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## REVIEW ARTICLE

### BIOREMEDIATION: AN INEXPENSIVE YET EFFECTIVE STRATEGY FOR REMEDIATION OF HEAVY METAL CONTAMINATED SITES

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#### *Abstract*

With the industrial progress, the presence of toxic heavy metals is increasing in the environment and polluting it to a great extent. Wastewater discharged due to sewage sludge applications and from industries is leaving detrimental effect on society as well as biological systems. Therefore, preventing heavy metal pollution is crucial for protecting the environment and cleaning contaminated sites. Applications of conventional technologies like ion exchange, reverse osmosis, chemical precipitation and evaporation recovery for this very purpose prove to be quite expensive and inefficient, so far. Research has demonstrated that bioremediation is the most effective and inexpensive method of cleaning up contaminated soil and water. This paper will throw some light on indigenous microbes that have high tolerance level to heavy metal and play cardinal role in restoration of sites. To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. The fundamental principles include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell and reduction of the heavy metal ions to a less toxic state. For getting insight about new isolates, bacterial diversity under unfavorable conditions and new genetic information on heavy metal resistance, it is pivotal to study the indigenous microorganisms at polluted sites.

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#### **Introduction:-**

Globally, environmental pollution is increasing ceaselessly on account of an unabated increment in population, industrialization, urbanization, anthropogenic activities and natural sources [1]. Heavy metals such as iron, manganese, mercury, lead, zinc, cadmium, uranium, cobalt, chromium and several others are cornerstones of human progress; they are quite literally the pillars of all the major civilizations, past and present because they are used widely as part of materials construction, agriculture, transportation and in processing of many industrial materials and commercial products. The recent expansion of human industrial activity, including mining, smelting, and synthetic compound creation has led to an exponential increase in the amounts of heavy metals released into the atmosphere, water and soil [2].

The metal pollution is of great concern as these hazardous pollutants are accumulated in living organisms including microorganisms, plants, animals and human (and are responsible for many metabolic and physiological disorders [3,4]. Unlike many other pollutants, heavy metals are difficult to remove from the environment [5]. Heavy metals are recognized to be powerful inhibitors of biodegradation activities. These metals cannot be degraded and are ultimately indestructible. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes. Each heavy metal has unique biofunctions or biotoxicities. For example, copper can enhance microbial growth at low concentrations but represses growth at high concentrations and cadmium has high toxicity even at low concentrations [6]. Uncontrolled discharges of large quantities of heavy

metals create not only a huge environmental and human health burden due to their high occurrence as contaminants and toxicity to all living beings [7,8], but they also increase the cost of wastewater treatment [9,10].

In recent years, different methods are being utilized for the removal of heavy metal ions from the aqueous disposed such as ion exchange, precipitation (Chemistry), membrane technologies, electrochemical treatments, activated carbon adsorption, etc. But, each of these procedures has some demerits that outweigh merits. Efforts are being made by applying biotechnological tools like bioremediation in managing and removal of metal ions to reduce contamination in the soil. Bioremediation is the “use of living organisms such as bacteria, fungi, yeast, and algae for removal of a pollutant from the biosphere”. It relies on biological processes to minimize an unwanted environment impact of the pollutants. The microorganisms in particular have abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds. Microorganism destroy organic contaminate in the course of using their chemical for their own growth and reproduction for new cell. Besides them, higher plants also been reported to remove such pollutants, primarily through their tissues.

Contamination through anthropogenic sources affect natural resources resulting in contamination in agricultural and other food products especially in a greater extent in underdeveloped countries. Many countries have regulatory guidelines for heavy-metal presence and exposure as well as remediation and treatment options. Screening of soil and water sources is conducted frequently to prevent overconsumption, but many of these programs and technologies are not readily available in developing nations, where the burden is the greatest [11,12,13]. The result is that people around the globe are exposed, and new approaches are required to reduce the adverse consequences of accumulation of these compounds.

#### **Heavy Metal and Biosystem:-**

Heavy Metals are defined as elements in the periodic table having atomic number more than 20 or densities more than  $5\text{g/cm}^3$ . It generally excludes alkali metals and alkaline earth metals. 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 etc.; (ii) the toxic metals and metalloids e.g. arsenic, mercury, lead, cadmium, chromium, silver etc.; (iii) The non-essential metals with no known biological effects e.g. rubidium, strontium, titanium etc [14].

A number of metals in a concentrations range is essential for biological system as they constitute cofactors for metalloproteins and enzymes. Most plants and animals are capable to regulate their metal content to a certain point, but metals that cannot be excreted build up in an organism over its lifetime. Heavy metals find their entry into food from natural sources like soil, air and water through wastewater irrigation, solid waste disposal, mining, smelting, sludge applications, vehicular exhaust, fertilizers, fungicides and industrial activities [15,16]. Consumption of food crops contaminated with heavy metals is a major food chain route for human exposure [17].

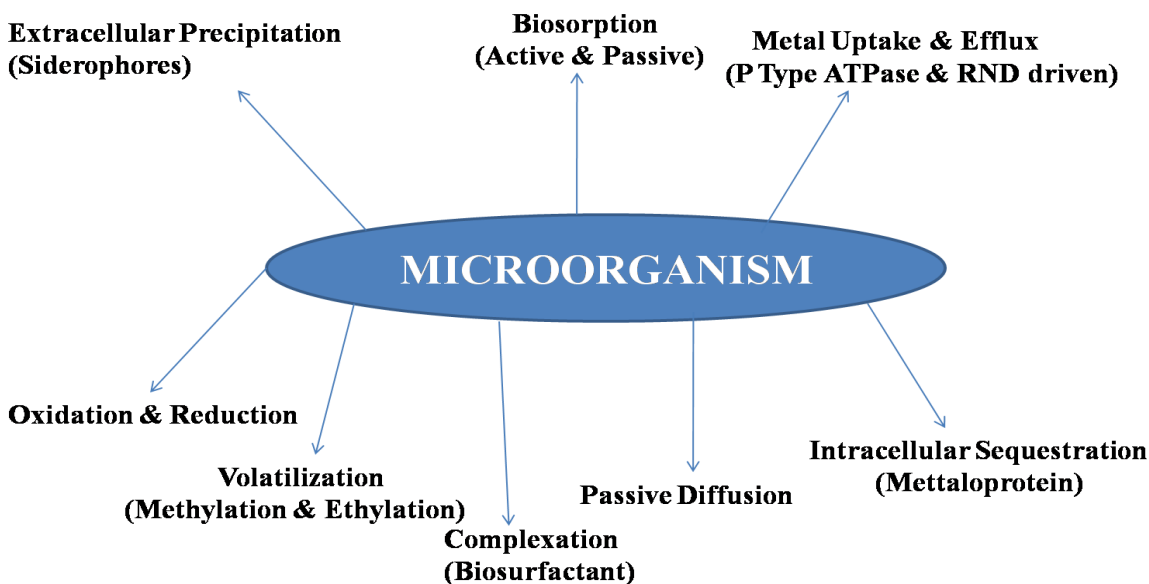
The environmental problems with heavy metals are undestroyable and the most of them have toxic effects on living organisms when exceeds certain concentration. Furthermore, some heavy metals are being subjected to bioaccumulation and may pose a risk to human health when transferred to the food chain [18]. For instance, Minamata disease with neurological damage and fatal deformity was developed in Minamata, Japan due to the effect of mercury toxicity when people consumed continuously mercury contaminated fish from Minamata bay. Metal toxicity is also associated to fatal diseases like birth defects, cancer, liver and kidney damage and may be a host of other maladies also [19].

#### **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 effective, economical and eco-friendly technology for industrial exploitation and pollution free environment. Microorganisms exert their heavy metal detoxification process by valence transformation, extracellular chemical precipitation, or volatilization. The extent of remediation varies noticeably with the metal as well as with the microorganisms. A list of metal resistant microorganisms, their source and metal tolerance is listed in Table 1.

### Microbial Metal Resistance Mechanisms:

When the bacterial cells are exposed to the high concentrations of heavy metals, the metals react within cells with various metabolites and form toxic compounds. Mechanisms for uptake of these metal species are present in the bacterial cell through which heavy metals enter the cell. Microorganisms pose their interaction with different heavy metals following different processes. Heavy metals are utilized by bacterial cells in small quantities in biosynthesis of various metabolic enzymes like cytochrome c oxidase, Kinases etc. However, bacteria in different ecosystems including soil and water are exposed to very high concentration of heavy metals, which exist in soil ecosystem due to its wide application in mining, industry processes and agricultural practices. Consequently, bacteria have evolved several types of mechanisms to defend against the high metal concentration and metal induced biotoxicity. Resistance to metal is the main mechanism of heavy metal remediation. From evolutionary point of view, it is believed that microorganisms could have been evolved heavy metal resistant. The major ways of heavy metal mobilisation and immobilisation by microorganism is shown in Fig.1.



**Fig.1:** Metal processing mechanisms of microorganism.

In general, biotechnological processes with the help of microbes can be categorized into three types through which remediation of heavy metal contaminations in soil can take place: The first one is biosorption (bioaccumulation) process through which microbes concentrate and bind metal contaminants onto its cellular structure [14]; the second is the process of extracellular precipitation and uptake by purified biopolymers [26]; and the third one may be through the process aided by other specific molecules derived from microbial cells [27].

Biosorption is most important process, both ecologically and practically. Extracellular materials immobilizes the metal through the binding of cell surface anionic functional groups with a large number of cationic metal including Cd, Pb, Fe, Zn. The anionic functional group presents in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria and the peptidoglycan, phospholipids, and lipopolysaccharides of gram-negative bacteria are the components primarily responsible for anionic character and metal-binding capability of the cell wall [28].

Active functional groups of extracellular binding materials play the central role in biosorption process. Metal ions become bound to the cell surfaces settled by the binding mechanisms those include electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions and extracellular precipitation or combination of these processes [29]. Functional groups under activated state like acedamido groups in chitin, amine groups in peptidoglycosides, sulfahydral and carboxyl groups in proteins, phosphate, phosphodiester and hydroxyl groups in polysaccharides take part in the biosorption process. 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 [30]. The advantage of biosorption is not only to be functioned under a broad spectrum of conditions like pH,

temperature etc. but also to be found economically feasible due to the cheap raw supplies that can be utilized as biosorbents. Table 2 summarizes basic information regarding the use of bacterial biomass for metal biosorption.

Another mechanism of microbial heavy metal remediation is mediated by the siderophore formation. Siderophores are low-molecular-weight chelating agents (200-2000 Da) produced by bacteria, fungi and plants to facilitate the uptake of iron [26]. 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 [41]. Siderophores can bind heavy metals and reduce bioavailability and metal toxicity. For instance, siderophores forms complex with copper and reduces copper toxicity in cyanobacteria.

Mechanisms of metal resistance in microbes include precipitation of metals as phosphates, carbonates and/or sulfides; volatilization via methylation or ethylation; physical exclusion of electronegative components in membranes and extra cellular polymeric substances (EPS); energy dependent metal efflux systems; and intra cellular sequestration with low molecular weight, cysteine-rich proteins [42,43].

Production and excretion of biosurfactants from microbial cells may render the bioremediation of heavy metals in heavy metal polluted area. Biosurfactant molecules are able to complex metals such as Cd, Pb and Zn [27]. Biosurfactant of anionic nature can capture the metal ions through electrostatic interactions or complexations [44]. 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 [14].

#### **Cadmium (Cd):-**

Cd, amongst all non-essential heavy metals, is perhaps the most attentively attracted due to potential toxicity to humans and relative mobility in soil-plant system [45]. The largest source of anthropogenic atmospheric Cd emissions is metal production followed by waste incineration and other sources including production of nickel-cadmium batteries, fossil fuel combustion and industrial dust generation. Cd pollution of arable soils is primarily caused by wastewater from mines being used to irrigate fields and by emissions from nonferrous metal refineries [46]. Because the risk of human exposure to heavy metals arises mainly from consuming crops grown in polluted soil and from drinking contaminated water, regulations for controlling heavy metal pollution are essential [47]. Exerting toxicity primarily to the kidney Cd can also cause bone demineralization and may impair lung function and increase the risk of lung cancer due to excessive exposure. For instance, Cd uptake is known to have caused itai-itai disease in Japan in 1950s.

Resistance to Cd in bacteria is based on Cd flux. Cyanobacteria have metallothionein like proteins and overexpression of this metallothionein *smt* locus increases the cadmium resistance and its deletion decreases resistance [48]. 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 [49]. Cd<sup>2+</sup> enters the cell of gram negative bacterial cell by CorA and NRAMP (Natural Resistance Associated Macrophage Protein) like uptake systems, binds to thiol compounds, exerting toxicity and is exported again by P-type ATPases, CBA (Cytometric Bead Array) and CDF (Cation Diffusion Facilitation) proteins [50]. In gram positive bacteria this takes place by RND driven trans-envelope and possibly also by CDF transporters [51]. *Zymomonas mobilis* showed resistance to Cadmium concentrations upto 5mM [52].

#### **Mercury (Hg):-**

Hg exists in nature in small amount as it is the sixteenth rarest element on the earth. But, the level of Hg is 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 [53]. The prevalence of Hg toxicity could be visualized by the Minamata disease stated above in this study. 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.

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 [43]. Once outside, however, metallic

Hg may be oxidized again by other bacteria. Hg transports and transformations are regulated by a tightly regulated genetic system named *mer* operon is consisted of four to five structural genes and of regulatory genes. The *mer* system and its regulation followed by a more extensive consideration of the utility of *mer* operon functions in environmental Hg remediation and in monitoring of Hg contamination [54].

#### **Arsenic (As):-**

Arsenic is one of the naturally occurring elements in the earth's crust and a notable element that is present in trace amounts in a healthy human body. The element is widely distributed in nature resulting from weathering, fire, dissolution, volcanic activity and anthropogenic input [55]. High levels of arsenic, however, can cause very grave health problems. Arsenic is used in pesticides, herbicides, wood preservatives and dyestuffs as well as production of arsenic-containing wastes during smelting and mining operations [56]. A major concern for arsenic-enriched environments is the potential for mobilization and transport of this toxic element to ground water and drinking water supplies. Human population can be exposed to arsenic in a number of ways which include ingestion of arsenic in drinking water or food. Major health hazard of As toxicity is hyperpigmentation or keratosis with an increased risk of skin, internal organ and lung cancer [57].

The +3 form Arsenite enters via aqua-glycerolporins (a major intrinsic protein under membrane channel family) and targets a broader range of cellular processes, binding to the thiol groups in important cellular proteins such as pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase [58].

Microorganisms can use methylation as a detoxification strategy for arsenic remediation from the local environment. For example, prokaryotes can produce volatile methylated arsines. "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 had been sequenced [59] and this number will be significantly higher now. ArsC protein is found in the cytoplasm of the microbial cell and mediates the reduction of arsenate to arsenite, with glutaredoxin, glutathione or thioredoxin. *Pseudomonas aeruginosa* (PAR3) showed resistance even at 200 ppm arsenic [60]. An arsenic-chelating metallothionein (fMT) from the arsenic-tolerant marine alga *Fucus vesiculosus* was expressed in *Escherichia coli*, resulting in 30- and 26-fold-higher As(III) and As(V) binding, respectively. *E. coli* cells coexpressing fMT and GlpF completely removed trace amounts (35 ppb) of As(III) within 20 min [61].

#### **Chromium (Cr):-**

Chromium is one of the most widely used metals in industry, such as steel production, alloy preparation, wood preservation, leather tanning, metal corrosion inhibition, paints pigments, metal plating, tanning, electroplating, steel manufacture and other industrial applications [62]. Chromium (Cr) is an essential trace element for all living organisms. Its valence state ranged from -2 to +6. The trivalent form of chromium ( $\text{Cr}^{3+}$ ) is an essential trace element which acts as cofactor for many enzymes in biological system e.g. activation of insulin receptor tyrosine kinase. Hexavalent chromium is easily soluble and 100-fold more toxic than trivalent one. High doses of  $\text{Cr}^{6+}$  have been associated with birth defects and cancer. Even, chronic exposure of  $\text{Cr}^{6+}$  in the form of lead chromate is also found to induce persistent or increasing chromosome damage [23].

Several bacteria have been reported to reduce  $\text{Cr}^{6+}$  that is toxic and mutagenic, to its trivalent form that is less toxic [63]. Bacterial resistance to chromate has been found in several *Pseudomonas* strains. Remediation of chromate ( $\text{Cr}^{6+}$ ) is mainly mediated by chromate reduction to non-toxic  $\text{Cr}^{3+}$  and chromate efflux. Efflux of chromate is regulated by sulphate uptake system and accumulation interferes with sulphate metabolism. Soils and marine sediments contain many facultative and strictly anaerobic bacteria are capable of reducing  $\text{Cr}^{6+}$  to  $\text{Cr}^{3+}$  [64]. Levels of  $\text{Cr}^{6+}$  resistance by other microbes are *Arthrobacter crystallopoites* (500 mg/L), *Pseudomonas spp.* CRB 5 (520 mg/L), *Bacillus maroccanus* Chr A21 (1040 mg/L), *Corynebacterium hoagie* Chr B20 (1144 mg/L), *Bacillus cereus* ES04 (1500 mg/L) [65], *E. coli* ASU7 showed relatively high minimal inhibitory concentrations (MIC) with 65 and 27 Kb and tolerated chromium ranged from 0.48 to 7.69 mM for  $\text{Cr}^{6+}$  and  $\text{Cr}^{3+}$ , respectively [62].

#### **Lead (Pb):-**

Pb has a diversified use in petrol fuel, food cans, cosmetics, paints, ceramics, batteries etc. So it is present in air, dust, soil and water to varying degrees which become exposed to humans through ingestion, inhalation and dermal absorption. Pb is cumulative toxicant that affects neurological, hematological, gastrointestinal, cardiovascular and renal systems of the human body. Microorganisms accumulate  $\text{Pb}^{2+}$  under general terms of biosorption. Import of

Pb<sup>2+</sup> into the cells of microorganisms by uptake system is common for other divalent metal cations and exported by ATP-hydrolyzing efflux systems. In that respect, the operon, *pbr* is the key system for lead resistance by microorganisms [66].

#### **Copper (Cu):-**

Cu is an essential metal for biological system. Except exposure to higher doses Cu does not create toxicity. Cu toxicity is based on its radical character leading to the production of hyperoxide radicals which interact with cell membrane through the binding with thiol compounds [50]. In gram positive bacteria, P type ATPases seem to detoxify Cu via efflux. In some microorganisms Cu resistance system encodes proteins which bind Cu in periplasm or close to the outer membrane [51]. In other study, Cooksey [14] reported that resistance against copper in the plant pathogen *Pseudomonas syringae* was because of the copper accumulation and compartmentalization in the cell's periplasm and the outer membrane and concluded that the protective mechanism against copper in *P. syringae* was due to four types of proteins (CopA, CopB, CopC and CopD). These proteins are encoded by the *cop* operon present on bacterial plasmid and proteins are found in the periplasm (CopA and CopC), the outer membrane (CopB), and the inner membrane and work together to compartmentalize copper away from bacterial cells. In contrast, copper resistance in *E. coli* is dependent upon efflux mechanism to overcome copper stress. The efflux proteins are expressed by plasmid-borne *pco* genes, which are in turn rely upon the expression of chromosomal *cut* genes. Moreover, two *cut* genes (*cutC* and *cutF*) encode a copper binding protein and an outer membrane lipoprotein. Most bacterial species in the metal stressed environment have acquired at least one of the above mentioned protective mechanisms. In addition, the evolution of the bacterial copper resistance occurred through the modification of copper uptake genes found on chromosomes [67].

#### **Manganese (Mn):-**

Manganese is an element essential to the proper functioning of both humans and animals, as it is required for the functioning of many cellular enzymes (e.g. manganese superoxide dismutase, pyruvate carboxylase) and can serve to activate many others (e.g. kinases, decarboxylases, transferases, hydrolases). Manganese can exist in 11 oxidative states; the most environmentally and biologically important manganese compounds are those that contain Mn<sup>2+</sup>, Mn<sup>4+</sup> or Mn<sup>7+</sup>.

This element is used principally in the manufacture of iron and steel alloys and in various products such as batteries, glass and fireworks. Potassium permanganate is used as an oxidant for cleaning, bleaching and disinfection purposes. Other manganese compounds are used in fertilizers, varnish and fungicides and as livestock feeding supplements. The neurological effects of inhaled manganese have been well documented in humans chronically exposed to elevated levels in the workplace [68]. The syndrome known as "manganism" is caused by exposure to very high levels of manganese dusts or fumes and is characterized by a "Parkinson-like syndrome", including weakness, anorexia, muscle pain, apathy, slow speech, monotonous tone of voice, emotionless "masklike" facial expression and slow, clumsy movement of the limbs. In general, these effects are irreversible.

The marine *Bacillus* sp. strain SG-1 produces dormant spores which enzymatically oxidize soluble Mn(II) to insoluble Mn(IV) oxides from coastal marine sediments in which Mn(II) oxidation is catalyzed by a multicopper oxidase, MnxG [69].

#### **Zinc (Zn), Cobalt (Co) and Nickel (Ni):-**

Zn can be found in large quantities in both soil and water and it's production in the world is still rising year-on-year basis. Zn is able to bio-magnify up the food chain through the water-bodies or soil. It is also to be noted that only a limited number of plants has a chance of survival on Zn rich soil. Zinc, an essential trace element is not biologically redox reactive. Hence, it is not used in cellular metabolisms like respiration. However, it is structurally, a vital constituent of several cellular enzymes. Furthermore, it also forms complexes in cells.

In addition, zinc actually, displays comparatively less toxicity to bacterial cells than other heavy metals and it is generally occurs in higher concentrations within bacterial cells. It is due to this reason, bacteria in heavy metal polluted environment accumulate zinc in a fast but unspecific uptake mechanism. Generally, uptake of zinc ions by bacterial cells is coupled with magnesium, and both ions may be transported by similar mechanism [14]. The zinc resistance in bacteria is achieved through two general efflux mechanisms: (I) mediated by a P-type ATPase efflux system and (II) mediated by an RND-driven transporter system. The P-type ATPase efflux system transports zinc ions across the cytoplasmic membrane by the energy released from ATP hydrolysis. In this regard, Garbisu [70]

isolate a chromosomal gene, *zntA*, from *E. coli* K-12 and inferred that the gene *zntA* might be accountable for the zinc and other cations transporting ATPase across cell membranes. In contrast to P-type ATPase efflux system, the RND-driven transporter system does not derive energy through ATP hydrolysis to transport zinc within the bacterial cells. As an alternative, it is powered by the proton gradient across the cell wall specifically, in gram-negative bacteria [51].

**Cobalt** toxicity is quite low compared to many other metals in soil. But, Co can cause health effects on the lungs, including asthma, pneumonia, and wheezing when exposed or breathed in very high levels.

Though **Nickel (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 [71].

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. The 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 *czc CBA* expression [72]. The structural gene region *cnr* which is based on cation efflux is the resistance determinant, composed of a *cnr CBA* structural region [73] that is preceded by a regulatory gene region. Another Co and Ni resistance determinant, *ncc*, was also characterized [49]. Similar to *cnr*, *ncc* is composed of a regulatory gene region followed by the structural region *ncc CBA*.

**Table 1:** Bacterial Metal Tolerance/MIC.

Metal	Bacterial Species	Source	Metal Tolerance/MIC	References
Cd (II)	<i>Pseudomonas aeruginosa</i>	Sludge of food factory	7 mM	[20]
	<i>E. coli</i>	Human faeces	200 µg/ ml	[21]
Co (II)	<i>Pseudomonas aeruginosa</i> PAL106 DQ464061	Garden soil tolerating pesticides	3000 ppm	[22]
Ni (II)	<i>Pseudomonas aeruginosa</i> PAL106 DQ464061	Garden soil tolerating pesticides	5000 ppm	[22]
Cr (VI)	<i>Pseudomonas aeruginosa</i> PAL106 DQ464061	Garden soil tolerating pesticides	10000 ppm	[22]
	<i>Brevibacterium casei</i> .	Chromite contaminated sites	500 mg/l	[23]
Mn (II)	<i>Pseudomonas psychrophila</i> (T); E-3 AB041885	Garden soil tolerating pesticides	6000 ppm	[22]
	<i>Planococcus rifietensis</i> (T); M8 AJ493659	Garden soil tolerating pesticides	6000 ppm	[22]
Cu	<i>Pseudomonas</i> spp.	Industrial waste/Rolling Mill	10mM	[24]
	<i>E. coli</i>	Human faeces	1750 µg/ ml	[21]
	<i>Bacillus subtilis</i>	Agricultural soil	3 mM	[25]
Zn	<i>Pseudomonas</i> spp.	Industrial waste/Rolling Mill	20mM	[24]
	<i>Bacillus subtilis</i>	Agricultural soil	5 mM	[25]
Pb	<i>E. coli</i>	Human faeces	3200 µg/ ml	[21]
	<i>Bacillus subtilis</i>	Agricultural soil	5 mM	[25]
Hg	<i>E. coli</i>	Human faeces	54.3 µg/ ml	[21]
	<i>Bacillus subtilis</i>	Agricultural soil	1 mM	[25]

**Table 2:** Biosorption by bacterial biomass ( $\text{mg g}^{-1}$ )

Metal	Bacterial Species	pH of Solution	Biosorption Capacity ( $\text{mg g}^{-1}$ )	References
Cr (III)	<i>Pseudomonas aeruginosa</i> AT18	7.72	200	[31]
	<i>Staphylococcus saprophyticus</i> BMSZ71	5	22.06	[32]
Cr (VI)	<i>Arthrobacter</i> sp.	5	175.87	[33]
	<i>Escherichia coli</i>	4.6-5.1	4.6	[34]
	<i>Pseudomonas fluorescens</i> TEM08	2	40.8	[36]
Cu (II)	<i>Arthrobacter</i> sp.	5	175.87	[33]
	<i>Geobacillus toebii</i>	4	48.5	[35]
	<i>Pseudomonas aeruginosa</i> AT18	6.25	86.95	[31]
	<i>Bacillus anthracis</i> PS2010	7-8	2.03	[37]
Cd (II)	<i>Geobacillus thermoleovorans</i>	4	38.8	[35]
	<i>E. coli</i>	5.6-6	10.3	[34]
	<i>Pseudomonas</i> sp.	9	0.078	[38]
	<i>S. saprophyticus</i> BMSZ71	7	54.91	[32]
	<i>Bacillus anthracis</i> PS2010	8	3.41	[37]
Fe (II)	<i>E. coli</i>	2.7-3.5	16.5	[34]
Hg (II)	<i>Staphylococcus saprophyticus</i> BMSZ71	6	78.17	[32]
Ni (II)	<i>Geobacillus thermoleovorans</i>	4	42	[35]
	<i>Pseudomonas aeruginosa</i> AT18	2	40.8	[36]
	<i>R. opacus</i>	5	7.63	[39]
Zn	<i>Geobacillus toebii</i>	5	21.1	[35]
	<i>Pseudomonas aeruginosa</i> AT18	7.72	56.4	[31]
	<i>Bacillus anthracis</i> PS2010	5	5.22	[37]
Pb	<i>Aeromonas hydrophila</i>	5	163.3	[40]
	<i>S. saprophyticus</i> BMSZ71	5	184.89	[32]
	<i>Bacillus anthracis</i> PS2010	5	6.44	[37]
Co	<i>Bacillus anthracis</i> PS2010	8	4.75	[37]

**Conclusion:-**

Bioremediation of heavy metal contaminated sites is essential for ensuring safe and secure environment that will promote healthy lifestyle around the globe. Bioremediation is considered as eco-friendly and cost-effective technique having high public acceptance. As compared to other techniques, this method is easy to carry out at any site. However, there are certain flip sides associated with it as well. For instance, it is effective on limited range of contaminations, the time scale involved for complete restoration of the environment is relatively long and the achieved level of residual contamination level may not always be appropriate.

It is noted that, though heavy metal resistant strains have to be isolated from environmental or clinical sources, microbial chromosomal mutation can be produced in the laboratory [74]. Thus, our hope for the large scale bioremediation of toxic metals resides on the further genetic manipulation of the metal resistant strains in hand for the development of hyper-absorber, hyper-accumulator or hyper-biosurfactant producing strains *etc.*, which in turn can contribute to a green and eco-friendly environment. Attempts have been made to use GEM to increase heavy-metal remediation in contaminated sites. However, strict regulatory guidelines by the Environmental Protection Agency make the use of GEM difficult, and a better understanding of how these microbes work and their safety and environmental containment is needed before they will be used for bioremediation [75,76,77].

A number of microbial metal bioremediation approaches to combat heavy metal pollution are established, but no wide spread and large scale use is on sight. With heavy metals accumulating alarmingly high in heavily populated areas of many parts of the world, upgradation of the existing microbial bioremediation processes to commercial level by making the processes faster, recyclable and taking control over them is the major challenge ahead. Polluted

environments often contain more than one metal. Therefore, complex approaches such as combination 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 life in our soils and water. It is prudent to carry out future research and development for using inexpensive, eco-friendly and easily available nutrients for cleaning up heavy metals from contaminated soil and for planning and executing a successful bioremediation program.

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