Microbes-mediated alleviation of heavy metal stress in crops: Current research and future challenges

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ABSTRACT

Heavy metals (HMs) pollute the environment on a global scale and have different harmful effects on ecosystems. Outstripping accumulation of diverse toxic HMs in soils has altered the diversity, structure, and function of microflora, degraded soils, reduces growth and yield of plant, and entered the food chain. HM treatment is necessary for maintaining the agricultural soil health. Many procedures and approaches have been used to recover contaminated soils in recent times, however, most of them were too expensive not environmentally friendly, and negatively affected soil properties. Usage of microbes was found as cost-effective and eco-friendly approach for biomediation of HMs. Microbes increased sustainability in agriculture soil health, which is essential to uninterrupted plant growth or improvement in stress full condition through mechanism like productions phytohormones, organic acids, biosurfactants, exopolymers, antioxidant enzymes; and solubilization of phosphorus. It is well known that plant growth-promoting microbes enhance crop productivity and plant resistance to HM stress. In this following review, deep insight have has provided on mechanism of alleviation of HM stress by microbes and enhancement of plant growth promotion.

1. INTRODUCTION

Pollution is a persistent global issue which is caused by various hazardous pollutants including heavy metals (HMs), xenobiotics, polyaromatic hydrocarbons, agro-chemicals, and industrial effluents. HMs, the metals and metalloids having a density more than 5 g/cm³ are one of the serious environmental pollutants due to their non-biodegradable characteristics. They are characterized into three classes' namely toxic metals (arsenic, cadmium, copper, nickel, chromium, cobalt, lead, mercury, tin, and zinc), precious metals (gold, platinum, ruthenium, palladium, and silver) and radionuclides (americium, radium, thorium, and uranium) [1]. They are mainly spread by various human activities such as mining, dye and pigment manufacturing, electroplating, and fossil fuel combustion [2,3]. HMs spread through diverse modes such as surface runoff, food chain, atmospheric deposition, and also adopt different chemical forms including carbonate-bound, exchangeable, iron manganese oxide-bound, residual, and organic-bound [4].

Around the globe, the HM accumulation in the environment poses a threat to the fertility of soil, plant, animal, and humans due to its highly toxic, mutagenic, carcinogenic, and teratogenic nature. In humans, HMs ingestion and inhalation may cause damage to the mental health, genetic makeup, and central nervous system. The HM direct contact could also escalate the cancer risk in the human population [5,6]. HMs have also a harmful impact on the ecological dynamics of the rhizosphere niche [7]. At the same time, they also have a negative impact on the growth of plants, biomass disposal, photosynthesis, and long-term production of food [2]. In plants HMs may result in the disruption of enzymatic function and nucleic acid structure; and hindrance of essential metals movement and absorption from their normal binding sites which leads to deficiency and nutrient imbalance [8]. After analyzing the serious and harmful impact of the HMs on the environment, environmentalists have come up with strategies such as physicochemical and biological approaches. The
biological approaches have proved to be more efficient due to cost-effective and eco-friendly approach. In biological approach, plant and microbes are used and among them, microbes are applauded more. Plant allied microbes; especially plant growth-promoting (PGP) beneficial rhizobacteria are playing a significant role in plant growth promotion, mobilization, and phyto remediation of HM from soil [9,10].

Recently, HM tolerant PGP microbes are known for their potential role in mitigating the HM stress for the plant growing in metal stressed soil and enhance their growth [11,12]. Multiple HM resistant-PGP rhizobacteria indicated multiple reactions to metal ions such as metal bioaccumulation, enzymatic transformation, deposition, complexation, precipitation, and oxidation/reduction, and, thus reducing HM ion toxicity towards plants [8]. In general, PGP rhizobacteria have caused a decrease in metal bioavailability on inoculation into different plants and different environments. The present review deals with the different mechanism of the microbes which helps in the mitigation of the HM stress in the plants for agro-environmental sustainability.

2. APPROACHES FOR REMEDIATION OF HMs

Mitigation of HM is an urgent need to reduce the pollutant spread via the food chain, thus decreasing the health-related risks. Removal of HM from the soil is a challenging task with respect to technical complexity and a variety of methods have been adopted for the elimination of toxic HM [13,14]. Several strategies such as physicochemical and biological are available to decontaminate the soil from HM [15]. Basic impurity immobilization and removal methods have evolved into current biological elimination approaches [16]. Traditional physicochemical treatment methods could not resolve the pollution problem due to their detrimental effects and intrinsic drawbacks including their cost, amendment of soil properties, secondary pollution, and structural and functional changes in microbial communities [17].

2.1. Physico-chemical Approaches

Soil vitrification and replacement both are included in physical remediation method [18]. Physio-chemical approaches include electro-reclamation, leaching, landfill, thermal treatment, excavation, soil physicochemical properties change such as redox and pH potential by the addition of organic matter, chemical reagents, and curing agents [19]. This strategy of HM removal is fast but expensive and moreover causes detrimental effect on physio-chemical and other biological properties of soil and results in increasing secondary pollution. These approaches fail to remove the complete pollutants [20].

2.2. Biological Approaches

The bioavailability and solubility of HM could be increased by microbiiota of soil [15,21]. The biological method of HM removal is a suitable approach due to an environmentally friendly process, reasonability, and higher public receipt. Moreover, the microbial use also conserves natural soil properties [22]. A biological approach includes bioleaching, bioaugmentation, biostimulation, bioreactors, bioventing, composting, bioremediation, phyto remediation, and land forming [23]. Phyto remediation is a biological method by which the efficacy of HM remediation could be increased if it is combined by microbes. Various microbes from diverse habitats have been reported for the effective removal of HMs from the soil. In a report, fungal species Phanerochaete chrysosporium, isolated from sewage contaminated soil have been reported for bioremediation of nickel and chromium up to 57% and 64.25%, respectively, in in vitro condition via biosorption mechanism [24]. In another report, a bacterium, Bacillus sp. isolated from HM contaminated soil was shown resistant to toxic HM such as Cr(VI), Cr(III), Cd²⁺, Co³⁺, Cu²⁺, Ni²⁺ and Zn²⁺. This bacterium was reported for effective detoxification of hexavalent chromium [25].

In an investigation, bacterial strain, Cupriavidus metallidurans from Pb-Zn mine showed to biosorbent the HM including Cr (4%), Cd (53%), Co (52%), Mn (74%), and Zn (48%) [26]. Bhattacharya and Gupta [27] reported Acinetobacter sp. for the detoxification of Cr (VI) up to 67% within 24h of incubation. In another report, Bacillus subtilis was reported for the immobilization of Cd metal through bioaugmentation technology. The inoculation of this strain in the carrot grown in cadmium contaminated soil was reported for alleviation of Cd stress along with enhancing the plant shoot (16%) and root (55%) [28]. Gan et al. [29] reported thermostable consortium of Acidithiobacillus caldus and Sulfo Bacillus thermotolerans for bioleaching of HM including, Zn, Cu, Mn, Cd, As, Hg, and Pb. Similarly, the bioleaching of HM was reported by fungus Aspergillus niger isolated from HM contaminated sediments. The fungus particularly leaches Pb by 11.5% from the polluted sediments along with Cd (93.5%), Cu (62.3%) and Zn (68.2%) [30]. Emenike et al. [31] reported the microbial blend of bacteria including Rhodococcus sp., Lysinibacillus sp., and Bacillus sp. for the bioremediation of HM such as Cu, Zn, and Pb by 86%, 73%, and 71% in leachate contaminated soil, respectively. The removal of HM was reported by the bacterial endophytes of Tridax procumbens identified as Pae nibacillus sp. This bacterium was reported for detoxifying Zn, Pb, and As [32].

In a different report, the phyto remediation of Cd was reported by the Cd resistant bacteria namely Arthrobacter sp., Micrococcus sp., and Pseudomonas sp. The phyto remediation of Cd metal through bacteria was assisted by biostimulation and bioaugmentation. The inoculation of these bacteria on the Glycine max L. growing in Cd contaminated soil was reported for increasing the growth parameters [33]. Satyapal et al. [34] noted that reported Pseudomonas for remediation of arsenic in water. The particular strain was reported for the attendance of aoxB, aoxR, and aoxC genes, which play a significant role in arsenic bioremediation. Moreover the bacterial isolate Pseudomonas was reported for the bioremediating other metals including Hg(II), Ag(I), Ni(II), Cr(IV), Co(II), Cd(II), Cu(II), and Pb(II). Similarly, Pseudomonas sp. sorted out from the Cd contaminated soil was reported for the Cd transformation at 6.0 pH and 50°C [35]. HM Cr bioremediation was reported by the soil bacteria namely, Pseudomonas fluorescens and Bacillus safensis by 84% and 72% respectively [36]. Diba et al. [37] reported halophilic bacteria Bacillus sp., Oceanobacillus sp. and Salinibacillus sp. sorted out from the Khara salt lake in Iran for detoxification of lead and nickel.

3. MECHANISMS OF HM RESISTANCE/TOLERANCE IN MICROBES

Resistance and tolerance of HM by the microbes are the most important mechanisms as the resistance helps in reducing and mobilizing the toxic HMs by 99.9%. Microbes show resistance in several environmental conditions including soil, water, and industrial and municipal waste. HMs resistance in microbes is majorly governed by the variety of transposon, chromosomal and plasmid-mediated resistance systems [Figure 1]. The maximum of the resistance mechanism is mediated by the plasmid which is highly effective to a particular anion and cation. Microbes using the various
systems tolerate the HM through mechanisms [38]. In the metal exclusion mechanism, microbes create modifications in the cell wall and envelop the membrane and surface layer. In this mechanism, the microbe protects its metals sensitive cellular components [39, 40]. Microbes protect the cellular component by passively absorbing the high level of the HM on the cell via charge-mediated attractions. The non-specific binding on the outer membrane, envelop, surface layer; and extracellular polymeric substances (EPS) of the HMs prevent the entry of these metals inside of the microbial cell and hinder the essential components interference of the cell [41]. Absorbing the HM on the surface of the microbes may cause the metal local detoxification due to the metal’s mobilization caused by to the coating of the polysaccharides on the surface layer of the microbes [42]. Another HMs tolerance mechanism in microbes is extracellular sequestration which is known to withstand the HM in the microbial cell. In particular type of mechanism, microbes produce several types of metabolites such as siderophores and biosurfactants that result in the metal precipitation. These secreted metabolites efficiently bind to the toxic HM and it subsequently detoxifies metals simply by complex formation or by the formation of the effective barrier around the cell of microbes [43].

In a report, endophytic bacteria *Bacillus thuringiensis* was reported for the siderophores production and reported for the detoxifying the toxic HM [44]. In a similar report, siderophores producing *Bacillus* sp. was reported for assisting phytoremediation of HM including iron, copper, zinc, cadmium, manganese, nickel, lead, and arsenic [45]. Detoxification of the metals through the mechanism of intracellular chelation refers to the metals deposition within the cytoplasm to prevent revelation to vital cellular components. A few of the microbes follow the cytosolic sequestration mechanism for protecting themselves from the toxic nature of the HM. Intracellular proteins can also decrease the concentration of free ion with the cytoplasm and sequester HMs which finally results in detoxification of the toxic HMs.

The detoxification of the metals through the enzymatic cation mechanism is another mechanism of the microbes which convert the toxic HM to less toxic forms. In this mechanism, various types of reaction such as oxidation, reduction, methylation, and demethylation are involved [46]. The reaction methylation of the HMs including Pb Sn, Se, and Te, could be converted into the gaseous state by the addition of the methyl group that helps in the methylated metal diffusion away from microbial cell due to its volatile nature. This mechanism results in less toxic environmental conditions for the microbial cell. Mechanism of HM alleviation was also reported by the active transports or efflux system. The system is considered the largest category of HM resistance systems in which microbes export the toxic metals out of the cytoplasm. This mechanism is plasmid or chromosomal encoded. The system works ATPase and non-ATPase-linked and highly specific for cations and anions and with the use of ATPase/chemiosmoticon/
proton pumps, microbes actively eliminate the toxic metals from its microbial cell [47].

4. MECHANISM OF MICROBIAL MEDIATED ALLEVIATION OF HMs IN PLANTS

4.1. Production of Phytohormones

Modification in the phytohormones content plays an essential role in the plant survivability. The changes in the level of phytohormones in the plant during the hostile environmental conditions may lead to perturbations of plant health and growth [48,49]. Microbes play a significant role in the mitigation of the HM stress by modulating the level of the phytohormones by producing different plant growth regulators (PGRs) such as auxin, gibberellins, and cytokinin [50]. In literature several types of the microbes have been known to produce the PGRs which also help in the alleviation of the plants stress caused by toxic HMs [51]. In a report, bacterium Burkholderia sp. sorted out from the metal contaminated soil was reported for the production of PGR, indole-3-acetic acid (IAA) along with 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophores. This bacteria was reported for alleviating the HM stress in plant Sedum alfredii after inoculation and also enhanced the plant biomass as compared to uninoculated control [52].

Similarly, bacteria Serratia marcescens and Rhodotorula mucilaginosa was reported for producing IAA, siderophores, and exhibits the activity of ACC deaminase which helps in remediating the toxic HM such as zinc, cadmium, and lead in the macrofungus Tricholoma lobsynys [53]. In an investigation, Polygonum pubeascens endophyte Rahneilla sp. was reported for alleviating cadmium, zinc, and lead in plant Brassica napus by producing, IAA, siderophores, and ACC deaminase He et al. [54]. In an investigation, Enterobacter sp. And Klebsiella sp. sorted out from the P. pubeascens gown in metal-polluted soil was reported for producing IAA, alleviating the cadmium, zinc, and lead toxicity and improving the growth parameters of rapeseed [55]. Singh et al. [56], have isolated Pseudomonas putida and B. safensis from the Phyllanthus urinaria rhizosphere, and these strains were reported for the production of IAA and alleviation of HM stress [Table 1].

In another investigation, Pseudomonas protegens have been reported for producing IAA, chitinase, polymer degrading enzymes, and siderophores which helps in the alleviation of HM stress [57]. In a report, Leifsonia xyli, the rhizospheric bacterium was reported for mitigating copper metal stress in tomato by the producing two different PGRs i.e. gibberellins and IAA [58]. In different report, microbial consortium of a fungus and bacterium namely, Paecilomyces formosus and Sphingomonas sp. was reported for producing gibberellins and the co-inoculation in soybean was reported for alleviating the stress caused by metal aluminum and zinc. The microbial consortium also reported for reducing the concentration of asbiscic acid and jasmonic acid (JA) which helps the plant to survive and grow well in stressful conditions [59 1788].

In another report, IAA producing fungal endophyte, Penicillium roqueforti was reported for alleviating HM stress in wheat crop growing in the HM contaminated soils [60]. Similarly, Bilal et al. [61], have concluded gibberellins and IAA producing endophytic fungus Penicillium funiculosum for alleviating the toxic effect of the HMs by hormonal modulation in the crop G. max L. Another report has reported phytohormones producing rhizobacterium identified as Staphylococcus arlettae alleviated the chromium stress in sunflower plant [62]. Bilal et al. [63] have reported two fungal endophytes i.e. P. formosus and P. funiculosum for the producing gibberellins and IAA which helps in mitigating multi-metal toxicity in soybean.

4.2. Synthesizing ACC Deaminase

Exposure of plant to HM stress causes an increase of ethylene biosynthesis which results in the declination of the root elongation, induction of apoptosis, and accumulation of hydrogen peroxide. The ACC deaminase synthesis by the microbes is one of the most important and accepted mechanisms for conferring the HM stress in plants. The production of the enzyme, ACCD enhances the root growth of the plants by hydrolyzing ACC which is the immediate precursor of the ethylene (produced during exposure to HM stress) to ammonia and α-ketobutyrate and reduces the ethylene level in the plant [64]. The inoculation of the ACC deaminase synthesizing diverse group microbes has reported for successfully alleviating the HM in stress [65,66]. In a report, bacterial endophyte namely, Pantoea agglomerans, Pseudomonas thivervalensis, and Ralstonia sp. were concluded for having the capability to synthesize the ACC deaminase enzyme and siderophores. These strains inoculation in the plant B. napus was reported for enhancing the growth of plant and alleviating the copper accumulation [67].

In another report, ACC deaminase enzyme-producing endophytic yeast Cryptococcus sp. isolated from ropes were reported as potential strain for mitigation of the multiple metal stress in plant Brassica albolabra growing in multiple-metal contaminated soil i.e. cadmium, lead, and zinc [68]. Guo and Chi [69], reported PGR rhizobacterium Bradyrhizobium sp. for alleviating HM stress in Lolium multiformum and G. max. In another report, ACC deaminase synthesizing Sinorhizobium meliloti was reported conferring Cu tolerance in Medicago lupulina [70]. Rizvi and Khan [71], have reported Pseudomonas aeruginosa for the production of ACCD enzyme along with hydrogen cyanide (HCN), ammonia, siderophores, and IAA. This strain was mitigating the HM stress in wheat plant and enhancing the crop root structure.

In a report, Pantoea sp. from the rhizosphere of Ziziphus nummularia was reported for synthesizing the ACC deaminase enzyme and it was alleviating copper stress for wheat crop [72]. Danish et al. [73], reported Agrobacterium fabrum and Leclercia adecarboxylata for the production ACCD enzyme and helps in alleviating the chromium stress in the crop Zea mays growing under Cr stressful conditions. In a report, Bacillus xiamenensis was reported for producing ACC deaminase enzyme and alleviating the toxic HM in soil contaminated by industries and enhancing the growth of flax plant [74]. In another report, copper accumulating bacteria Pseudomonas sp. was reported for producing ACCD and enhancing Helianthus annus L. plant growth [75].

4.3. Production of Siderophores

Siderophores, the iron-chelating compounds are produced by both microbes and plants for the sequestration of the mineral iron from the surrounding soil. This compound helps in combating the nutrient (fulfilling the iron mineral requirement), biotic (pest and pathogens), and abiotic stresses such as HM stress. Siderophores confer the stress exerted by abiotic factors by reducing the accumulation of the HM in plant that helps in enhancement [76]. The exposure of the HM to plant may affect plants negatively which leads to lower siderophores production. On the other hand, microbial siderophores help in alleviating the stress for plant [77,78]. Numerous microbial species have been found producing siderophores and combating the metal stress.
in the plants [79]. In a report, rhizospheric bacterium *Burkholderia* sp. was reported for having siderophores production along with IAA and ACCD. The inoculation of the strain in the *Salix caprea* plant growing under the Cd and Zn contaminated soil was found to lower the uptake of metal in roots and promoted the plants growth [80].

Similarly, Zhang *et al* [81], have reported bacterial endophytes *Agrobacterium tumefaciens* and *Bacillus* sp. of *Commelina communis* plants for the production of iron chelating compound, ACC deaminase, and IAA which helps in mitigating the effects of lead in the plant. In another report, siderophores producing *Pseudomonas* sp. were reported for alleviating Pb and Cd stress in *Oudemansiella radiata* plant [82]. Gaonkar and Bhosle [83] have reported *Bacillus amyloliquefaciens* for the production of iron chelating agent, siderophores which were having a capability for mitigating arsenic, lead, aluminum, and cadmium stress. Similarly, *Acinetobacter* sp. and *P. putida* having attributes of producing siderophores, IAA, and phosphorus solubilization was reported for alleviating copper stress in maize and enhanced its growth by increasing the biomass and chlorophyll content [84]. In another report, HM stress in soil is mitigated by siderophores producing microbe *Streptomyces lividans* and *Streptomyces mirabilis* [85].

In a report, siderophores producing isolate *Brevundimonas diminuta* from rice crop was reported for alleviating arsenic stress in crop rice via increasing biomass, chlorophyll, and MDA content [86]. In a report, Chen *et al* [87] reported *Enterobacter* sp. for the alleviating cadmium and iron stress in *Hibiscus cannabinus* through exhibiting PGP activity of siderophores production and IAA. In another report, siderophores producing *Klebsiella pneumoniae* were reported for the alleviation of cadmium stress in *Vigna mungo* [88]. Similarly, an investigation reported *Pseudomonas orientalis* and *Chaetomium cupreum* for the siderophores production, mitigating metal stress, and growth promotion of *Eucalyptus globulus* [89]. In another report, consortium of ACCD and IAA producing strain *Pseudomonas* and *Bacillus* was reported for alleviating the cadmium, lead, and zinc content and improving the spinach growth [90]. In another report, siderophores producing bacterium *Rhizobium* with a combination of root endophytic fungi *Piriformospora indica* was reported for alleviating cadmium stress in alfalfa plant [91].

### 4.4. Phosphorus Solubilization

Phosphorus (P) solubilization is another microbial mediated mechanism for the HM stress alleviation in the plants. Microbes have the capability to solubilize the mineral phosphate complexes such as Al-P Ca-P and Fe-P present in soil which enhances the phosphorus availability that rapidly immobilizes the HMs in soil [92]. The immobilization of the HM enhances the plant tolerance towards the HM stress through the formation of the insoluble HM phosphate complex. In literature, various microbial species have been found for the exhibiting this

| Table 1: PGP bacteria enhanced phytoremediation of heavy metals. |
|------------------|------------------|------------------|------------------|------------------|
| **PGP bacteria**  | **Associated Plant (s)** | **Heavy Metal** | **PGP attributes** | **References** |
| *Achromobacter xylosoxidans* A×10 | *B. juncea* | Cu | ACC deaminase, IAA, P solubilization | Ma *et al*. [154] |
| *Azotobacter chroococcum* | *Zea mays* | Pb | IAA, decrease soil pH | Hadi and Bano [155] |
| *Bacillus macroligenous HKK-1* | *B. juncea* | Cd | IAA, Gibberellins | Wu *et al*. [156] |
| *Bacillus pumilus E2S2* | *Sedum plumulzincicola* | Cd | IAA, ACC deaminase, siderophores | Ma *et al*. [157] |
| *Bacillus thuringiensis DGB-1* | *Ainsa firma* | As | ACC deaminase, IAA, Siderophores | Baba *et al*. [158] |
| *Burkholderia sp. 362* | *Lycopersicum esculentum* | Pb | Phosphate solubilization, IAA, ACC | Jiang *et al*. [159] |
| *Cupriavidus taiwanensis* | *M. pudica* | Pb | Biodegradation, biosorption | Chen *et al*. [160] |
| *Cupriavidus taiwanensis* | *M. pudica* | Cd | Biodegradation, biosorption | Chen *et al*. [160] |
| *Cupriavidus taiwanensis* | *M. pudica* | Cd | Biodegradation, biosorption | Chen *et al*. [160] |
| *Enterobacter sp. JYX7* | *Polygonum pubescens* | Cd | IAA, siderophores, ACC deaminase | Jing *et al*. [55] |
| *Enterobacter cloacae CAL2* | *B. napus* | As | IAA, ACC deaminase | Nie *et al*. [161] |
| *Mesorhizobium huakuii B3* | *Astragalus sinicus* | Cd | Production of metallothioneins | Sriprang *et al*. [162] |
| *Micrococcus sp. MU1* | *Helianthus annus* | Cd | IAA, ACC deaminase | Prapagdee *et al*. [163] |
| *Paeenbacillus macerans NBRFT5* | *B. juncea* | Cu | Siderophores, organic acids, protons | Tiwari *et al*. [164] |
| *Pseudomonas aeruginos* | *Orychophragmus violaceus* | Cd | Improve plant growth | Liang *et al*. [165] |
| *Pseudomonas fluorescens G10* | *B. napus* | Pb | IAA, ACC deaminase | Sheng *et al*. [166] |
| *Pseudomonas jessenii PM15* | *Ricinus communis* | Zn | Biosorption, ACC deaminas, IAA, | Rajkumar *et al*. [167] |
| *Pseudomonas putida 06909* | *Helianthus annuus* | Cd | Production of metal-binding peptide | Wu *et al*. [168] |
| *Pseudomonas putida KT2440* | *Triticum aestivum* | Cd | Production of phytochelatins | Yong *et al*. [169] |
| *Pseudomonas sp. LK9* | *Solanum nigrum* | Cd | Biosurfactants, siderophores | Chen *et al*. [170] |
| *Pseudomonas tolerui ACC23* | *B. napus* | Cd | ACC deaminase, siderophores and IAA | Dell’Amico *et al*. [153] |
| *Pseudomonas veronii* | *S. alfredi* | Zn | IAA, decrease soil pH, supply P and Fe | Long *et al*. [171] |
| *Psychrobacter sp. SRA1* | *B. juncea* | Ni | ACC deaminase, IAA, P solubilisation | Ma *et al*. [172] |
| *Rahnella sp.* | *Amaranthus* | Cd | IAA, siderophores, ACC deaminase | Yuan *et al*. [173] |
| *Rhizobium leguminosarum* | *B. juncea* | Zn | Metal chelation | Adebanjo *et al*. [174] |
| *Staphylococcus arlettae* | *B. juncea* | As | IAA, siderophores, ACC deaminase | Srivastava *et al*. [175] |

mechanism and alleviating HM stress [47,93]. In a report, microbial endophytes were sorted out from the Solanum nigrum L. which were Acinetobacter sp. Enterobacter sp., Enterobacter aerogenes and Serratia nematodiphila. These isolates were found to solubilize the phosphorus along with synthesizing siderophores, ACCD, and IAA which was efficiently mitigating cadmium stress in the plants S. nigrum and enhances its growth [94,95].

In another study, phosphorus solubilizing fungus Trichoderma harzianum were reported for mitigation of the cadmium stress in chickpea [96]. El-Deeb et al. [97], reported Enterobacter sp. an endophytic bacterium of aquatic hyperaccumulator plant, Eichhornia crassipes for alleviating cadmium and zinc metal stress. This strain was reported for solubilizing the mineral P and producing siderophores and IAA. In another report, tricalcium phosphate solubilizing, IAA, and exopolysaccharides producing P. aeruginosa was reported for the mitigating the toxicity of chromium metal stress in chickpea [98].

Fungus, Trichoderma virens from rhizospheric soil of plant growing in the mine tailing soil was reported for phosphorus solubilization and exhibiting the phytase activity. The strain was also reported for synthesizing siderophores for the sequestration of the iron and ACCD enzyme. All these activities exhibited by the microbe were reported for the bioleaching of the cadmium, arsenic, zinc, copper, and lead and enhance maize physiological and growth parameters such as chlorophyll, total soluble sugar, starch, protein, root, and shoot length [99]. In a report by Sukweeneyadi et al. [100], Paenibacillus yongiensis, phosphate solubilizer, and siderophores producer were reported for ameliorating of aluminum, drought as well as salinity stress in plant Arabidopsis thaiiana. Marzban et al. [101] have reported P. putida as efficient solubilizer and producer of phosphorus and IAA, respectively. The strain inoculation in maize crop growing in lead, copper, and cadmium contaminated soil was reported for mitigating metal stress and increased dry weight of the plant.

In a similar investigation, bioaccumulation and biosorption of the HM was reported by the phosphorus solubilizing bacterium namely, Ensifer adhaerens. The strain was also reported for secreting IAA, siderophores, HCN, and ammonia under in vitro conditions [102]. Similarly, Mitra et al. [103] have reported Enterobacter sp. for solubilizing phosphorus and also mitigating cadmium stress in rice crop growing in the cadmium stressed soil. In another report, phosphate solubilizing bacteria Achromobacter xylosoxidans was reported for reducing chromium metal stress in HM contaminated soil [104]. In a report, HM chromium immobilization was found to be done by the phosphorus and potassium solubilizing microbes B. thuringiensis, B. cereus, B. subtilis and Stenotrophomonas maltophilia [105]. Pramanik et al. [106] have reported Pseudomonas sp. from a metal contaminated rice rhizosphere for solubilizing phosphorus, nitrogen fixation and IAA production under cadmium stress.

4.5. Production of Organic Acids

HMs have the ability to produce organic acid such as citric, gluconic, oxalic and succinic acid in many PGR microbes have been reported [107,108]. It was certified that the organic acid-producing PGPMs can reduce metal-induced stress in plant and help to increasing the survivability of plant in HM polluted soil [109]. The complex form of an organic acid-like metal oxalate crystal can turn and deactivate cytology and reduce the free metal ion effect, later plants provide to toxic metals. Microbial organic acids are known to have a higher affinity for chelating HM than essential nutrients. Stimulate the production of organic acid via plants uncovered to HM that help the formation of more complexes using HM [110,111]. The acetate, citrate, malate, and oxalate are incorporated by a complex reaction (HM detoxification) to dissolve the HM of the soil’s solid phase (mineral weathering) and thus make them unavailable for stressed plant regeneration. “Conferring benefits in the sequestration of important nutrients” [112]; “modifying antioxidant enzyme reactions shown through plants” [109]; and “dissolve mineral phosphate and releasing P” [113], the resulting insoluble HM phosphorus precipitates (eg, Cd and Pb) [8].

Several microbes have been reported for the remediating HM via the production of organic acids such as Enterobacter sp. and Pantoea sp. was reported immobilizing Pb and P solubilizing [114]. In a report, tartaric acid-producing bacterium Burkholderia cepacia was reported for alleviating Cd stress in S. alfredii plant [115]. Similarly, Enterobacter asburiae PS13 was reported and producing gluconic acid and remediating Cd [109]. In a report, rhizobacteria B. cepacia, Enterobacter cancerogenes, and Microbacterium sepedae were reported for producing organic acid including oxalic acid, acetic acid, formic acid and tartaric acid which helps in the mobilization of Cd and Zn metal [116]. Gao et al. [117] reported citric acid producing fungus Purpureocillium lilacinum for detoxification of Cd and Pb. The fungal strain inoculation on the S. nigrum L. growing under HM stressed soil. Similarly, the organic acid, oxalic acid-producing endophytic bacteria Sphingomonas was reported for the detoxification of Pb and Zn. The inoculation of this strain in the S. alfredii was enhancing the plant growth parameters in HM stress [118]. Khanna et al. [119] reported Burkholderia gladioli and P. aeruginosa for remediating HM stress via producing the organic acids such as succinic acid, malic acid, fumaric acid, and citric acid. The strains were also reported for increasing the seedling growth of tomato in HM stressed plants.

4.6. Production of Bio-surfactants

Bio-surfactants are the extensive group of structurally different surface-active compounds composed of fatty acids, lipoprotein, glycolipids, mycolic acid, and phospholipid. These are amphiphilic compounds with hydrophobic and hydrophilic portions [120]. They are synthesized as metabolites by different groups of microbes in the exponential or stationary phase of the microbial growth. Microbial bio-surfactants are found to be having a countless advantages in the biodegradability of pollutants including HM [121]. Biosurfactants-mediated toxic metal bioremediation is promoted in two basic ways in solid phases. The first way is mediated by the complexation of metals free forms in a solution which is followed up by desorption based on Le Chatelier’s principle. Another way is the metal accumulation with the bio-surfactants under the reduced interfacial tension conditions which allow direct contact between the sorbed metal and bio-surfactants. HM extraction through the microbial bio-surfactants is achieved by counter binding, precipitation-dissolution, and ion exchange. In the ion exchange mechanism, bio-surfactants carrying negative charge form a stronger bond with positive charge carrying metal ions which make them non-toxic and reduces the toxicity of the metals [122]. Some scientist has investigated that HM was alleviated from soil via bio-surfactants of di-rhamnolipids, rhamnolipids, and sophorolipids secreted by B. subtilis, P. aeruginosa, and Torulopsis bombicola [123,124].

In a report, bacterium isolated from the marine environment Bacillus sp. was reported for detoxifying the effect of hexavalent Cr through the biosurfactants production and extracellular enzyme reductase [125]. Sriram et al. [126] isolated bacterium Escherichia fergusonii from oil-contaminated soil. This bacterium was reported for having a
potential to produce the lipopeptide biosurfactants which successfully remediated HM such as Cu, Mn, Pb, Fe, Ni, and Zn. In an investigation report, biosurfactant mediated HM bioremediation was reported by the yeast, namely, Candida lipolytica. The lipoprotein produced by the yeast was reported for removing 96% of Cu and Zn and also helped in the reducing the concentration of Pb, Cd, and Fe [127]. Similarly, lipopeptide biosurfactant consisting of fengycin and surfactin obtained from B. subtilis was reported for biodegrading the HM including Cd, Cu, Co, Pb, Ni, and Zn by 44.2%, 26.2%, 35.07%, 40.3%, 32.2%, and 32.07% respectively [128]. de França et al. [129] reported HM detoxifying bacterium B. subtilis for producing biosurfactant. Similarly, remediation of Cr was mediated by the biosurfactant marine bacterium B. subtilis [130]. In another report, Bacillus sp. was reported for producing biosurfactants which help in the alleviation of HM stress [131]. Bacterium Citrobacter freundii was also reported for the producing biosurfactant and bioremoval of HM [132]. In an investigation, biosurfactant mediated HM detoxification was reported by the bacterium B. subtilis. The bacterium was able to produce the lipopeptide biosurfactant [133].

4.7. Production of Exopolymers

EPS are high-molecular weight natural polymers liberated via microbes into the environment. They are made up of homo and heteropolysaccharides (such as mucopolysaccharides, proteins humic, and substances) that adhere to the bacterial cell surface. The polysaccharides composition which makes EPS varies in diverse species of microbes, but galactose, glucose, and mannose may be mentioned as monomers. Other EPS molecules are amino sugar, natural sugars, organic ester-linked substituents, pyruvate ketals, and uronic acid [134-136]. The ionic property of exopolymers is attributed to its acyl group, which increases the lipophilicity of the compound and, consequently, alters its interactions with other cations and polysaccharides [137]. Microbial EPS plays a vital role in the complexing of toxic HM and reducing their mobility and plant accessibility [138]. EPS generated by PGP rhizobacteria has been demonstrated to bind potentially harmful trace elements and trap precipitated metal oxides and sulfides, resulting in the organic metal complexes development and increased trace element resistance [139]. In this study, Xu et al. [139] was reported EPS secreted by bacterium P. putida converted the bioavailable Cd\(^{2+}\) into organic species through complexation. Cd\(^{2+}\) binding capacity of EPSs released by P. putida was shown to be primarily due to phosphate and carboxyl groups [140]. PSB could immobilize HM via absorption of metal to EPS. In another study, EPS producing Azotobacter spp. were found to inhibit Cd and Cr absorption by wheat via binding Cd (15.2 mg g\(^{-1}\)) and Cr (21.9 mg g\(^{-1}\)) of., and subsequently, immobilizing them [141]. Similarly, Wang et al. [142] reported Desulfovibrio desulfuricans for detoxification of HM by EPS. The bacterium was reported for alleviating HM such as Cu\(^{2+}\), Cu\(^{3+}\)and Zn\(^{2+}\).

4.8. Induction of Plant Production of Antioxidant Enzymes

Toxic HM could cause oxidative stress by releasing oxygen-based radicals, known as reactive oxygen species (ROS). ROS generally considered existing in this form like “oxygen-derived free radicals including alkoxyl (RO•), hydroxyl (HO•), peroxide anion (O\(^{2-}\))”, and peroxy (RO\(^{2-}\)) or oxygen-derived nonradical such as hydrogen peroxy (H\(_{2}\)O\(_{2}\)), organic hydroperoxides (ROOH), and singlet oxygen (\(\frac{1}{2}O\(_{2}\)) [143,144]. All of these species which are oxygen-based toxics are ROS; however, not all ROS species are not oxygen radicals. ROS are reactive molecules with unpaired valence shell electron, which are short-lived, unstable, and chemically reactive [145]. ROS is reactive in nature because ROS such as hydroxyl radicals, (H\(_{2}\)O\(_{2}\)), superoxide radicals (O\(^{2-}\)) and peroxides (O\(_{2}\)), radicals, and singlet oxygen quickly attack all types of biomolecules such as lipids, amino acids, nucleic acids, and proteins, causing irreversible metabolic dysfunction and cell death [146]. HMs toxicity, such as other abiotic stressors, causes plants to produce ROS [147]. To defend against oxidative stress, plant cells produce antioxidant enzymes including ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, peroxidase, and superoxide dismutase and nonenzymatic antioxidants such as ascorphol, assacorbate, glutathione, and proline [148]. According to certain research, PGP microbes cause plants to produce antioxidant enzyme in response to stress exerted by abiotic factors. In this study, P. aeruginosa were reported under Zn stress an uptake of N and P and increases in total soluble protein, leaf chlorophyll, and biomass of wheat [149]. In another study, Afridi et al. [150] were reported microbes Kocuria rhizophila and Cronobacter sakazakii, carry SOD and CAT higher activity under salt stress condition in wheat.

4.9. Induction of HM Resistance Genes

HM resistance genes in microbes can respond to the encouragement of HM. The co-occurrence of HMs resistance gene in long-term HM contamination area is still poorly assumed. Some of the genes have been identified that gene involved in induced systematic tolerance (IST) and stimulating IST in plant host. It has been known that few microbes have the capability to trigger IST in plant host, analogous to those for triggering ISR in plants. Microbes are involved in the ISR induction produces some metabolites such as antibiotics, biosurfactants, siderophores, and lipopolysaccharides from bacterial outer membranes, and volatile organic compounds. These metabolites have led to the stimulation of resistance against their pathogens in many plants. Systemic defense responses emanating from bacteria are controlled via signal network.

These metabolites result in the resistance initiation in several plants against their plant pathogens. Systemic defense responses from microbes are controlled via a signal network. In this network, phytohormones including ethylene, JA, and salicylic acid play a significant role [150,151]. Some of the phenomena have linked with IST. Plant-associated microbe is affecting the appearance of few genes coding for proteins associated with HM tolerance. In an investigation by Ouziad et al. [152], Glomus intraradices increased the tomato growth cultivated under Zn stress condition. However, the expression of LeNramPl (for HM transporter) and Lem2 (coding for MTs) genes was reduced in the inoculated plant. This indicates that G. intraradices reduced the HM concentration in plant. A large group of plants has been reported for their potential to deposit substantial level of HM. However, a lot of plants associated with phytoremediation (hyper accumulating plants) do not produce enough biomass to make this method effective. As a result, using PGPMs to facilitate phytoremediation is more suitable. A wide variety of PGPMs has been molecularly identified that successfully aid in phytoremediation of HM. As an example, Jing et al. [55] was isolated metal resistance PGPMs from P. pubescens grown in metal contaminated soil. These isolated strains were recognized as Enterobacter sp. and Klebsiella sp. that were inoculated into B. napus (Canola/Rapeseed) for HM accumulation and improve growth of plant and the accumulation of Cd and Zn. The same plant was also reported by Dell’Amico et al. [153] produced Pseudomonas tolaasii, P. Fluorescens, and Mycobacterium sp. and uptake Cd successfully.
5. CONCLUSION
The pollutant, HMs is one big concern of every environmentalist due to its harmful effect on whole global system. Anthropogenic activities such as industrialization, mining, and afforestation are the major cause of this environmental contaminant which is very hard to stop. As a result, remediation is one technique that could be used for mitigating this contaminant. Remediation through biological methods is a more effective method of mitigating HM in comparison to physical and chemical methods and biological methods phyto-microbial system is more preferred. Bioremediation through microbes’ weather bacteria or fungi has reported as an effective approach to eliminate the HM or lowering the toxicity. According to the literature, microbes used a specialized mechanism of alleviating the HM that also helps in enhancing the plant growth promotion. Microbes enhance the plant growth by various mechanisms such as availing the nutrients by solubilizing, or chelating. Solubilization of phosphorus, chelation of iron through siderophores, production of organic acids, exopolysaccharides are some of the mechanism which helps in the enhancement of the plant growth. The use of microbes for the alleviation of HMs and enhancement of plant is an effective method and more effective microbial strain should be explored in future research.

6. AUTHORS’ CONTRIBUTIONS
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

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