SciMedCentral

JSM Biotechnology & Biomedical Engineering

Review Article

# Recent Advancements and Challenges in Microbial Bioremediation of Heavy Metals Contamination

**Banik S[1], Das KC[2], Islam MS[3] and Salimullah M[4]***

[1]*Banik S, Environmental Biotechnology Division, National Institute of Biotechnology, Bangladesh*

[2]*Keshob Chandra Das, Molecular Biotechnology Division, National Institute of Biotechnology, Bangladesh*

[3]*Saidul Islam, National Institute of Biotechnology, Bangladesh*

[4]*Salimullah, Molecular Biotechnology Division, National Institute of Biotechnology, Bangladesh*

***Corresponding author**

Salimullah, Chief Scientific Officer, Molecular Biotechnology Division, National Institute of Biotechnology, Ganakbari, Bangladesh, Tel: 880-2-7788443; E-mail: salim@nib.gov.bd, salim2969@gmail.com

**Submitted:** 20 March 2014

**Accepted:** 19 May 2014

**Published:** 21 May 2014

**ISSN:** 2333-7117



**Keywords**

- Bioremediation
- Microbes
- Metal resistance
- Heavy metal contamination

**Abstract**

Heavy metal contamination is potentially a significant environmental issue and certainly a major health concern in many parts of the world, primarily due to various anthropogenic activities. Amid various eco-friendly remediation processes, bioremediation- specifically microbial bioremediation- appears to be the consensus method of choice. This article describes the severe consequences of heavy metals contamination, available microbial bioremediation processes and their limitations and future research directions in the field.

## INTRODUCTION

Heavy metals create hazardous problems when pollution takes place in the environment. Heavy metals are metals with densities above 5 g/cm$^3$. Weast (1984) designated 53 out of 90 naturally occurring elements as heavy metals [1]. Heavy metals can be loosely defined based on density, atomic weight, atomic number or other chemical properties and are sometimes synonymously referred to as toxic or harmful metal species. In reality, biologically it is very difficult to differentiate 'heavy metals' from genuinely toxic metals. The cations of heavy metals play vital roles in various sophisticated and essential biochemical reactions. At higher concentrations, however, these heavy metal cations form unspecific complexes which lead to toxic effects and can be very dangerous for normal physiological functions. Exposure to heavy metals is alarmingly increasing in some parts of the world, particularly in less developed countries, though emissions have declined in most developed countries over the last hundred years [2].

Contamination of heavy metals into the environment may appear due to both natural and anthropogenic sources. Natural sources of heavy metals contamination include seepage from rocks into water, volcanic activity, forest fires, and partitioning of polluting elements between sedimentary rocks and their precursor sediments and water [3]. For instance, a natural copper concentration as high as 10% was found in surface peat filtering copper-rich spring water in New Brunswick, Canada [4].

Anthropogenic sources of heavy metals contamination include industrial wastes, mining activity, agricultural practices, automobile emissions and military activity. [5,6]. Contamination through anthropogenic sources affects natural resources resulting in contamination in agricultural and other food products particularly in underdeveloped countries. Heavy metal contamination in such countries can be exemplified by the contamination of nearly 20 million hectares of arable soils (approximately one fifth of the total areas of farmland) in China [7]. However, developing countries such as South and Southeast Asian countries (Malaysia, Vietnam, India, Thailand, Philippines, Indonesia, Bangladesh and Pakistan) have become more attentive to heavy metal contamination in agricultural soils and crops due to their potential effects on human health [8].

The aquatic or soil system of heavy metal contamination can be remediated or ameliorated with the help of *ex-situ* and *in-situ* techniques. *Ex-situ* bioremediation involves removal of waste materials and their collection at a place to facilitate microbial degradation [9]. *Ex-situ* techniques include excavation and landfill, thermal treatment, acid leaching and electro reclamation.

These techniques have a higher cost and are more complex in their implementation when comparing to *in-situ* techniques. Considering these disadvantages of *ex-situ* techniques, *in-situ* strategies which involve contact between microorganisms and the absorbed contaminants at the place of contamination, such as amendments, sand capping and phytoremediation can be promising. Above all, use of microorganisms is the potential strategy that may contribute very effectively to heavy metal remediation process in an eco-friendly manner [6].

## Heavy metals in the environment and biosystem

Based on biological functions and effects, metals have been divided into three classes: (i) the essential metals with known biological functions e.g. calcium, cobalt, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, zinc; (ii) the toxic metals and metalloids e.g. arsenic, mercury, lead, cadmium, chromium, silver; (iii) The non-essential metals with no known biological effects e.g. rubidium, strontium, titanium [10]. A number of metals in a concentrations range essential for biological systems act as cofactors for metalloproteins and enzymes. However, at high concentrations these same metals deleteriously block essential functional groups, displace other metal ions or modify the active conformation of biological molecules [11].

Pollution in the aqueous and solid systems of the environment leads to the bioaccumulation and biomagnifications of the toxic forms of bioavailable heavy metals. Most plants and animals are capable of regulating their metal content to a certain extent, but metals that cannot be excreted build up in an organism over its lifetime. This bioaccumulation process leads to cumulative effects on the occurrence of biomagnifications through the food chain (Figure-1). For example, Minamata disease with neurological damage and fatal deformity was developed in Minamata, Japan due to the effects of mercury toxicity when individuals consumed mercury-contaminated fish from Minamata bay. Metal toxicity is also linked to fatal diseases like birth defects, cancer, liver and kidney damage and possibly a host of other maladies [12].

## Heavy metals remediation

Heavy metals cannot be degraded to harmless by-products by any biological, physical or chemical means. However, they can be transformed from one oxidation state or organic complex to another [13]. Heavy metals are present in soil and aqueous streams as both natural components or as a result of human activity [14]. In an aqueous environment, heavy metals are usually distributed as water-soluble species, colloids, suspended forms and sedimentary phases. The influencing factors regulating the distribution of heavy metals in aquatic environment are $P^H$, oxidation-reduction potential, organic matter species, salinity, temperature, etc. Of these factors $P^H$, oxidation-reduction potential and organic matter species are the most important influencing factors [15]. On the other hand, metals in a soil matrix need to be removed by solubilization in a liquid phase; afterwards they can be concentrated in the desolubilization phase [16].

The remediation strategy may be adopted through both

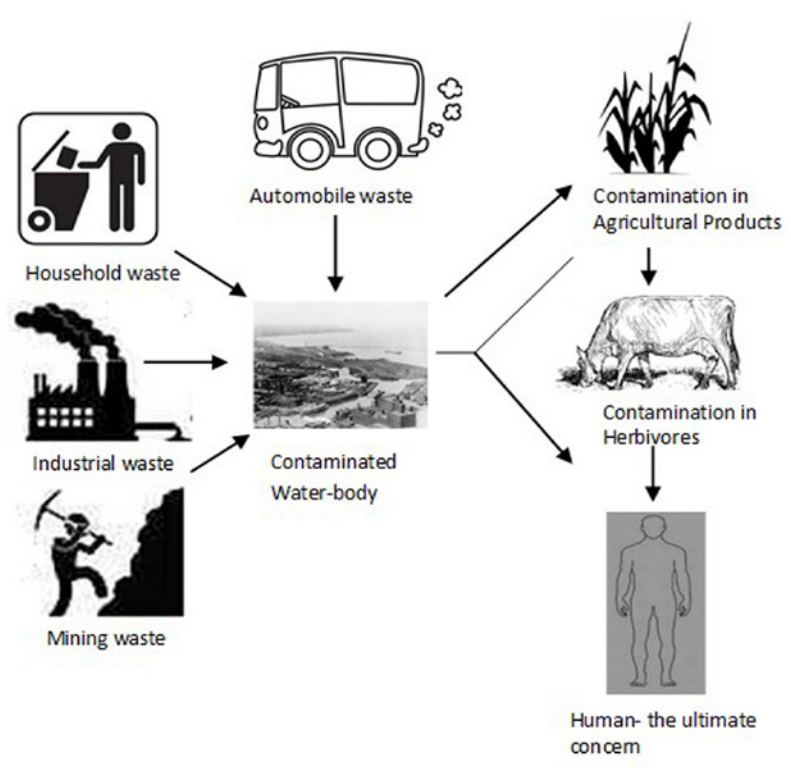


**Figure 1** Bioaccumulation and biomagnification of heavy metals in plants and animals. Heavy metals sourced from various natural and anthropogenic sources contaminate the environment and thus ultimately affect biological systems through the food chain.

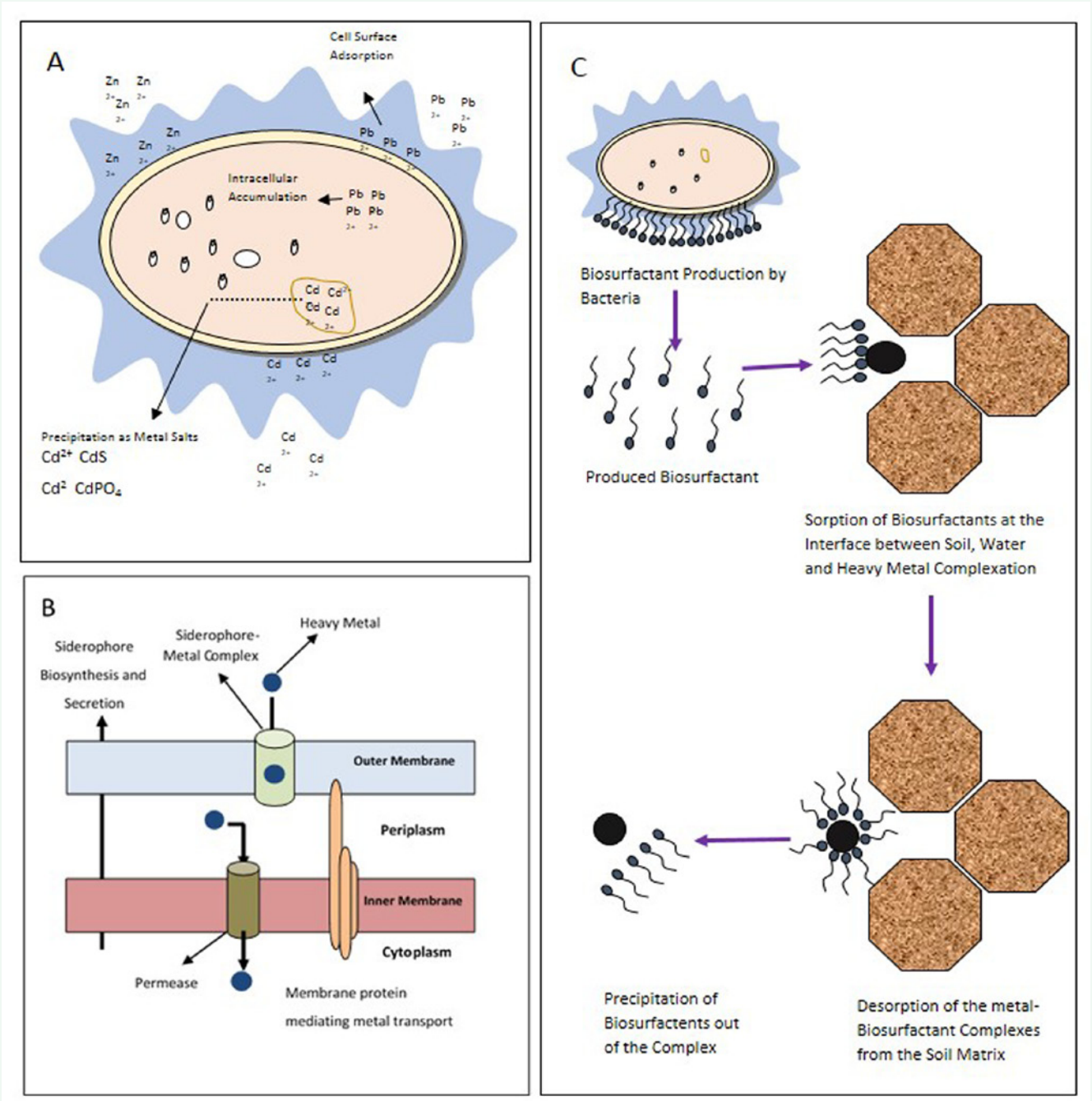


**Figure 2** Bacterial bioremediation of heavy metals contamination. (A) Bacterial biosorption of heavy metals through cell surface adsorption, extracellular precipitation and intracellular accumulation. (B) Bacterial heavy metal remediation through siderophore formation. This is aided by the formation of siderophore-metal complexes and membrane protein-mediated metal transport. (C) Bacterial heavy metal remediation through biosurfactant production. Biosurfactants undergo sorption and desorption at the soil-water-heavy metal matrix and lead to the precipitation of heavy metals.

*in-situ* and *ex-situ* techniques. The *in-situ* strategy focuses on metal stabilization (immobilization) which can be achieved by enhancing metal sorption, precipitation and complexation capacity on sediment and hence the potential mobility or bioavailability of the toxic metals to the environment are lowered [17].This strategy is employed by amendments, sand cap and phytoremediation techniques. Immobilization of heavy metals, usually using inexpensive amendments such as apatite, zeolites, steel shot or beringite, is a promising remediation method. Amendments through apatite minerals can effectively immobilize almost all lead (Pb), Manganese (Mn), Cobalt (Co), Copper (Cu), Cadmium (Cd), Zinc (Zn), Magnesium (Mg), Barium (Ba), Uranium (U) and Thorium (Th) in sediment [14,17,18]. Another choice of *in-situ* heavy metal remediation is capping the contaminated sediment with sandy materials, such as clean sediment, sand, or gravel. Phytoremediation is the heavy metal remediation method which is an ecologically responsible alternative to the environmentally destructive chemical method

that uses plants to extract, sequester or detoxify pollutants [19]. Phytoremediation can be achieved either by plants themselves or by the root colonizing microbes.

Practically, remediation methods such as excavation and landfill, thermal treatment, acid leaching and electro reclamation are not suitable due to their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the specific conditions of the contamination, soil properties, site condition, and so on [20,21]. In light of this, the development and application of the more broadly-applicable phytoremediation or microbial remediation techniques for heavy metal contamination are necessary.

## Microbial remediation of heavy metals

Microbial cells have a significant effect on the distribution of heavy metals in the environment. Microbial bioremediation of heavy metals is an effective, economical and eco-friendly technology to reduce industrial exploitations of chemical methods of bioremediation and to achieve pollution free environment [6]. Microorganisms exert their heavy metal detoxification process by valence transformation, extracellular chemical precipitation, and/or volatilization. Few heavy metals can also be detoxified during metabolic processes of microbes by enzymatic reduction [22]. The extent and efficiency of remediation varies noticeably with the metal as well as with the microorganism. A list of metal resistant microorganisms, their source and uptake efficiency is listed in (Table-1). Although mostly contributed by bacteria, fungi have also been reported to have heavy metal detoxification properties (Table-1). Fission yeast in particular has a well-developed system for heavy metal detoxification which is constituted by pathways common to both fungi and plants.

## Mechanism of microbial remediation

Microorganisms interact with different heavy metals utilizing different processes. Resistance to metal is the main mechanism of heavy metal remediation. From an evolutionary point of view, it is believed that heavy metal resistance in microorganisms may have emerged in response to heavy metals exposure. While microbial heavy metal resistance property is activated by metal stress, it is also possible for microbes to contain independent resistance mechanisms that do not require metal stress for activation. In some cases, activation of resistance may be dependent on exposure to a specific metal.

In general, biotechnological processes relying on the help of microbes can be categorized into three types through which remediation of heavy metals contamination in soil can take place: the first is the biosorption (bioaccumulation) process through which microbes concentrate and integrate metal contaminants onto its cellular structure [10]; the second is the process of extracellular precipitation and uptake by purified biopolymers [32]; and the third may involve assistance by other specific molecules derived from microbial cells [33].

Biosorption is the most important process in both ecological and practical terms. Extracellular materials immobilize the metal through the binding of cell surface anionic functional groups which contain a large number of cationic metals including Cd, Pb, Fe, and Zn. This extracellular binding is usually accomplished by slime layers composed of carbohydrates, polysaccharides and sometimes nucleic and fatty acids [10]. Active functional groups of extracellular binding materials play the central role in the biosorption process. Metal ions become bound to cell surfaces via a range of binding mechanisms involving electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions and extracellular precipitation or some combination of these processes [34]. Functional groups in an activated state like acedamido groups in chitin, amine groups in peptidoglycosides, sulfhydral and carboxyl groups in proteins, phosphate, phosphodiester and hydroxyl groups in polysaccharides take part in the biosorption process [6]. Bacteria are excellent biosorbents due to their high surface-to-volume ratios and a good number of potentially active chemosorption sites e.g. teichoic acid in the bacterial cell wall [35].

Another mechanism of microbial heavy metal remediation is mediated by siderophore formation. Siderophores are low-molecular-weight chelating agents (200-2000 Da) produced by bacteria, fungi and plants to facilitate the uptake of iron [32]. Along with their capacity to feed microorganisms with iron, siderophores can also chelate numerous other metals with variable affinities. Metals other than iron can activate the production of siderophores by bacteria, thereby implicating siderophores in the homeostasis of metals other than iron and especially heavy metal tolerance [36]. Interaction of siderophores with other metals having chemistry similar to that of iron, such as Al, Ga and Cr form trivalent ions similar in size to iron. Thus, siderophores, by binding heavy metals, can reduce both

**Table 1:** List of some comprehensively investigated heavy metal accumulating microorganisms. Metals, microorganisms and their source, MRL/uptake efficiency and removal percentage with removing time has been shown.

| Metals | Microorganisms | Source | MRL/Uptake Efficiency | Removal (%) | References |
|---|---|---|---|---|---|
| Cd | *Bacillus* strain H9 | Metal-contaminated soil | 275 µg/ml | 36 (48h) | [23] |
| | *Aspergillusterreus* | Industrially polluted sediments | 122 ppm/g | 70 (13 days) | [24] |
| Cr | *Pseudomonas aeruginosa* | Wastewater | 0.08 mg/ml | 46 (2 days) | [25] |
| | *Aspergillusniger* | Acquired | 5.1– 6.6 mg/g | 21– 36 (7 days) | [26] |
| Pb | *Pseudomonas aeruginosa* PU21 (Rip64) | - | 0.5 mg/ml (110 mg/g) | 80 (2 days) | [27] |
| | *Aspergillusniger* | Acquired | 5.3– 34.4 mg/g | 13– 88 (7 days) | [26] |
| Cu | *Thiobacillus ferrooxidans* | Adopted | 0.02 mg/ml (700 mg/g) | 25 (15 min) | [28] |
| | *Schizosaccharomyces pombe* | Acquired | 0.6– 1.3 mg/g | 11 – 25 (4 days) | [29] |
| Ni | *Pseudomonas spp.* | Local isolate | 74.9 mg/g | 98 (4 days) | [30] |
| | *Candida spp.* | Sewage | 10.3– 46.8 mg/g | 29– 57 (5–15 days) | [31] |

**Abbreviations:** MRL: Maximum Resistance Level

bioavailability and metal toxicity; e.g. siderophore-mediated complexation reduces copper toxicity in cyanobacteria [4] and in *Pseudomonas aeruginusa* and *Alcaligenes eutrophus* siderophore synthesis is induced by heavy metals in the presence of high iron concentrations [37].

Production and excretion of biosurfactants from microbial cells may spur the bioremediation of heavy metals in polluted areas. Biosurfactant molecules are able to complex metals such as Cd, Pb and Zn [33]. Biosurfactants of anionic nature can capture metal ions through electrostatic interactions or complexations [38]. In turn, complexations formed by biosurfactants increase the apparent solubility of metals. Thus, metal bioavailability can be influenced by common metabolic by-products that results in metal reduction resulting in the formation of less soluble metal salts including sulfide and phosphate precipitates [10].

**Cadmium (Cd):** Cd and Cd-compounds are more mobile in soil, more bioavailable and tend to bioaccumulate due to their higher relative solubility [39]. Amongst all non-essential heavy metals,Cd is perhaps the most attentively tracked due to potential toxicity to humans and its relative mobility in soil-plant systems [40]. The largest source of anthropogenic atmospheric Cd emissions is metal production, followed by waste incineration and other, more minor sources including production of nickel-cadmium batteries, fossil fuel combustion and industrial dust generation. Water bodies are largely contaminated by Cd through processed water from smelters, phosphate mining and related fertilizer production, and electroplating wastes. The major route of Cd entrance into the human body from the environment is ingestion, especially of plant-based foodstuffs [41]. Exerting toxicity primarily to the kidney, Cd can also cause bone demineralization and may impair lung function and increase the risk of lung cancer following excessive exposure [42]. For instance, in the 1950s Cd contamination led to renal impairment and bone disease (Itai-itai disease) in exposed populations in Japan [43].

Resistance to Cd in bacteria is based on Cd flux. Cyanobacteria have metallothionein-like proteins and overexpression of this metallaothionein *smt* locus increases the cadmium resistance and its deletion decreases resistance [44]. Cadmium seems to be detoxified by gram negative bacteria with the help of RND (Resistance Nodulation Cell Division) systems like *czc*, which is mainly a zinc exporter [45]. $Cd^{2+}$ enters the cell of a gram negative bacterial cell by CorA and NRAMP (Natural Resistance Associated Macrophage Protein)-like uptake systems, binds to thiol compounds, exerts toxicity and is exported again by P-type ATPases, CBA (Cytometric Bead Array) and CDF (Cation Diffusion Facilitation) proteins [46]. In gram positive bacteria this takes place by RND-driven trans-envelope and possibly also by CDF transporters [47]. In yeast (*S. cerevisiae*), glutathione binds cadmium and the resultant cadmium biglutathionate complex is transported by YCF1P and ABC transporters into the vacuole [48].

**Chromium (Cr):** Anthropogenic spread of hexavalent Chromium ($Cr^{6+}$) is caused by wide applications in various industries such as stainless steel production, electroplating of chrome, dyes, leather tanning and wood preservatives [49], and its high solubility and toxicity makes its remediation a priority. Chromium exists in the environment as the highly toxic $Cr^{6+}$ anion and the less-soluble toxic $Cr^{3+}$. The trivalent form of chromium ($Cr^{3+}$) is an essential trace element which acts as cofactor for many enzymes in biological system e.g. activation of insulin receptor tyrosine kinase [50]. Plants and animals do not bio accumulate $Cr^{3+}$ but $Cr^{6+}$ is a well-known group A human carcinogen and is also associated with birth defects [51]. Chronic exposure to $Cr^{6+}$in the form of lead chromate is known to induce persistent or increasing chromosome damage [49]. An epidemiological study in America among chromate industry workers who worked just for 1 year between 1931 and 1949 showed that the percentage of death due to lung cancer was 18.2% over the same time frame [52].

Several bacteria have been reported to reduce $Cr^{6+}$ that is toxic and mutagenic, to its less toxic trivalent form [53]. Bacterial resistance to chromate has been found in several *Pseudomonas* strains and also with a plasmid in *Streptococcus lactis* [54]. Remediation of chromate ($Cr^{6+}$) is mainly mediated by chromate reduction to non-toxic $Cr^{3+}$ and chromate efflux. Efflux of chromate is regulated by the sulphate uptake system as accumulation interferes with sulphate metabolism [55]. Soils and marine sediments contain many facultative and strictly anaerobic bacteria capable of reducing $Cr^{6+}$ to $Cr^{3+}$ [56]. Anaerobic bacteria which reduce sulphate and iron can indirectly reduce $Cr^{6+}$ via hydrogen sulphide (HS-) and Fe (II) respectively [57,58]. It is also reported that a blue-green algae *Nostoc* exists in soil chronically polluted by chromium (about 5000mg/kg of soil) from leather tannery. Levels of $Cr^{6+}$ resistance by other microbes are *Arthrobacter crystallopoites* (500 mg/L), *Pseudomonas spp.* CRB 5 (520 mg/L), *Bacillus maroccanus* ChrA21 (1040 mg/L), *Corynebacterium hoagie* ChrB20 (1144 mg/L), and *Bacillus cereus* ES04 (1500 mg/L) [59].

**Mercury (Hg):** Hg exists in nature in small amounts as it is the sixteenth rarest element on earth. However, levels of Hg are rising due to industrialization and other anthropogenic activities such as the burning of coal and petroleum, the use of mercurial fungicides in agriculture, the papermaking industry, and mercury catalysts in industries [60]. The prevalence of Hg toxicity is exemplified by the Minamata disease mentioned above. The toxicity of both organic and inorganic Hg compounds is due to their strong affinity for sulfur containing organic compounds, such as enzymes or proteins. All chemical forms of Hg are toxic, but most research has focused on methyl mercury (MeHg) due to its links to large-scale public health issues.

Microorganisms like bacteria, yeast and protozoa play a vital role in the cycling of Hg in the global natural environment. Microorganisms are able to reduce Hg to the metal, which does not remain inside the cell with the potential of becoming oxidized again, but leaves the cell by passive diffusion [61,62]. Once outside, however, metallic Hg may be oxidized again by other bacteria. Hg transport and transformations are regulated by a tightly regulated genetic system named the *mer* operon, consisting of four to five structural and regulatory genes. The *mer* system functions in environmental Hg remediation and more extensive consideration of the utility of *mer* operon helps in monitoring of Hg contamination [63].

**Arsenic (As):** In the environment, As compounds have

been abundant at near toxic levels since the origin of life [64]. Arsenic exposure to human may occur from food, air and water; amongst these water is the predominant route of exposure and all major chronic As poisoning have stemmed from water [65]. Bangladesh is an example where As contamination is very common and history of arsenicosis patients have been traced. It has been estimated that About 57 million peoples in Bangladesh are experiencing exposure to As in their drinking water above the level of WHO drinking water guidelines for As (10 µg/l) [66]. The major health hazards of As toxicity are hyperpigmentation or keratosis leading to an increased risk of skin, internal organ and lung cancer [65].The predominant forms of inorganic arsenic are +5 (arsenate: H2AsO4-and $HAsO4^{2-}$) and +3 (arsenite: H3 $AsO_3^0$ and $H2AsO_3^-$). Arsenate mimics phosphate and can enter the microbial cell via transporters, from there interfering with phosphate-based energy-generating processes and ultimately inhibiting oxidative phosphorylation. The +3 form, arsenite, enters via aqua-glycerolporins (a major membrane channel family protein) and targets a broader range of cellular processes, binding to thiol groups in important cellular proteins such as pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase [67].

Microorganisms can use methylation as a detoxification strategy for arsenic remediation from the local environment. For example, through methylation fungi can produce monomethylarsonic acid (MMA) or dimethylarsonic acid (DMA) and prokaryotes can produce volatile methylated arsines. The ArsC arsenate reductase protein can also act in arsenic remediation by bacteria and yeasts. The genes for ArsC and other proteins required for arsenic detoxification are often encoded on plasmids. More than 100 Ars operons have been sequenced [64] and this number will be significantly higher now. The ArsC protein is found in the cytoplasm of the microbial cell and mediates the reduction of arsenate to arsenite, via glutaredoxin, glutathione or thioredoxin cofactors.

**Lead (Pb):** The toxic nature of lead has been recognized for millennia, with the earliest published reports dating back to 2000 BC [68]. Pb has a diversified use in petrol fuel, paints, ceramics, food cans, make up, batteries etc. It is therefore present in air, dust, soil and water to varying degrees with human exposure occurring through ingestion, inhalation and dermal absorption [69]. Pb is cumulative toxicant that affects neurological, hematological, gastrointestinal, cardiovascular and renal systems of human body. It is estimated that 0.6% of the global burden of disease, with the highest burden in developing region is accounted for by Pb exposure [70].

Pb does not create any extraordinary toxicity to microorganisms which accumulate $Pb^{2+}$ through general biosorption processes. Import of $Pb^{2+}$ into the cells of microorganisms occurs via uptake systems which belong to the protein family of the divalent metal cation-transporting P-type ATPases while export is mediated by ATP-hydrolyzing efflux systems. The operon *pbr* contains the components of this key system of microorganism lead resistance [71,72].

**Copper (Cu):** The world's copper production is rising daily, leading to more and more copper in the environment. Anthropogenic use of Cu includes as a component in electrical equipment, construction, industrial machinery, and phosphatic fertilizers. Copper normally leaches into drinking water from Cu pipes and from additives designed to control algal growth. Cu is an essential metal for biological systems. Cu strongly complexes with organic materials in the soil, implying that only a small fraction of copper will be found in solution as ionic copper, Cu (II). Except in exposure to very high doses, Cu does not create toxicity. However, long term exposure to Cu can cause anemia, liver and kidney damage and stomach and intestinal irritation [73].

Cu toxicity is based on its radical character leading to the production of hyperoxide radicals which interact with the cell membrane through binding with thiol compounds [46]. In gram positive bacteria, P-type ATPases seem to detoxify Cu via efflux. In some microorganisms, Cu resistance systems encode proteins which bind Cu in the periplasm or close to the outer membrane [47].

**Zinc (Zn), Cobalt (Co) and Nickel (Ni):** Zn can be found in large quantities in both soil and water as the world's Zn production continues to rise. Zn is able to bio-magnify up the food chain in water-bodies or soil. It is also notable that only a limited number of plants have a chance of survival in Zn-rich soil [74]. Acute toxicity to humans by zinc arises from the ingestion of excessive amount of zinc salts either incidentally or deliberately as an emetic or dietary supplement.

Cobalt toxicity is quite low compared to many other metals in soil. Co is present in nature as the metal and in two valence states- Co (II) and Co (III) which form various organic and inorganic salts. Cobalt functions as cofactors for several enzymes in biological systems and is also very important for the synthesis of vitamin $B_{12}$.However, Co can contribute to adverse health effects in the lungs, including asthma, pneumonia, and wheezing when exposure occurs in very high levels [75].

Nickel can find its way into the human body indirectly, e.g. through food which has been handled, processed or cooked by utensils containing large quantities of nickel. Though Ni and Ni-compounds belong to the classic noxious agents encountered in industry, the general population may be exposed to nickel in the air, water and food. The toxicity and carcinogenicity of some Ni-compounds in experimental animals and in the occupationally exposed population are well-documented [76]. For instance, Ni-carbonyl is the most acutely toxic nickel compound and may cause frontal headache, nausea, vomiting, insomnia and irritability in its immediate toxic effect [77].

The major determinant of Zn, Co and Ni resistance is the *czc* structural gene. This structural gene region contains the genes for the OMF CzcC, the MFP CzcB and the CzcA protein of the RND family. These three genes form an operon, czcCBA, that is transcribed tri-cistronically and is flanked by a multitude of genes involved in metal-dependent regulation of czcCBA expression [78]. A second structural gene region, *cnr,* which is based on cation efflux as the resistance determinant is composed of a *cnrCBA* structural region [79,80,81] preceded by a regulatory gene region. Another Co and Ni resistance determinant, *ncc*, was also characterized [82]. Similar to *cnr, ncc* is composed of a regulatory gene region followed by the structural region *nccCBA.*

## PROSPECTS AND CHALLENGES

Remediation of heavy metal through microbial treatment has numerous advantages including eco-friendliness, specificity, adaptability, self-reproducibility, recycling of bioproducts *etc*. Major drawbacks to this method of treatment are the slowness of the processes and difficulty in controlling the processes. However, since safe removal of high levels of heavy metals is a concern matched by increased global cognizance of the environmental problems brought on by other methods, microbial processes represent the most logical, long-term solution for remediation. As discussed earlier in the manuscript, while a number of microbial metal bioremediation approaches to combat heavy metal pollution are established, wide spread and large-scale remains relatively rare [6]. Additionally, long-term sustainability of microbial remediation remains a question of great import as studies into long-term effectiveness remain scarce [10]. With heavy metals accumulating at alarmingly high in heavily-populated areas of the world, upgrading existing microbial bioremediation processes to a commercial level by making the processes faster, recyclable and more easy to control is a major challenge going forward.

Polluted environments often contain more than one metal. Therefore, complex, combinatorial approaches of more than one metal-resistant mechanism in one bacterium through genetic manipulation or symbiotic approaches will be necessary for large scale remediation of toxic metals and to regenerate healthy, thriving soils and water. Recently, a number of approaches have been developed to manipulate bacterial genetics in order to design multi-metal resistant bacterial strains. For instance, Peitzsch *et. al*. constructed a chromate sensor plasmid pEBZ141 and transferred the plasmid into the *A. eutrophus* CH34 strain and showed that these mutant strains were resistant to chromate ions and/or to $Co^{2+}$ and $Ni^{2+}$ [55].

The microbial community is still often treated as a "black box" when considering its contribution to bioremediation and its impact on the ecosystem [83]; largely due to the number of bacteria in the environment which cannot be cultured in the laboratory [84]. This situation does not permit scientists to discern the means for molecular manipulation of microbial genetic systems which could solve problems in heavy metal contamination remediation. It is notable that,-although heavy metal resistant strains have to be isolated from environmental or clinical sources, microbial chromosomal mutation can be produced in laboratory [54]. Various genetic manipulation studies such as the engineering of bacterial strain to express metal transport systems, insertion of metal resistant genes or operons to bacteria, and the insertion of hexa-histidine peptides to the surface layerprotein RsaA of *C.crescentus* showed that these modifications can significantly change the resistance of microbes to heavy metals [85-88].Thus, our hope for large-scale bioremediation of toxic metals resides on further genetic manipulation of metal resistant strains in hand for the development of hyper-absorbing, hyper-accumulating or hyper-biosurfactant producing strains which can act as eco-friendly bioremediation methods.

## ACKNOWLEDGEMENTS

Dr. Max Burroughs, Research Scientist, National Center for Biotechnology Information (NCBI), National Institute of Health (NIH), USA for critical reading and editing of the manuscript. Abu Hashem, Senior Scientific Officer, Microbial Biotechnology Division and Md. Shafiquzzaman, Scientific Officer, Molecular Biotechnology Division; National Institute of Biotechnology, Bangladesh.

## REFERENCES

1. Weast RC. CRC Handbook of chemistry and physics, 64th ed. CRC, Boca Raton: Fla; 1984.
2. Järup L . Hazards of heavy metal contamination. Br Med Bull. 2003; 68: 167-182.
3. Shani SSK. A position paper. Hazardous metals and minerals pollution in India. Indian National Science Academy, New Delhi. 2011 Aug.
4. Stone EL, Timmer VR. Copper content of some northern conifers. Can J Bot. 1975; 53: 1453-1456.
5. Pazirandeh M, Wells BM, Ryan RL . Development of bacterium-based heavy metal biosorbents: enhanced uptake of cadmium and mercury by Escherichia coli expressing a metal binding motif. Appl Environ Microbiol. 1998; 64: 4068-4072.
6. Rajendran P, Muthukrishnan J, Gunasekaran P . Microbes in heavy metal remediation. Indian J Exp Biol. 2003; 41: 935-944.
7. Wei CY, Chen TB. Hyperaccumulators and phytoremediation of heavy metal contaminated soil: a review of studies in China and abroad. ActaEcologicaSinica. 2001; 21: 1196-1203.
8. Luo Y, Wu L, Liu L, Han C, Li Z. Heavy metal contamination and remediation in Asian agricultural land. Chinese Academy of Science. Nanjing 210008, PR China. 2009.
9. Dubey RC. A text book of biotechnology, New Delhi; 2005.
10. Maier RM, Pepper IL, Gerba CP. Environmental Microbiology, 2nd ed. San Diego. CA: Academic Press; 2009.
11. Garbisu C, Alkorta I. Basic concepts on heavy metal soil bioremediation. EJMPEP. 2003; 3: 58-66.
12. CERCLA Priority list of hazardous substances. ATSDR–2001.
13. Jaiswal S. Role of rhizobacteria in reduction of arsenic uptake by plants. Bioremed. Biodegrad. 2011; 2: 4.
14. Raskin I, Kumar PBAN, Dushenkov S, Salt DE. Bioconcentration of heavy metals by plants. Curr Opin Biotechnol. 1994; 5: 285-290.
15. Peng JF, Song YH, Yuan P, Cui XY, Qiu GL .The remediation of heavy metals contaminated sediment. J Hazard Mater. 2009; 161: 633-640.
16. Diels L, De Smet M, Hooyberghs L, Corbisier P . Heavy metals bioremediation of soil. Mol Biotechnol. 1999; 12: 149-158.
17. Raicevic S, Wright JV, Veljkovic V, Conca JL . Theoretical stability assessment of uranyl phosphates and apatites: selection of amendments for in situ remediation of uranium. Sci Total Environ. 2006; 355: 13-24.
18. Raicevic S, Kaludjerovic-Radoicic T, Zouboulis AI . In situ stabilization of toxic metals in polluted soils using phosphates: theoretical prediction and experimental verification. J Hazard Mater. 2005; 117: 41-53.
19. Meagher RB . Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol. 2000; 3: 153-162.
20. Chaney RL, Brown SL, Li YM. Progress in risk assessment for soil metals, and in-situ remediation and phytoextraction of metals from hazardous contaminated soils. Phytoremediation: State of Science. 2000 May 1-2; US-EPA, Boston, MA: 2000.

21. Cheng S, Vidakovic-Cifrek Z, Grosse W, Karrenbrock F . Xenobiotics removal from polluted water by a multifunctional constructed wetland. Chemosphere. 2002; 48: 415-418.

22. Lovley DR . Dissimilatory metal reduction. Annu Rev Microbiol. 1993; 47: 263-290.

23. Roane TM, Josephson KL, Pepper IL. Dual-bioaugmentation strategy to enhance remediation of cocontaminated soil. Appl Environ Microbiol. 2001; 67: 3208-3215.

24. Massaccesi G, Romero MC, Cazau MC, Bucsinszky AM. Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). World J Microbiol Biotechnol. 2002; 18: 817–820.

25. Hassen A, Saidi N, Cherif M, Boudabous A. Effects of heavy metals on Pseudomonas aeruginosa and Bacillus thrungiensis. Bioresour Technol. 1998; 65: 73– 82.

26. Dursun AY, Ulsu G, Cuci Y, Aksu Z. Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing Aspergillusniger. Process Biochem. 2003; 38: 1647–1651.

27. Chang JO, Law R, Chang CC. Biosorption of lead, copper and cadmium by biomass of Pseudomonas aeruginosa PU21. Water Res. 1997; 31: 1651–1658.

28. Boyer A, Magnin J-P, Ozil P. Copper ion removal by Thiobacillus ferrooxidans biomass. Biotechnol Lett. 1998; 20: 187 –190.

29. Donmez G, Aksu Z. The effect of copper(II) ions on growth and bioaccumulation properties of some yeasts. Process Biochem. 1999; 35: 135– 42.

30. Magyarosy A, Laidlaw RD, Kilaas R, Echer C, Clark DS, Keasling JD. Nickel accumulation and nickel oxalate precipitation by Aspergillus niger. Appl Microbiol Biotechnol. 2002; 59: 382-388.

31. Dönmez G, Aksu Z . Bioaccumulation of copper(II) and nickel(II) by the non-adapted and adapted growing Candida sp. Water Res. 2001; 35: 1425-1434.

32. Chu BC, Garcia-Herrero A, Johanson TH, Krewulak KD, Lau CK, Peacock RS et al . Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. Biometals. 2010; 23: 601-611.

33. Maier RM, Soberón-Chávez G. Pseudomonas aeruginosa rhamnolipids: biosynthesis and potential applications. Appl Microbiol Biotechnol. 2000; 54: 625-633.

34. Blanco A. Immobilization of nonviable cyanobacteria and their use for heavy metal adsorption from water in Environmental biotechnology and cleaner bioprocesses. Oluguin EJ, Sanehez, Hernandez E, editors. Philadelphia Taylor & Amp, Francis. 2000; 135.

35. Beveridge TJ . Role of cellular design in bacterial metal accumulation and mineralization. Annu Rev Microbiol. 1989; 43: 147-171.

36. Schalk IJ, Hannauer M, Braud A . New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol. 2011; 13: 2844-2854.

37. Höfte M, Dong Q, Kourambas S, Krishnapillai V, Sherratt D, Mergeay M. The sss gene product, which affects pyoverdin production in Pseudomonas aeruginosa 7NSK2, is a site-specific recombinase. Mol Microbiol. 1994; 14: 1011-1020.

38. Rufino R, Luna J, Campos-Takaki G, Ferreira SRM,Sarubbo L. Application of the biosurfactant produced by Candida lipolyticain, the remediation of heavy metals. Chem Eng Trans. 2012; 27: 61-66.

39. Cadmium Review. Nordic Council of Minister. 2003 Jan.

40. Tran TA, Popova LP. Functions and toxicity of cadmium in plants: recent advances and future prospects. Turk J Bot. 2013; 37: 1-13.

41. Pollution prevention and abetment handbook. World Bank Group. 1998 Jul.

42. Bernard A . Cadmium & its adverse effects on human health. Indian J Med Res. 2008; 128: 557-564.

43. Kaji M. Role of experts and public participation in pollution control: the case of Itai-itai disease in Japan. Ethics Sci Environ Polit. 2012; 12: 99-111.

44. Gupta A, Morby AP, Turner JS, Whitton BA, Robinson NJ . Deletion within the metallothionein locus of cadmium-tolerant Synechococcus PCC 6301 involving a highly iterated palindrome (HIP1). Mol Microbiol. 1993; 7: 189-195.

45. Schmidt T, Schlegel HG . Combined nickel-cobalt-cadmium resistance encoded by the ncc locus of Alcaligenes xylosoxidans 31A. J Bacteriol. 1994; 176: 7045-7054.

46. Nies DH . Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev. 2003; 27: 313-339.

47. Nies DH . Microbial heavy-metal resistance. Appl Microbiol Biotechnol. 1999; 51: 730-750.

48. Li ZS, Lu YP, Zhen RG, Szczypka M, Thiele DJ, Rea PA. A new pathway for vacuolar cadmium sequestration in Saccharomyces cerevisiae: YCF1-catalyzed transport of bis(glutathionato)cadmium. Proc Natl Acad Sci U S A. 1997; 94: 42-47.

49. Das AP, Mushra S. Hexavalent chromium(IV): environment pollutant and health hazard. JERAD. 2008; 2: 386-392.

50. Davis CM, Vincent JB. Chromium oligopeptide activates insulin receptor tyrosine kinase activity. Biochemistry. 1997; 36: 4382-4385.

51. Meegoda JN, Kamolpornwijit W, Vaccari DA, Ezeldin AS, Noval BA, Mueller RT et al. Practice periodical of hazardous, toxic and radioactive waste management. 1999; 3: 124-131.

52. Agency for toxic substances and disease registry.Chromium toxicity. Case Studies in Environmental Medicine (CSEM).Course WB 1466; 2008.

53. Garbisu C, Alkorta I, Llama MJ, Serra JL . Aerobic chromate reduction by Bacillus subtilis. Biodegradation. 1998; 9: 133-141.

54. Shukla OP, Rai UN, Dubey S, Mishra K. Bacterial resistance: a tool for remediation of toxic metal pollutants. Environews. 2006; 12.

55. Peitzsch N, Eberz G, Nies DH . Alcaligenes eutrophus as a bacterial chromate sensor. Appl Environ Microbiol. 1998; 64: 453-458.

56. Francis AJ. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. Experimentia. 1990; 46: 840-851.

57. Sedlak DL, Chan PG. Reduction of hexavalent chromium by ferrous iron. Geochim. Cosmochim. Acta. 1997; 61: 2185-2192.

58. Pettine M, Barra L, CampanellaL, Millero FJ. Effect of metals on the reduction of chromium (VI) with hydrogen sulfide. Water Res. 1998; 32: 2807-2813.

59. Carlo V, Luciana G. Bioremediation of soils polluted with hexavalent chromium using bacteria: a challenge. Environ Bioremed. Technol Springer. 2006; 57 –76.

60. Dash HR, Das S. Bioremediation of mercury and the importance of mer genes. IBBS. 2012; 75: 207-213.

61. Silver S . Bacterial resistances to toxic metal ions--a review. Gene. 1996; 179: 9-19.

62. Silver S, Phung LT . Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol. 1996; 50: 753-789.

63. Barkay T, Wagner-Döbler I. Microbial transformations of mercury:

potentials, challenges, and achievements in controlling mercury toxicity in the environment. Adv Appl Microbiol. 2005; 57: 1-52.

64. Mukhopadhyay R, Rosen BP, Phung LT, Silver S . Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev. 2002; 26: 311-325.

65. Kapaj S, Peterson H, Liber K, Bhattacharya P . Human health effects from chronic arsenic poisoning--a review. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2006; 41: 2399-2428.

66. Appelo T. Arsenic in groundwater: A world problem. Proceedings of seminar, Utrecht; National committee of the IAH; Deltares, Utrecht, the Netherlands. 2006.

67. Lloyd JR, Oremland RS. Microbial transformations of arsenic in the environment: from soda lakes to aquifers. Elements. 2006; 2: 85–90.

68. Needleman HL. History of lead poisoning in the world: lead poisoning prevention and treatment: implementing a national program in developing countries. George AM, editor. The George Foundation, Bangalore; 1999.

69. Ezzati M, Lopez AD, Rodgers A, Murray CJL, editors. Comparative quantification of health risks. Vol. 2 (chapter 19), World Health Organization, Geneva; 2004.

70. WHO. Global health risks: Mortality and burden of disease attributable to selected major risks, Geneva, World Health Organization, 2009.

71. Tsai KJ, Lin YF, Wong MD, Yang HH, Fu HL, Rosen BP. Membrane topology of the p1258 CadA Cd(II)/Pb(II)/Zn(II)-translocating P-type ATPase. J Bioenerg Biomembr. 2002; 34: 147-156.

72. Borremans B, Hobman JL, Provoost A, Brown NL, van Der Lelie D . Cloning and functional analysis of the pbr lead resistance determinant of Ralstonia metallidurans CH34. J Bacteriol. 2001; 183: 5651-5658.

73. Wuana RA, Okieimen FE. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology. 2011; 20.

74. Lenntech. Environmental effects of zinc. Technical university of Delft, the Netherlands.

75. Factsheet. Cobalt in the environment. Ministry of the environment programs and initiatives. c2001.

76. Cempel M, Nikel G. Nickel: A review of its sources and environmental toxicology. Polish J Environ Stud. 2006; 15: 375-382.

77. Nickel toxicological overview, c2009.

78. Grosse C, Grass G, Anton A, Franke S, Santos AN, Lawley B et al. Transcriptional organization of the czc heavy-metal homeostasis determinant from Alcaligenes eutrophus. J Bacteriol. 1999; 181: 2385-2393.

79. Nies DH, Silver S . Plasmid-determined inducible efflux is responsible for resistance to cadmium, zinc, and cobalt in Alcaligenes eutrophus. J Bacteriol. 1989; 171: 896-900.

80. Sensfuss C, Schlegel HG. Plasmid pMOL28-encoded resistance to nickel is due to species efflux. FEMS MicrobiolLett. 1988;55:295-298.

81. Liesegang H, Lemke K, Siddiqui RA, Schlegel HG . Characterization of the inducible nickel and cobalt resistance determinant cnr from pMOL28 of Alcaligenes eutrophus CH34. J Bacteriol. 1993; 175: 767-778.

82. Schmidt T, Schlegel HG . Combined nickel-cobalt-cadmium resistance encoded by the ncc locus of Alcaligenes xylosoxidans 31A. J Bacteriol. 1994; 176: 7045-7054.

83. Iwamoto T, Nasu M . Current bioremediation practice and perspective. J Biosci Bioeng. 2001; 92: 1-8.

84. Kogure K, Simidu U, Taga N . A tentative direct microscopic method for counting living marine bacteria. Can J Microbiol. 1979; 25: 415-420.

85. Patel J, Zhang Q, McKay RM, Vincent R, Xu Z . Genetic engineering of Caulobacter crescentus for removal of cadmium from water. Appl Biochem Biotechnol. 2010; 160: 232-243.

86. Deng X, Li QB, Lu YH, He N, Jiang J. Genetic engineering of E. coli SE5000 and its potential for Ni2+ bioremediation. Process Biochem. 2005;40:425–430.

87. Selifonova O, Burlage R, Barkay T . Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. Appl Environ Microbiol. 1993; 59: 3083-3090.

88. Brim H, McFarlan SC, Fredrickson JK, Minton KW, Zhai M, Wackett LP et al. Engineering Deinococcus radiodurans for metal remediation in radioactive mixed waste environments. Nat Biotechnol. 2000; 18: 85-90.

**Cite this article**

*Banik S, Das KC, Islam MS, Salimullah M (2013) Recent Advancements and Challenges in Microbial Bioremediation of Heavy Metals Contamination. JSM Biotechnol Bioeng 2(1): 1035.*