

MITIGATION OF HEAVY METAL POLLUTION-AN OVERVIEW OF MICROBIAL REMEDIATION AND PROSPECT IN BANGLADESH

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In the era of science and modern technology, environmental pollution is still the threat to survival of mankind. With the increasing urbanization process, anthropogenic sources of toxic heavy metals like mercury, lead, arsenic, chromium are also rising rapidly. Inadequate measures have been taken to mitigate the detrimental effects of such pollution. Among many mitigation process like physical, chemical and biological methods; microbial remediation is eco-friendly and cost-effective process and converts the toxic substances to the least toxic forms. By the blessing of modern science, various advanced molecular techniques (i.e. genetically modified organisms) can also be utilized. This review article summarizes the mitigation techniques of toxic heavy metals with emphasis to microbial remediation. However, more research is required to make them more expedient for mass use. Above all the remedies, cognition and circumspection are the exigent demands.

Keywords: Toxicity, Heavy metal, Microbial remediation

INTRODUCTION

Environment pollution is rapidly increasing due to the advancement of technologies, unplanned urbanization, unsafe agricultural practices and rapid industrialization. Metals are essential elements for the biological functions of plants and animals within a limited concentration unless they interfere with metabolic systems of organisms (1). Lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), zinc (Zn), uranium (Ur), selenium (Se), silver (Ag), gold (Au), nickel (Ni) and arsenic (As) are commonly known as heavy metal can cause toxicity at higher concentration. These metals can accumulate in the body through the food chain and pose risks to the health of living organisms which may even lead to cancer. Recently it was estimated that Hg, Pb, Cr, and Cd from different sources has posed a serious threat to 66 million people and water contamination by As has alone affected >150 million people globally (2, 3, 4). Not only the human life, these heavy metal pollutions can also affect plants by reducing plant growth, photosynthetic activities, plant mineral nutrition, and activities of essential enzymes (4, 5). Heavy metals produce reactive oxygen species (ROS) using free radical mechanism, cause oxidative stress as well as break DNA molecules (6, 7, 8). Some protective components for cells like Glutathione, superoxide dismutase etc. antioxidants are also got decreased (1).

Source and Effects of Heavy Metals on Environment

The high density and detrimental toxic effects of heavy metals are the matter of great concern among all other pollutants. They can persist and cause long term effect on biological system by their non-biodegradable nature. the major sources of heavy metals (9, 10) are presented in Figure 1.



Figure 1: Sources of heavy metal pollution in the environment.

Natural sources: Various natural disasters like volcanic activity, erosion, forest fires contribute in the increasing quantity of heavy metals. Weathering of minerals, sea-salt sprays, aerosol particulates, and biogenic sources (9, 10) also increase the heavy metal concentrations (Hg, As). Manmade activities are indirectly influence these sources as we disrupt the natural environment.

Agricultural source: Fertilizer, pesticide, fungicide uses are necessary to improve the cultivation quality. Uncontrolled use of these chemicals' harms the

environment by increasing heavy metal concentrations (As, Cd, Pb) (10, 11). Their wash-off mismanagement also affects the environment.

Industrial sources: In the era of industrialization, environment is under threat due to the improper management of industrial run-off and untreated waste which contains various pollutants like heavy metals (9, 11, 12). Thermal power plant, leather tanning, coal and mining industries, refineries discharge, battery and electroplating industries (Cd, Hg, Cr, Pb) pollute the environment.

Miscellaneous: Various daily activities of human life can cause the environmental deterioration. Traffic emission, smoking, biomass burning, cooking smoke, e-waste increases the heavy metal (Cd, Cr, Hg) concentrations in environment (11, 12).

Effects of heavy metals: Bioaccumulation speed the entrance of heavy metals in food chain easily. Their maximum permissible limit (13,14) and their effects (9, 11) are shown on Table 1.

Table 1: Harmful effects of heavy metals and their permissible limit in the environment.

Heavy metals	Permissible limit (in mg/L)	Harmful effects
Arsenic (As)	0.05	Carcinogenic, damages cardiovascular, renal, endocrine, reproductive system
Chromium (Cr)	0.05	Bronchitis, cancerous
Cadmium (Cd)	0.005	Renal cancer, lung disorder, breast cancer, prostate cancer
Mercury (Hg)	0.002	Carcinogenic, effects fertility and immune system, Minamata disease, sclerosis, colorectal cancer
Lead (Pb)	0.3	Alzheimer's disease, nervous system failure, hematological effect, intestinal cancer

Heavy metals are the reason behind many life-taking diseases in recent era. From Alzheimer's disease, atherosclerosis, Parkinson's disease to lethal cancer (15); heavy metals are causing many life-threatening diseases and also damages internal organs of the body such as the kidney, liver and cardiac tissues (16). The worst side of these effects are, they kill slowly and silently. The duration of exposure to these heavy metals determine the degree of toxicity. Not only the human life, plants are not safe from their exposure. Inhibiting cytoplasmic enzyme, various physiological activities of plants like the processes of respiration, photosynthesis, electron transport chain and cell division are interrupted due to oxidative stress (17, 18). It is very important to eradicate the heavy metals from the ecosystem due to their adverse effects.

Nervous system: Cognitive impairment of central nervous system including neurodevelopmental changes, neurodegenerative diseases and changes in synaptic transmission and neurotransmitter balance

occur upon arsenic pollution (19). Cadmium causes neurodegenerative defects, including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and multiple sclerosis along with peripheral neuropathy, olfactory dysfunctions, neurological disturbances, learning disabilities, and mental retardation, also the impairment of motor function and behavioral changes in both adults and children (20). The neurotoxicity of cadmium arises from neural cell death via apoptosis; providing plenty of apoptosis-induction factors, including impairment of neurogenesis, inhibition of neuron gene expression, offering epigenetic effect, endocrine disruption, etc. (21).

Renal system: Lead has the greatest influence on the kidneys. Acute lead nephropathy causes proximal tubular dysfunction, resulting in Fanconi like syndrome, also hyperplasia, interstitial fibrosis, atrophy of the tubules, renal failure, and glomerulonephritis (20).

Hepatic system: Chronic lead exposure causes the oxidative stress on liver cells, glycogen depletion, resulting in cirrhosis (22). Cadmium accumulates in the liver and causes hepatocellular damage (20).

Reproductive system: Several studies shown that risk of infertility increases due to the exposure of arsenic, lead, mercury (19, 20). This infertility condition prevails mostly in women than men nowadays. Heavy metal exposure disturbs ovulation cycle, disrupts reproductive hormones.

Cardiovascular system: Mercury, cadmium, lead are great threat for cardiovascular system. Their chronic exposure cause hypertension, thrombosis, cardiac diseases like heart failure (21, 22, 23).

Carcinogenesis: Heavy metals disrupt transcription by shifting zinc from certain regulatory proteins, causes damage to the DNA repair mechanism, cellular tumor regulating genes, and chromosomal structure and sequence by releasing ROS (22). Mercury's peroxidative activity generates a significant quantity of reactive oxygen species (ROS), which can contribute to carcinogenesis by damaging cellular proteins, lipids, and DNA, resulting in cell damage (23). Several toxic effects are summarized in the Figure 2 below.

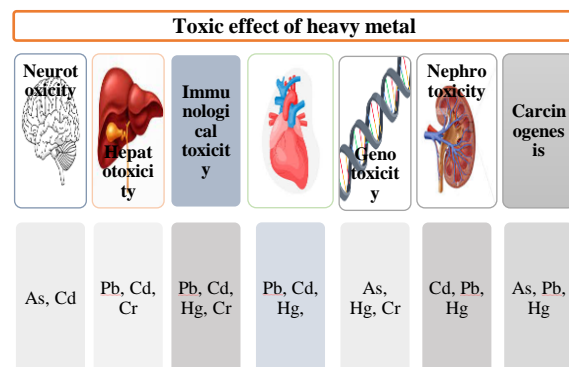


Figure 2. Effect of heavy metal on various systems.

Methods for Detection of Heavy Metals

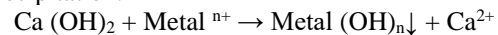
Atomic absorption spectroscopy (AAS) (24), inductively coupled plasma-mass spectroscopy (ICP-MS) (25), and atomic fluorescence spectrometry (AFS) (26) are used for the determination of heavy metal concentration. All of them obey the basic principle of spectroscopy; excitation of electron of the sample by a beam of light and then detect the concentration in a detector screen. AAS and AFS can detect up to the metal concentration of microgram level ($0.1-1 \mu\text{g l}^{-1}$), whereas the ICP-MS can detect up to picogram level (0.03-11 pg). The development of sensors to detect the traces of heavy metals in various environmental sources offers a systematic procedure for environmental monitoring (4, 27). A portable biosensor technology utilized engineered *E. coli* which selectively produced 4-amino phenol upon selective metal interaction exhibiting a detection potential of 1.5 ppb (LOD), 5 ppb (LOQ), and 0.122 $\mu\text{A/ppb}$ (sensitivity) for arsenic and 0.1 ppb (LOD), 0.34 ppb (LOQ) and 2.11 $\mu\text{A/ppb}$ (sensitivity) for mercury (28). Bio-char, bio-surfactant, and bio-catalytic removal (27-37) techniques are recent advancement. Xenobiotic pollutants including heavy metals can be solubilized by secondary metabolite of microorganism; bio-surfactants are secondary metabolites produced by microorganisms (32-35). Despite of the current advancement in modern technologies, Bangladesh having increasing rate of pollution are not utilizing them widely. Many articles have been published on the recent scenario of toxicity level, i.e. Cr found in river water was 86.93 mg/L whereas the permissible limit is 0.05 mg/L (36, 37). In this review we aim to draw the attention of respective authority to take initiative to utilize the eco-friendly microbial remediation.

Different Approaches for Heavy Metal Remediation

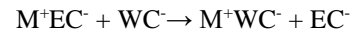
Heavy metals are deteriorating our environment via different natural and anthropogenic reasons. They are being accumulated into our food chain. From the primary consumers to quaternary consumers, bio magnification gets continued. Several physical, chemical and biological methods can be utilized to remove the pollutants (38-41). Ultrafiltration, coagulation, flocculation, adsorption, membrane filtration, and ion exchange are commonly used physical methods for heavy metal remediation. Chemical methods include neutralization, solvent extraction, chemical precipitation, and electrochemical treatment.

Precipitation methods: Coagulation and flocculation methods use chemical substance (aluminum, ferrous sulfate, ferric chloride as coagulant and polyaluminium chloride (PAC), polyacrylamide (PAM) as flocculants) to destabilize the solid waste particles. They are considered as most effective and widely used in industries (38). Lime and limestone precipitates heavy metal ions. Sulfide, carbonate

precipitations are also used with hydroxide precipitation.



Ion exchange method: Synthetic organic ion exchange resins, inorganic zeolite are commonly used matrices for ion exchange. They exchange heavy metal ions from their surroundings. They utilize the mechanism as followed, where EC^- is the exchange anion, M^+ is fixed cation, WC^- is the waste water anion.



Adsorption: Carbon based nano-adsorbent (carbon nanotubes, graphenes), Cellulose based adsorbent (Lignin, chitosan) are used as adsorbent for heavy metal ions (38). Adsorption is a mass transfer process in which a substance is transferred to different phase by physical and chemical interactions. Heavy metals get adsorbed on the surface and become separated. Various mineral adsorbents like zeolite, silica, clay are also used. The adsorption efficiency gets decreased after a certain period of separation. Magnetic nanoparticles (Fe_3O_4) act as good adsorbents using magnetic field-based method (38).

Membrane filtration: Silicon carbide made polymeric ceramic membrane can separate heavy metal ions depending on their size. Nano filtration technique can extract cadmium, copper like ions (38).

Electro-chemical method: Applying the basic concept of electrochemistry; electroflotation, electrocoagulation methods can separate heavy metal ions (38). Electric charge potential separates the ions. Carbon-based or sulfur mixture made cathode with different ratios in acidic conditions can remove mercury, cadmium, lead ions from wastewater (38).

Biological methods: The chemical mitigation of these heavy metals produce harmful by products and also, they are not convenient to apply in wide range. Source controlling has also been troublesome procedure due to lack of concern. Therefore, biological techniques employing the use of plants and microorganisms are being preferred owing to their environmentally friendly and economical approaches (2,3). Various bacterial and fungal species such as *Pseudomonas aeruginosa*, *Paenibacillus jamilae*, *Bacillus subtilis*, *Aspergillus* sp., *Botrytis* sp., *Neurospora* sp., *Saprolegnia* sp., *Penicillium* sp., and *Trichoderma* sp. have been reported to actively metabolize and reduce different heavy metals (42-45). Plants remove heavy metals by different processes such as *Typhalatifolia*, *Brassica juncea*, and *Characanesens* (phytovolatilization) (45, 46) *Morus alba* and *Populus alba* (phytoaccumulation/phytoextraction) (7) *Helianthus annuus* and *Phaseolus vulgaris* (rhizofiltration) (7, 45). Various in-situ and ex-situ physiochemical and biological treatments can be used for the mitigation

of heavy metals.

Microbial Remediation:

The commonly used remediation process for heavy metal degradation includes methods such as; coagulation, chemical precipitation, electro dialysis, evaporative recovery, floatation, flocculation, ion exchange, nano-filtration, reverse osmosis, ultrafiltration, as well as physiochemical methods such as extraction, stabilization, immobilization, soil washing, etc. These methods, even if effective, are generally expensive as a result of high energy and chemical reagent requirements, apart from production of secondary noxious end-products (1). The eco-friendly bioremediation techniques involve the utilization of microbes- bacteria, algae, fungi and yeast which is known as the microbial remediation (47). Various factors e.g. physical, chemical, biological, soil-type, nutrient source, presence of other types of microorganisms can affect the process of microbial remediation (48, 49). Microbial remediation utilizes the transformation of toxic heavy metals, especially As, Cr, Hg and Se in soils and sediments into less toxic or innocuous forms by redox reaction mechanism (50, 51). The mechanisms of microbial degradation of heavy metals are mainly bioaccumulation, bioaugmentation, bioprecipitation, bio-simulation, biotransformation, bioleaching, bioventing and biosorption. The degradation mechanism which depends on the special structure of the cell wall (52-55). *Escherichia coli* K-12 adsorb the majority of heavy metal ions, and the adsorption capacity of *Pseudomonas* and *Bacillus* are strong though their degradation capacity depends on their structural factors (52). Several microbial remediation processes are shown schematically in Figure 3. Bio char- the product of pyrolysis of biomass obtained from sources such as crop residue, manure and solid wastes which can be used to stimulate microorganisms by changing the pH, decreasing the solubility of heavy metals and increasing microbial biomass and available nutrients (56). It has the ability to donate, accept or transfer electrons within their environments abiotically or through biological pathways (55-61).

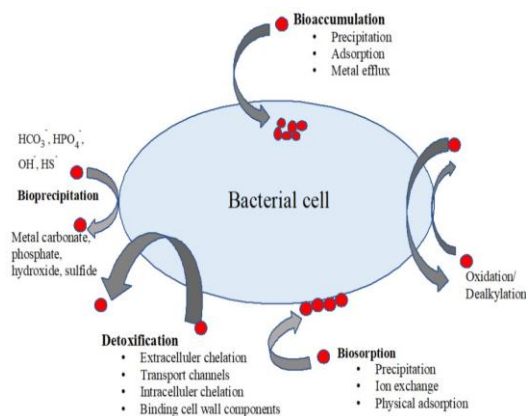


Figure 3. Microbial remediation process of heavy metal

Bioaccumulation: Bacterial cell can accumulate the heavy metals inside their cell. It can act as an exposure indicator since the heavy metals are not metabolized (55). Microorganisms recycle heavy metals by synthesizing proteins. They can be used further in cellular processes such as enzyme catalysis, signaling, and stabilization of charges in biomolecules (53). Bioaccumulation occurs via the similar pathways of nutrient intake. Further biomagnification occurs when they enter the food chain and the concentration gets increased (55).

Biosorption: Biological adsorbents facilitate the binding of heavy metals to non-living biomass of microorganisms. Functional groups of cell wall play the vital role in sorption components. Various bacterial strains like *Paenibacillus jamilae*, *Bacillus firmus*, *Bacillus licheniformis*, *Herbaspirillum* sp., and *Paenibacillus peoriae* secrete Exopolysaccharides (EPSs), composed of carbohydrates and proteins, which jointly facilitate the biosorption of Zn, Cd, Hg and Pb ions (53). The cell structure of microorganisms, extracellular precipitation, intracellular accumulation influences the biosorption process.

Bio-oxidation: Sulfur-oxidizing and iron-oxidizing bacteria can remove heavy metals by bioleaching which is an oxidation process (53). It occurs through the direct metabolic activity of leaching bacteria or indirectly through the by-products of bacterial metabolism. Heavy metals get bound to the bacterial cell and then oxidized to their soluble metal forms.

Bioprecipitation: Various solid minerals (carbonates, phosphates, silicates, and sulphates) are formed from heavy metal ions that are subsequently precipitated by microorganisms (53, 55). It is also termed as biomineralization. It is governed by the pH, and temperature. Photosynthetic microorganisms and sulfate-reducing bacteria, which contribute significantly to mineral precipitation through autotrophic and heterotrophic pathways (53).

Biotransformation: Biotransformation involves the conversion of toxic metal (like chromium, arsenic) forms into less toxic forms. It involves oxidation-reduction or mineralization-demineralization reactions (53). Fungi can form coordination compounds by generating oxalate which convert soluble metals into insoluble metal oxalates. They accumulate the transformed metals in their hyphae, so the toxic metals cannot enter into the roots of plants.

Utilization of natural microorganisms in the environment for *in situ* microbial remediation is affected by the non-availability of suitable nutrient levels (62, 63) where ex-situ microbial remediation involves taking the contaminated media from its original site to a different location for treatment based on the extent of pollution, geographical locality and geology of the contaminated site (63).

Bacillus sp., *Flavobacterium*, *Mycobacterium* sp., *Nitrosomonas* sp., *Pseudomonas* sp., *Penicillium* sp., and *Xanthobacter* sp. are commonly used microorganisms for bioremediation procedure and *Bacillus licheniformis*, *Bacillus firmus*, *Bacillus coagulans*, *Bacillus megaterium*, *Enterobacter* sp. *II*, *Bacillus licheniformis*, *Bacillus licheniformis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhi*, *Bacillus cereus*, *Desulfovibrio desulfuricans*, *Enterobacter cloacae*, *Kocuriarhizophila*, *Micrococcus luteus*, *Lactobacillus* sp., *Pantoea agglomerans*, *Alcaligenes* sp., *Ochrobactrum intermedium*, *Cupriavidus metallidurans* are considered as most effective for heavy metal bioremediation (54).

Microorganism remediates heavy metals by active and passive methods. Due to the presence of phosphate, hydroxyl, carboxylate, amino groups in the biomolecules (lipid, carbohydrate, proteins) of microorganisms, heavy metal ions can be adsorbed in the biological cells. Ion exchange between heavy metals and teichoic acid and peptidoglycan of cell wall also occur. Gram positive bacteria thus can adsorb more heavy metals than the gram negative (54). By intracellular and extracellular sequestration process microorganisms can absorb complex metal ion inside the cell (intracellular) and accumulate insoluble metal complex present in the periplasm (extracellular). *Pseudomonas putida* sequesters cadmium and zinc by intracellular process forming metal binding site on the cell surface and then transport them into the cytoplasmic membrane. Copper ions are sequestered by *Pseudomonas syringae* (in periplasm), zinc ions by *Synechocystis* PCC 6803 (periplasm), *Geobacter* sp. and *Desulfuromonas* spp. manganese and chromium ions by *G. metallireducens*, chromium ions by *G. sulfurreducens*, lead ions by *Vibrio harveyi* (54).

Fungi can also mitigate heavy metals facilitated by their hyphae made of carbohydrates, amino acid, triglycerides, phosphate etc. Remediation, decomposition, or conversion of toxic elements in the ecosystem which is termed as Mycoremediation. The fungal cell wall is rich in mannuronic and guluronic acids in large quantities facilitating heavy metals sequestration (54). *Penicillium* spp., *Trametes versicolor*, *Cladosporium resinae*, *Aspergillus niger*, *Funaliatrogii* sp., *Rhizopus arrhizus*, *Aureobasidium pullulans*, *Ganoderma lucidum* sp., *Aspergillus versicolor*, *Aspergillus fumigatus* etc. mitigate heavy metals (54).

Cyanoremediation is another microbial remediation process where algal species are involved. Their polysaccharide made cell wall structure binds the heavy metals (54). Green and blue algae act as a good heavy metal remediate. Genetically modified blue algae *Synechocystis* sp. PCC6803 bioaccumulate arsenic (54). *Oscillatoria* sp., *Synechococcus* sp., *Calothrix* sp., *Nostoc* sp., and *Anabaena* sp. are used for cyanoremediation of heavy metals. Few microbial

organisms are listed in Table 2 (65-80).

Table 2: Microorganisms used in heavy metal remediation.

Microorganisms		Heavy metals removed
Bacteria	<i>Escherichia coli</i>	Hg, Cd, Pb
	<i>Staphylococcus aureus</i>	Cr
	<i>Bacillus cereus</i>	Cr
	<i>Bacillus subtilis</i>	Cr
	<i>Pseudomonas veronii</i>	Cd, Zn, Cu
Algae	<i>Spirulina</i> spp.	Cr, Cu, Mn, Zn
	<i>Hydrodictyon</i>	As
	<i>Oedogonium</i>	As
Fungi	<i>Aspergillus niger</i>	Zn, Cu, Cr, Pb, Th, Co, Mn
	<i>Aspergillus fumigatus</i>	Pb
	<i>Gloeophyllum sepiarium</i>	Cr
	<i>Rhizopus arrhizus</i>	Cd, Th, Cu, Zn
	<i>Aspergillus versicolor</i>	Ni, Cu
Yeast	<i>Sacharomyces cerevisiae</i>	Pb, Cd, Cu

Bacterial metabolites and transporters can detoxify heavy metals. Inside the bacterial cells they are detoxified by the sequestration method (79, 80). A significant reduction on the concentration of Cd and Pb were found from heavy metal tolerant isolated bacteria (82). The presence of diverse metal binding functional groups in cell wall elevates the fungal efficiency to mitigate the heavy metal. In another study it was found that four different fungi remove the heavy metals effectively, 10–20% of Cd (100 mg/L) and Hg (50 mg/L), 34–62.74% of As (10 mg/L) whereas more than 99% removal was recorded for Pb (50 mg/L) (83). Furthermore, the treatment of *Pinus massoniana* tree with ectomycorrhizal fungi significantly contributed to the survival of the plant while reducing the translocation of heavy metals in rhizosphere (84,85). Alternatively, different factors like, type and abundance of ectomycorrhizal fungi, heavy metal type, and plant adaptation to fungi exhibited different effects on the transport and absorption of heavy metals (85-87).

Factors Affecting Microbial Remediation

Temperature is a vital factor in microbial remediation. Microbes can sustain in an optimum temperature only. Their growth, metabolism, survival is pH and temperature dependent mainly. Elevated temperature interrupts the protein synthesis, whereas lower temperature also disturbs the growth and intercellular transportation processes. Optimum temperature (20-40°C for mesophilic and 55-80°C for thermophilic bacteria) maintenance is very important for microbial remediation (54).

Bacillus sp., *Acinetobacter junii*, *Cellulosimicrobium funkei*, *Escherichia coli*, *Micrococcus luteus*, *Pannonibacter phragmitetus*, *Pleurotus platypus*, *Lentinula edodes*, *Pseudochrobacter umsaccharolyticum*, *Vigribacillus* sp. *Pseudomonas aeruginosa* and *Trichoderma* sp. are reported for maximum bioremediation in optimum pH (5.5-8.5) (54). Any change in pH will disturbs the enzyme regulation, formation of metal complexes, solubility of metal ions.

Nutrients uptake by microorganism (nitrogen, phosphorous, potassium), characteristics of pollutants (liquid/semi-solid/solid/gaseous, inorganic/organic, level of toxicity etc.) available oxygen content (aerobic/anaerobic), climatic factors, light (for photosynthetic organisms), enzymes influence the bioremediation process.

Recent Advancement in Microbial Remediation and Future Prospects

Microbial remediation aims at comprising of the data of chemical structure and composition, RNA/protein expression, organic compounds, catalytic enzymes, microbial degradation pathways, and comparative genomics to interpret the underlying degradation mechanism carried out by a particular organism for a specific pollutant (88). Computers are used to store, manipulate, and retrieve information linked to the DNA, RNA, and proteins of the genome (88, 89). A variety of bioinformatics tools are used to interpret all of these sources in order to study microbial remediation to develop more effective environmental safeguard. Bioinformatics map up the metabolic pathways of pollutant mitigation-efficient microbes (89). The study of pollutant-degrading protein structure using polyacrylamide gel electrophoresis, microarrays, and mass spectrometry in proteomics are widely been using (88). Microbial remediation Tools Based on Omics- genomics, transcriptomics, metabolomics, and proteomics; can correlate DNA sequences with the abundance of metabolites, proteins, and mRNA (90, 91). Genomic tools such as PCR, analysis of isotope distribution, DNA hybridization, molecular connectivity, metabolic foot-printing, and metabolic engineering are used to study the genetic information for better understanding of biodegradation process. A PCR-based quantitative analysis of soil microbial communities can be used to determine the gene markers in the soil. Some more recent advanced tools like engineered polymeric nanoparticles (92, 93), gene editing (94) and synthetic biology (95) can also be utilized for the pollutant mitigation process. Genetically-engineered plants can be able to bio remediate specific pollutants (96). *R. eutropha* CH34, *Alcaligenes eutrophus* AE104 (pEBZ141), *Rhodospseudomonas palustris*, *M. huakuii* subsp. *Rengei* strain B3, *Astragalus sinicus* are a few genetically modified microbial strains used for Cr, Hg, Cd (54) heavy metal removal. Biosurfactant (secondary metabolite and one kind of

biological micelle) act efficiently to eliminate heavy metals. Studies show that rhamnolipid produced by *Bacillus* sp. strains, eliminated around 65% and 18% of copper and zinc respectively and *Bacillus* sp. MTCC 5514 released biosurfactant reduced 2000 mg/l Cr⁶⁺ to Cr³⁺(54).

There are several technical and financial issues which have made the mitigation of heavy metals a challenging task. To overcome the drawbacks of traditional approaches used for the heavy metals removal microbial remediation will be a great prospect. The development of biosensors and advance detection methods has made it possible to monitor and quantify the level of heavy metals in biotic and abiotic environments with better efficiency and reliability. The recent advancements with the use of waste derived bio-char for the remediation of heavy metal polluted environments have opened new avenues toward sustainable approach in heavy metal removal (9). Furthermore, employing transcriptomic approach can enhance the microbial remediation effectiveness and site implementation. The introduction of genes technology can further increase the bioremediation potential (9). The knowledge of the genome is crucial using omics approaches to develop engineered microorganisms with enhance synthesis of specific enzymes needed for mitigation of pollutants (98). Genomics, transcriptomic, proteomics, and metabolomics can be utilized together to detoxify the heavy metals.

Heavy metal pollution in Bangladesh

In the era of globalization, Bangladesh is keeping pace with the global race with increasing industrialization. Along with urbanization, infrastructural improvement; we are losing the natural balance of our environment. Our breathing air, food, water is being contaminated badly due to our lack of concern. Studies shown in Table 3, portray the pollution level in various sources (99-104).

Table 3: Studies on current scenario of heavy metal concentration in Bangladesh.

Experiment al sample	Heavy metal studied	Salient findings	Reference
Roadside dust	Cr, As, Pb	Cr, Pb were found in higher concentration in winter season than the summer.	Kabir <i>et al.</i> (99)
Fruits and vegetables	As, Cd, Pb,Cr	Pb and Cd were higher in concentration. Carcinogenic health risk for As and Pb were in Threshold level.	Shaheen <i>et al.</i> (100)
Fish	Cd, Cr, Pb, As	<i>O.mossambicus</i> showed higher content of As though other metal concentrations were found in no risk level.	Ahmed <i>et al.</i> (101)

Food stuff grown in soil (cereal, pulses, vegetables, food)	As, Cd, Pb, Cr	Carcinogenic health risk was found for As. Significant anthropogenic contributions were found for Cr, as, Pb.	Kormoker <i>et al.</i> (102)
Ground water	Pb, Cr, As	Pb and As were in higher content than WHO guideline value 100 and 6.4% respectively.	Sharmin <i>et al.</i> (103)

Heavy metals have been detected in a concerning amount in various food products like cereal, vegetables, fruits, fish (104). The trophic transfer causes the entrance of heavy metals to the food web from primary source. This indicates inhaling the contaminated air, we are also up taking the heavy metals in our body through our regular dietary. Most Commonly consumed fish by mass people in Bangladesh are *Labeo rohita*, *Oreochromis mossambicus* and *Pangasius pangasius*. Among them tilapia (*Oreochromis mossambicus*) contained a higher concentration of arsenic (101). It indicates the potential risk for carcinogenic and chronic toxicity due to the continuous consumption. Moreover, food stuff grown in soil (cereal, pulses, vegetables, food) were found to be contaminated above tolerance level and showed a carcinogenic risk for children and infants (102).

Surprisingly lead is found in higher concentration in ground water than arsenic (103); which was considered as the most common fatal pollutant for drinking water in Bangladesh. Researchers assumed the adult and children of central-western areas of Dhaka are in threat of carcinogenic and non-carcinogenic health risk. Similar study by other researchers also showed the 95% of drinking water in Jamalpur district in Bangladesh are not suitable according to WHO guideline value of chromium and cadmium (105). Several studies also found a transport of heavy metals from soil to plants (106). Thus, the farming lands near industrial zone and their grown foods are also get contaminated. Therefore, it is badly needed to be concerned more not only about the quantitative measurement of heavy metals but also their transport pathways and adapt a suitable remediation process. Overall, the areas which are mostly affected with heavy metals like arsenic, cadmium, chromium, arsenic (104) are indicating below in Figure 4.

Microbial remediation on Bangladesh perspective

The adequate knowledge about heavy metal pollution in a densely polluted country like Bangladesh is very uncommon where the mass population are not properly educated. Lack of proper management along with the poor economic condition, heavy metal detoxification processes are not widely used. Following figure shows schematic presentation on research fields on heavy metals (Figure 4) (106).

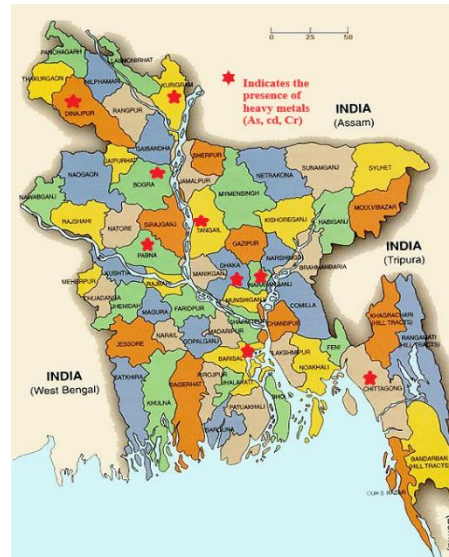


Figure 4: Heavy metal polluted areas in Bangladesh

Typical focused area	Less discussed area	Governing body
<ul style="list-style-type: none"> Heavy metal in environment (water, air, solid waste) Risk assessment 	<ul style="list-style-type: none"> Bioremediation Phytoremediation Waste water treatment Tropic transfer of heavy metal Marine food toxicity Policy and regulation 	<ul style="list-style-type: none"> Government authorities like Ministry of Food, Bangladesh Standard and Testing Institute-BSTI, Bangladesh Food Safety Authority-BFSA, and non-government authorities

Figure 5: Gross presentation of heavy metal research aspects in Bangladesh.

Despite of so many limitations, many researches are conducting on the detection of heavy metal concentration and their possible microbial remediation (106-108). *Lysinibacillus sphaericus* was found as arsenic resistant microbe from the contaminated soil in Bangladesh (109, 110). Another work has been conducted on proliferation and propagation of drug-resistant bacteria of clinical importance from the pharmaceutical waste treatment strategy (111). In a previous study researchers reported chromium resistant bacteria (*Staphylococcus aureus* and *Pediococcus pentosaceus*) isolated from environmental samples. They collected the industrial discharge of tannery industries which are mixed with the nearest river and canals causing chromium contamination. Their study found the bacteria were capable of reducing Cr (VI) to Cr (III) (108). In another study, the *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 were found to possess high potential for heavy metal microbial remediation in river water (107). Improper management of industrial discharge, poor enforcement of standard guidelines and regulations, unavailability of research data concerning sustainable

management, and limited public awareness were marked as the threat to food chain (106). In serial enrichment cultures of drinking wells of Bangladesh researchers showed transfer-persistent arsenite oxidation activity under four conditions (aerobic/anaerobic; heterotrophic/autotrophic) (112). The enriched microbiomes contained genes highly similar to the arsenite oxidase (*aiiA*) gene of chemolithoautotrophic (e.g., *Paracoccus* sp.) and heterotrophic arsenite-oxidizing strains along with 16S rRNA gene sequences (*Hydrogenophaga*, *Acinetobacter*, *Dechloromonas*, *Comamonas*, and *Rhizobium/Agrobacterium* species). Most of the researchers suggest widespread application of microbes in pollution management. Microbial population, suitable environment for microbial growth conditions, nutrient supply affect the remediation process. The microbial remediation techniques might sound complex but it the most environmentally friendly and sustainable approach which needs to be explored for human welfare.

CONCLUSION

The complete elimination of contaminants via chemical and physical methods of remediation is not cost-effective and eco-sustainable. Contrary to these techniques, microbial remediation is the suggested solution to remove various persistent contaminants by relying on eco-friendly biological processes. The microbial remediation process has a number of advantages like it converts the toxic substance to its less toxic form, less expensive, hazardous substances can be handled safely etc. Undeniable fact regarding this remediation that it is very specific for biodegradable pollutants and often it is very time-consuming. Proper genetic modification might be helped to mitigate the pollutants as per requirement. Advanced molecular techniques can also speed up this process. Above all, prevention is better than cure and public awareness regarding pollution, appropriate treatment of waste and waste water before those are released in the environment can lower most of the pollution and support healthy living.

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