. 2012; Mukhopadhyay and Maiti, 2010). Further, Limmer and Burken (2016) categorized phytovolatilization into two types.

1. **Indirect phytovolatilization:** Plant roots usually burrow deeply into large regions of soil, which ultimately increases the flux of volatile pollutants from the subsurface (belowground).
2. **Direct phytovolatilization:** When volatilization of contaminants occurs from leaves (stomatal opening, cuticle) or stems (trunk), it is termed direct phytovolatilization, involving uptake, translocation, and volatilization of the toxicants.

Phytoaccumulation

Plants that grow naturally on contaminated sites are termed excluders and hyperaccumulators based on their survival strategies (Baker, 1981). Excluders can tolerate high levels of heavy metals and limit their toxicity to the roots, where they get detoxified into less harmful ionic forms. However, hyperaccumulators are known to accumulate toxic heavy metals in their shoots (Van der Ent et al. 2013). A hyperaccumulator can uptake HMs up to 100–1000 times faster than non-hyperaccumulators, as stated by Reeves (2006) and Rascio and Navari (2011). The maximum allowed concentration of different heavy metals in dry biomass for plants to qualify as hyperaccumulators is given (Table 2).

Heavy metals	mg/kg dry mass
Cadmium (Cd)	100
Selenium (Se)	100
Cobalt (Co)	1000
Copper (Cu)	1000
Nickel (Ni)	1000
Lead (Pb)	1000
Zinc (Zn)	10000
Manganese (Mn)	10000

Phytofiltration

Rhizofiltration, caulofiltration, and blastofiltration are the three subtypes of phytofiltration processes where contaminants are removed from liquid systems (sewage water, industrial effluents, wastewater, and wetlands) via plant roots, shoots, and seedlings, respectively (Mesjasz-Przybyowicz et al. 2004). Rhizofiltration is the pollutant's adsorption or absorption on the root surface due to exudate secretions that facilitate the binding of pollutants (by changing rhizospheric pH) and further reduce the metal leaching (Timalsina et al. 2022). Aquatic species such as *Hyacinthus orientalis* (hyacinth), *Lemna minor* (duckweed), *Typha latifolia* (cattail), and *Populus alba* (poplar) are frequently utilized for remediation of contaminated water as they possess high accumulation and tolerance capacities towards heavy metals, with rapid growth producing high biomass (Hooda, 2007).

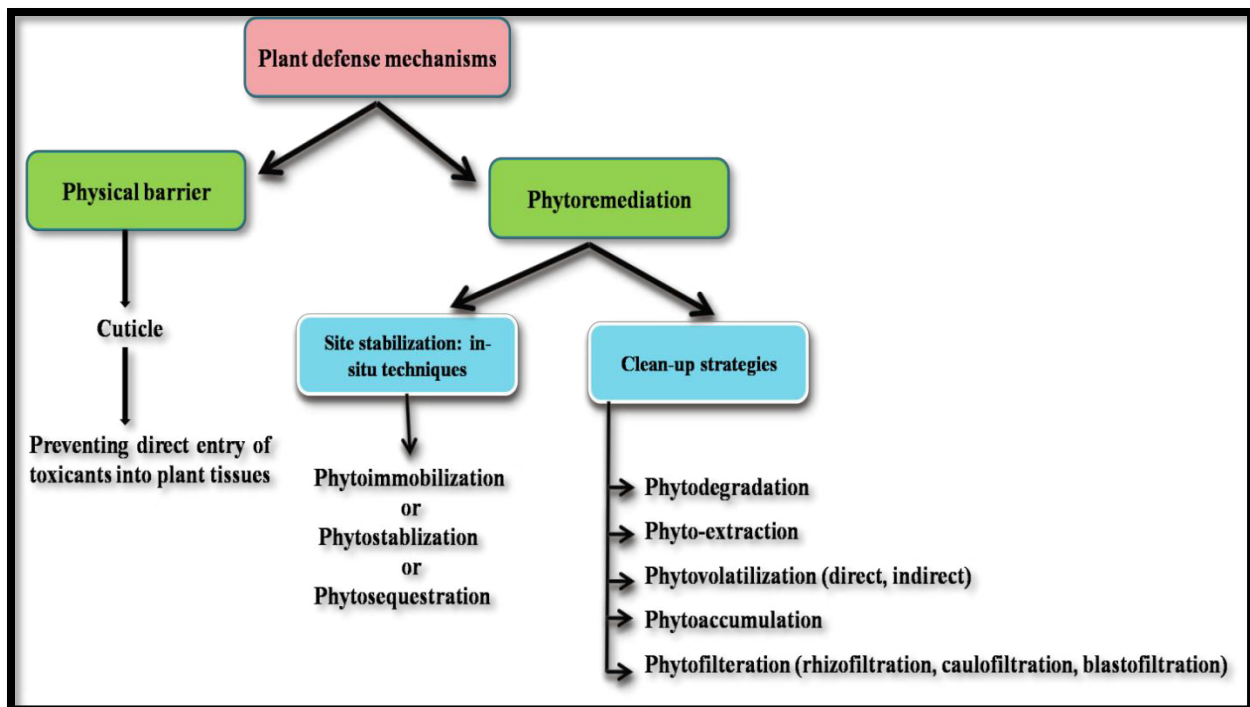


Fig. 3: Different strategies adopted by plants to cope up toxicant's effects.

5.2 Microbial strategies for heavy metal mitigation

The process of utilizing microorganisms to reduce heavy metals from the contaminated site is termed "microbial remediation" (Su, C. 2014). Microorganisms have evolved a variety of mechanisms for maintaining homeostasis and resistance in order to accommodate to HMs' toxicity because of their distinctive degradative enzymes for a particular contaminant (Wei et al. 2014). The microbial capability of

flourishing in adverse conditions (extremely hot & cold environments) also makes them suitable agents for performing bioremediation, and aerobic (*Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Nocardia*, *Flavobacterium*, *Rhodococcus*) as well as anaerobic ones can be utilized for pollutants degradation (Sharma, I. 2020). Different microbial approaches for heavy metal mitigation are discussed further.

Table 3: Tabular representation of different plants species' defence mechanism under heavy metal stress with suitable examples (Kafle et al. 2022).

Strategies	Heavy Metals	Plantspecies	References
Phytoextraction	Cd, Zn	<i>Thlaspi caerulescens</i>	Cosio et al. (2004)
		<i>Arabidopsis halleri</i>	
	Cu	<i>Commelina communis</i>	Wang et al. (2004)
	Pb	<i>Pelargonium hortorum</i>	Manzoor et al. (2019)
		<i>Sedum alfredii</i>	Ning et al. (2019)
Cd, Pb, Ni, Zn	<i>Xanthium strumarium</i>	Khalid et al. (2019)	
Ni, Co, Fe		<i>Lolium perenne</i>	Hernandez et al. (2019)
		<i>Lactuca sativa</i>	
Phytostabilization	As	<i>Eupatorium cannabinum</i>	Gonzalez et al. (2019)
	Cd, Zn	<i>Kosteletzkya pentacarpos</i>	Zhou et al. (2019)
		<i>Solanum nigrum</i>	Li et al. (2019)
	Cd	<i>Salix sps</i>	Yang et al. (2019a)
Phytovolatilization	Se	<i>Brassica juncea</i>	Banuelos et al. 1997
		<i>Scirpus robustus</i>	Arthur et al. 2005
	As	<i>Polypogon monspeliensis</i>	Ruppert et al. 2013
Phytodegradation	Cu, Cd, Zn	<i>Populus</i>	Guerra et al., 2011
Rhizodegradation	Pb	<i>Sesbania cannabina</i>	Maqbool et al. 2012
Rhizofiltration	As	<i>Azolla caroliniana</i>	Favas et al. 2012
		<i>Callitriche lusitanica</i> <i>Callitriche brutia</i>	
	Fe, Cr, Cu, Cd,	<i>Eichhornia crassipes</i>	Rai, 2019

5.2.1 Bioaccumulation

It is the migration of heavy metals from the cell's surface into its cytoplasm (Ramasamy et al. 2007), comprising two steps: adsorption (metal ions becoming adsorbed on the cell surface) and heavy metal entry into the cytoplasm, due to which metallic ions become immobilized within the cytoplasm of the cell, termed as metal sequestration (Mishra and Malik 2013).

5.2.2 Biotransformation

Microbes reclaim the polluted environment by acting as detoxifiers and converting the toxic pollutant into soluble and less toxic forms. Complexation, precipitation, and solubilization of are some examples of metal resistance mechanisms performed by microbes during transformation (Roane et al. 2015). *Agrobacterium* sp. and *Pseudomonasaeruginosa* are some identified metal-reducing species which transform the toxic hexavalent chromium (Cr^{+6}) to (Cr^{+3}) (Kamaludeen et al. 2003). Mercury (Hg) has also been reported to be converted from its dangerous form into a harmless form by *Hymenoscypheus*, *Neocosmospora*, and *Verticillium terrestris* (Kelly et al. 2006). *Aspergillus parasitica* and *Cephalosporium aphidicola* also aggressively remediate lead (Pb) from contaminated sites (Akar et al. 2007).

5.2.3 Biosorption

Some microorganisms release extracellular polymeric substances (EPS) such as polysaccharides, glycoproteins, lipopolysaccharides, and soluble peptides for self-defence under unfavourable conditions (Sedarat and Taylor-Robinson, 2022). Microbial exopolysaccharides (EPS) lead to biofilm formation during heavy metal exposure, which acts as a protective sheath and enables the heavy metal tolerance of microbial cells. Rhizobial EPS (succinoglycan, galactoglucan) can remove Cd, As and Hg by bioabsorption (Gupta and Diwan, 2017). *Gemella*, *Micrococcus*, and *Hafnia* sp. can be used for the bioremediation of Cr, Cd and Pb from tannery effluents (Marzan et al. 2017). Microbial groups with biosorption of different heavy metals are also illustrated further (Table 4).

Heavy Metal	Bacteria	Fungi	Algae
Cd	<i>Bacillus laterosporus</i> , <i>Bacillus Licheniformis</i>	<i>Asparagopsis armata</i>	<i>Codium vermilar</i> , <i>Cystoseira barbata</i>
Cu	<i>Micrococcus luteus</i> ,	<i>Aspergillus niger</i> ,	<i>Codium</i>

	Desulfovibriodesulfuricans	Phanerochaetechryso sporium	vermilara
Pb	Enterobacter cloacae, Micrococcus luteus	Botrytis cinereal, Asparagopsis armata	Cystoseirabarba ta, Spirogyra sp.
As	Sulphate-reducing bacteria	---	---
Ni	Desulfovibriodesulfuricans, Pseudomonas aeruginosa	Asparagopsis armata	Codium vermilara Cystoseirabarba ta
Zn	Pseudomonas sp.	Phanerochaetechryso sporium	Codium vermilara
Hg	Bacillus cereus	---	---
Sb	---	---	Sargassum muticum

5.2.4 Biomineralization

It is the natural capacity of microorganisms to bind the metal and create minerals to efficiently collect heavy metal in a stable solid phase, preventing heavy metal from leaching. Microbial cells produce urease enzymes, leading to an increase in pH and encouraging carbonate production, aiding in the mineralization of heavy metal ions (Li et al. 2013). Bacillus sp. KK1 has been identified as Pb bioremediation agent using induced calcite precipitation (Govarthanan et al. 2013). SporosarcinaginsengisoliCR5's remove As by generating the urease enzyme throughout the biomineralization process (Achal et al. 2012).

5.2.5 Volatilization

It is the direct release of ionic forms of heavy metals into the atmosphere after turning into a volatile state. For e.g., in mercury-resistant bacteria, the enzyme organomercurial lyase is the product of the mer B gene responsible for the conversion of methyl mercury to mercuric ions, which are further converted into 100-fold less toxic elemental mercury by mercuric reductase, a product of the mer A gene' (Fig 4). Metal-binding peptides, phytochelatin, and metallothionein are some examples of chelator molecules used for metal binding. In addition to facilitating microbial uptake and the transit of heavy metals, these chelators bind to ionic forms of metals.

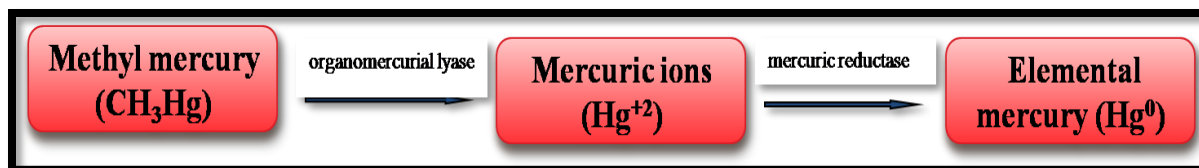


Fig. 4: Conversion of toxic mercury ionic form into less harmful elemental mercury by mercury-resistant bacteria (Krout et al. 2022).

6. Conclusion

Bioremediation is a slow process that takes years to complete. On the other hand, the remediation of polluted soils is also a laborious operation. High quantities of heavy metals may be hazardous to livestock if they eat vegetation growing on remediation sites. The process of bioremediation is intriguing and demonstrates that there is still much to learn about the special skills of microorganisms and plants concerning the mitigation of polluted sites. Researchers are constantly seeking hybrid methods to enhance and use this more successfully. Therefore, to take the necessary measures against extended exposure to them, a thorough awareness of heavy metals is essential.

Conflict of Interest : None

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