



Heavy metal toxicity and sustainable interventions for their decontamination

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The elements with more than 5 g cm^{-3} relative density are commonly defined as “heavy metals”. Heavy metals (HMs) which are commonly associated with poisoning of humans, plants and other organisms include arsenic (As), lead (Pb), chromium (Cr), cadmium (Cd), nickel (Ni) and mercury (Hg). Heavy metal toxicity causes serious threat to all life forms on earth that results in severe contamination of food chain. High tissue concentrations of HMs are toxic to humans, animals and plants. Increased concentration of HMs in soil is because of both natural sources as well as human activities. Major ways through which HMs penetrate the human body are water, food and air. These toxic metals bind with cellular structures of organisms and disrupt the biological functions. The HMs vary in toxicity depending on various factors such as exposure time, reactivity of metal species, concentration of metals and health status of people exposed. Contamination of HMs is one of the most serious environmental concerns worldwide. Ground water contamination by HMs is also linked with expansion of cities, development of industries and intensive use of chemicals in agriculture.

In the environment, HMs are present in trace amounts i.e. $< 10 \text{ ppm}$ and considered as trace elements. In context to public health significance, As, Pb, Cd, Hg and Cr rank among the priority metals due to their higher toxic nature. Heavy metals are also classified as potent human carcinogens by United States Environmental Protection Agency (USEPA). Some essential HMs are important constituents of various important enzymes and play vital role in different redox reactions but their excessive exposure is linked with several harmful effects and causes various diseases (WHO 1996). For example, copper (Cu) acts as an essential

cofactor for different enzymes that are involved in reactive oxygen species (ROS) homeostasis because of its ability of inter-conversion from Cu II to I oxidation states. However, this property of Cu also makes it highly toxic due to the production of ROS and causes oxidative stress. Similar to Cu, many other HMs are also required for active functioning of biological pathways. HMs such as As, aluminium (Al), antimony (Sb), barium (Ba), Ni, Cd, beryllium (Be), bismuth (Bi), Pb, Hg, indium (In), lithium (Li), vanadium (V), silver (Ag), tellurium (Te), platinum (Pt), tin (Sn), strontium (Sr), and uranium (U) have not been reported to be involved in biological functions so are termed as non-essential metals (Yedjou et al. 2012).

Millions of people in several countries like India, Bangladesh, Mexico, Chile, Taiwan and Uruguay are in chronic exposure to As. Arsenic is considered as class I human carcinogen. The safe limit of As in drinking water is $10 \mu\text{g L}^{-1}$ (WHO 2004). Concentration of As may be between $20\text{--}140 \text{ ng kg}^{-1}$ in various foods. Application of pesticides, chemical fertilizers and waste disposal leads to increase of As concentration in soil that normally ranges between 1 and 40 mg kg^{-1} . According to USEPA (USEPA 2020), the limit for HMs in soil and for oral dose are 0.77 mg kg^{-1} and $0.33 \mu\text{g Kg}^{-1} \text{ day}^{-1}$ for As, 78 and 1 for Cd, 0.31 and 3 for Cr, 400 and N/A for Pb, 11 and N/A for mercury, and 1600 mg kg^{-1} and $20 \mu\text{g Kg}^{-1} \text{ day}^{-1}$ for Ni (USEPA 2020). Similarly, in both contexts, environmental as well as occupational, Cd contamination is a serious issue. Cd is commonly present at an average concentration of 0.1 mg kg^{-1} soil. Continuous application of Cd in industries has dramatically increased the human exposure and environmental pollution. To prevent environmental pollution, use of Cd at commercial level has declined in many developed countries. Example is United States (US), where the daily intake of Cd is approximately $0.4 \mu\text{g kg}^{-1} \text{ day}^{-1}$, which is lesser than that of oral reference dose recommended by USEPA (USEPA 2006). National Toxicology Program of US and International Agency for Research on Cancer (IARC) declared Cd as a

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human carcinogen. Mercury is also a ubiquitous pollutant whose exposure leads to several health hazards. Inorganic, organic and elemental form of Hg is present in the environment and each has its own mode of toxicity. Methylmercury is the most abundantly present organic form of Hg in the environment and is formed as a consequence of methylation by the action of microorganisms present in soil and water.

Chromium is a naturally occurring element and health hazards associated with its exposure depend on oxidation states (high toxicity of the hexavalent form). The recommended permissible limit of Cr (CrVI) in surface and drinking water is $50 \mu\text{g L}^{-1}$ (WHO). In atmosphere, concentration of Cr lies between 1 and 100 ng cm^{-3} but can be found to exceed in areas where industries use Cr. Chromium level in soil is reported in the range between 1 and 3000 mg kg^{-1} , 5 and $800 \mu\text{g L}^{-1}$ in sea water and $26\text{--}5.2 \text{ mg L}^{-1}$ in lakes and rivers (Yedjou et al. 2012). Carcinogenicity of Cr in humans is reported but the mechanism needs to be explored in depth. Key factors responsible for toxicity of Cr mainly include solubility of its compound and the oxidation state. Thus, the carcinogenicity of HMs is the global interest of research in reference to public health concern.

Rocks, soils, water, and the atmosphere are the key sources for the HMs exposure of humans and animals. Plants exploit these resources for their survival, and are unable to avoid contaminants due to their sessile nature. Thus, plants have evolved their own detoxification machinery and tolerance mechanisms. Plants take up HMs and sequester them in different tissues, to maintain the concentration below toxic levels. Consumption of these contaminated plants by humans and animals causes accumulation of these toxic pollutants. Plant roots provide a way for the entry of HMs present in the environment and translocate them in above ground parts that hampers the normal functioning and physiology of plants and leads to stunted growth (Chauhan et al. 2020). Loss of soil fertility, marked decrease in agricultural yield and reduction of microbial diversity and activity of microbes is the result of HMs pollution in soil (Kushwaha et al. 2015). Therefore, in depth analysis and understanding of pathways and mechanisms in plant is necessary to avoid food chain contamination.

In present time, several methods have been applied to control HMs pollution in soil but these are not sufficient. The unaffordable cost and low efficiency of the methods viz., thermal treatment, excavation and landfill and acid leaching, makes them not much suitable for commercial application and practices. Use of plants, “the green solution i.e. phytoremediation” is an eco-friendly and cost effective method for the amelioration of toxic HMs in soil.

For HMs contaminated sites, plantation proved to be an efficient method for reclamation of soil. Phytoremediation helps in restoration of natural habitat and for amelioration of HMs stress and environmental contamination. Plants have

evolved mechanisms like ROS homeostasis, chelation and sequestration of HMs and exclusion of metal ions that help them to avoid HMs stress. Phytochelatins (PCs) are cysteine rich thiolic ligands which are produced by plants and well reported for the elimination of toxic metals in cytosol. Phytochelatins are composed of glutamic acid, glycine and cysteine with chemical formula $(\text{-Glu-Cys-})_n\text{Gly}$ where n is 2 to 11. Phytochelatins bind with HMs like As and Cd and transport these complex molecules into vacuole (Tripathi et al. 2013). Another metal binding peptide, metallothioneins (MTs) are cysteine rich and of low molecular weight and their role in metal tolerance is well reported.

Rhizospheric microbes alter the bioavailability of HMs by the release of acids, chelating agents, phosphate solubilization and changes in redox potential (Mishra et al. 2017). Oxidation of As to AsV and reduction of CrVI–III by the action of *Alcaligenes faecalis* and *Pseudomonas fluorescens* is reported (Kushwaha et al. 2015). Rhizospheric soil is rich in microbial diversity and well known to affect the mobility and availability of HMs to plants. Supplementation of plant growth promoting rhizospheric microbes in soil and treatment of seeds with them exhibits several growth promoting traits and also improves nutritional status of plants. Microbes exert their beneficial effects due to production of siderophores, nitrogen fixation, and release of phytohormones and elevation of nutrient levels. There are wide spread reports on utilization of rhizospheric microbes along with plants, a technique known as rhizoremediation.

Amelioration of HMs through phytoremediation include; phytoextraction (use of metal accumulators for the removal of toxic HMs in soil), phytovolatilization (production of volatile metal derivatives and evaporation through aerial parts), phytostabilization (plants decrease the bioavailability of toxic metals in soils) and rhizofiltration (exclusion of toxicants from polluted water either through roots of plant or microorganisms associated with the rhizosphere). Exposure of HMs induces plants to activate their defense machinery in several ways like immobilization, compartmentalization of the complexed metal ions, exclusion, and the expression of stress responsive proteins and hormones (Chauhan et al. 2020). Plant–microbe interactions make the process of phytoremediation more efficient. Microorganisms present in soil and particularly in the rhizosphere play vital roles in maintaining soil structure, prevention of nutrient loss, detoxification of toxicants, improved plant growth and productivity as well as control of plant pests. Thus the presence of rhizospheric microbes increases the capacity of plants to remediate HMs stress. Plants and rhizospheric microbes show direct interaction in which plants are the source of carbon for microbes that help later to tide over HMs contamination in polluted soil. Plants and rhizospheric microbes also show indirect interaction in which plants favour increased microbial diversity and microbes degrade contaminants in

soil due to their metabolic activity. Mycorrhizal associations are known to improve the efficiency of phytoremediation in HMs contaminated soil, as these fungi have evolved tolerance to HMs. Fungal hyphae provides large absorption surface area to plants for increased water and minerals uptake. Mycorrhizae also restrict uptake and accumulation of HMs in plants by providing an exclusion barrier. Bioremediation is considered as most efficient and eco-friendly for remediation of toxic metals. Use of PGPRs in HM polluted soil is important to avoid use of excessive chemical fertilizers and to maintain nutrient properties and structure of soil. This is one of the most promising methods for remediation of metalliferous environment, for safe agricultural practices and for improved microbes mediated metal tolerance (Mishra et al. 2017). Microbes help in reduction of bioavailability of metals to plants through diverse mechanisms. These resistance mechanisms of microbes include conversion of toxic metals into their less toxic or non-toxic forms, enzymatic redox reactions, metal chelation, bioaccumulation and exclusion of HMs for the better survival in HMs polluted environment. The positive impact of microbial supplementation to HMs exposed plants are because of their beneficial direct and indirect mode of actions viz., production of exopolysaccharides, formation of biofilm, phytohormones and siderophores production. Nowadays, genetically transformed microbes i.e. novel phytomicrobial strategy are being extensively exploited to enhance the amelioration of HMs and to increase stress tolerance in plants.

The day by day increase in concentration of HMs in soil and water has received a marked attention globally due to the persistent and non-degradable nature of HMs. Heavy metal contamination and remediation is still one of the great challenges for the researchers. In HMs polluted soil, use of plant growth promoting microorganisms (PGPMs) equipped with HMs tolerant machinery along with supplementation of nutrients is suggested to be efficient for remediation. The use of non-food crops which are not consumed by humans and other animals can be another important strategy for removal of HMs and to protect food chain contamination. Additionally, application of microbes in consortium is another potent tool for mitigation of HMs stress in highly contaminated sites. Treatment of plants growing in HMs polluted soil with PGPMs and nutrient additives lead to dual benefits as they not only result in detoxification of HMs induced toxicity but also lead to biofortification of nutrients. Use of genetic engineering to develop “microbial biosensors” is an emerging and promising technology for HMs remediation

and detection of contaminated sites. In spite of findings till date, various aspects of regulatory networks, in-depth molecular mechanisms of microbe assisted HMs tolerance in plants still needs to be explored to unravel the cross-talk between plant and microbes in soil for mitigation of HMs stress. Though, there are several aspects that need to be investigated, advancement in genetically engineered techniques to develop transgenic varieties for HMs tolerance of plants is also needed. Research has shown that sustainable interventions including bioremediation and phytoremediation are promising for the reduction of HMs toxicity in the ecosystems and need to be explored further.

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