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HEAVY METAL TOLERANCE MECHANISMS IN MICROORGANISMS - A REVIEW

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ABSTRACT

Contamination of environment because of use of organic and inorganic chemicals and their accidental leakage has reached up to the level of ecosystem. In this regard several conventional techniques have been put to use but for environmental restoration microbial bioremediation has been the most understood biotechnological process. In biotransformation of heavy metals their role to convert heavy metals to innocuous forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal- contaminated sites. Microbes have various mechanisms of metal sequestration which are involved in the resistance and their tolerance to excess concentrations of heavy metals in the environment thus aiming to avoid the buildup of excess metal levels. Studies have been carried out throughout the world and new bacterial strains that have plasmid linked degradation / reduction ability are discovered. Research carried out in this area, regarding the role of microorganisms in remediation of heavy metals is discussed in this review paper.

KEY WORDS : Heavy Metal, Bioremediation, Microbial mechanisms, Heavy metal

INTRODUCTION

Considerable irremediable damage to environment has been caused because of its contamination due to various anthropogenic and industrial activities. In recent years, discharges of large volumes of hazardous waste, heavy metals, metalloids and organic contaminants into the environment due to Industrialization and technological advancement have resulted in increasing burden on the environment. The build-up of such contaminants in environment continues to create serious global health concerns. Since they cannot be degraded and have the tendency to bioaccumulate, they can pose adverse impacts on all segments of environment including biosphere, atmosphere, hydrosphere and lithosphere. Heavy metal Contamination of the environment has increased to a level beyond the recommended limit therefore has adversely affected the natural ecosystem to the detriment of man (Gaur *et al*., 2014; Dixit *et al*., 2015; Tak *et al*., 2013). Linked inextricably to the overall quality of the environment contamination of soil, surface and groundwater has affected the quality of life on earth is. Also in the urban environment of the developing countries, like China (Wong *et al*., 2003) and India (Khillare *et al*., 2004; Sharma *et al*., 2008)unorganized and fast industrialization and urbanization have contributed to the elevated level of heavy metals.

Since they do not biodegrade and bioaccumulate in nature these heavy metals are the subject of concern due to their high toxicity (Gautam *et al*., 2014; Wai *et al*., 2012). Heavy metals like lead, cadmium, arsenic, mercury etc can result in acute or chronic damage to nervous system, bone defects, heart disease, cardiovascular disease, cancer etc.. Apart from being hazardous to human health, a number of environmental pollutants cause high-level degradation impacts on various ecosystem components as well. in ensuring a functional and balanced ecosystem environmental protection is an important factor Many examples from past can be taken where heavy metal contamination has resulted into disaster like an entire Japanese town , Minamata, got affected by mercury poisoning due to consumption of fishes from water contaminated by a petrochemical plant. Such incidents have increased awareness among people ultimately leading to development of new approaches to minimize or even eliminate metals present in the environment.

Before the heavy metals are discharged discharge into the environment many different physicochemical and biological processes are usually employed to remove them from industrial wastewaters (Fomina and Gadd, 2014). Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, osmosis, evaporation, and sorption are expensive to put into operation and use, particularly when the metal concentration is low, technically difficult to operate and harmful to soil microbial diversity (Hinchman *et al*., 1995). On the other hand bioremediation which is eco-compatible and uses the inherent biological mechanisms of microorganisms and plants to eradicate hazardous contaminants even when present in very low concentrations where physicochemical removal methods fail to operate proves to be an efficient method. Since microorganisms can adapt to extreme conditions bioremediation by microbes is considered very useful. As an alternative strategy to conventional treatments several studies have been conducted which reveal that for the uptake of heavy metals in polluted waters microorganisms play an important role and that this action involves accumulation or resistance (Tsezos and Volesky, 1981; Gadd and White, 1993; Texier *et al*., 1999).

Heavy metals sources and impacts

The term "heavy metals" according to the definition, applies to the group of metals and metalloids with atomic density is greater than 4 g/cm^3, or is 5 times or more, greater than water density (Hawkes, 1997). Based on their biological functions and effects metals have been classified into three classes (Roane and Pepper, 2000):

- Essential metals with known biological functions eg. Na, K, Mg etc.
- Toxic metals eg. Cd, Hg, Ti, Pb, Al etc.
- Nonessential, nontoxic metals with no known biological effects Rb, Cs, Sr, and Ti (Gadd, 1988).

Also can be either nutritionally or non nutritionally essential, depending on their involvement in normal cell functions (Overhoff and Forth, 1978; Chang *et al*., 1996).

Although heavy metals such as Cu, Cd, Hg, Pb, etc., are natural components of the earth's crust but these become concentrated due to anthropogenic activities and through various means like inhalation, diet and manual handling can enter plant, animal and human tissues. They interfere with various cellular components. Originating from diverse sources the availability or entry of heavy metals into the ecosystem may either be by natural or anthropogenic sources.

Apart from georganic and atmospheric sources most of the environmental contamination and human exposure result from other sources like industrial, agricultural, pharmaceutical, domestic effluents, etc. (Bradl, 2002). In point source areas such as mining, foundries and smelters, and other metal-based industrial operations environmental pollution has been reported very prominent (Bradl, 2002; He *et al*., 2005; Fergusson, 1990). Metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water are also responsible for contamination of Environmental (Nriagu, 1989). Accumulation of one or more of the heavy metals might occur above defined limits high enough to cause adverse effects to human health, plants, animals, ecosystems, or other media in most soils of rural and urban environments due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by anthropogenic activities (D'Amore *et al*., 2005).

As heavy metals are present in trace concentrations (ppb range to less than 10ppm) in various environmental matrices these are also considered as trace elements (Kabata- Pendia, 2001). Even though they are present in traces they can pose serious threat to all organisms. Due to their toxic effects in plants, human and food these are kept under environmental pollutant category. For their potential to inflict acute organ damage, among the 10 listed chemicals by WHO of major public concern are Cadmium, lead, mercury and arsenic (Tchounwou *et al*., 2012). The impact of some toxic metals has been studied on human health (Forstner, 1995). Usually toxic metals cells by acting as anti metabolites or forming precipitates or chelates with essential metabolites are. Sources of some of the heavy metals in the environment with their toxicity to life forms are enlisted below:

Bioremediation and its need

The overall quality of the environment largely affects and is inseparably related to the quality of life on earth. A tremendous variety of byproducts are produced by human activities like mining, manufacturing, agriculture, and other industrial processes. Organic and inorganic residual compounds are released by such processes. These compounds may be inert and harmless, or can be highly destructive and toxic to the environment. A considerable irreparable damage to all ecosystems has been caused by heavy metals from such activities which have led to environmental contamination. To people living near these contaminated sites these metal pollutants pose adverse health effects. Diarrhea, anemia, diminution mental capabilities, headaches etc. can be caused on exposure to metals such as mercury, lead, and arsenic. Permanent kidney and brain damage can be caused due to chronic contact to these pollutants. These are not only hazardous to human health, but also adversely affect fauna and flora.

The problems associated with contaminated sites as their numbers are continuously increasing have become a worldwide issue. These contaminated sites have become a matter of concern as these are becoming potential threat to human health. Hence for the enhancement of the various ecosystems the importance to get rid of heavy metal pollution is understood.

Table 1. Heavy metals, their sources and impacts on humans

Metal	Sources	Impact on Humans	References
Arsenic (As)	Atmospheric deposition, smelting, thermal power plants, fuel burning, Pesticides, mining, combustion of coal, tobacco smoke	Leads to severe vomiting, diarrhea, causes birth defects, gastrointestinal damage, brain damage, cardiovascular and respiratory disorder,	(ATSDR, 2007)
Beryllium (Be)	Volcanic dust,Coal and oil combustion, nuclear power,	Cancer, Allergic reactions, heart diseases, affects lung	(Gordon and Bowser, 2003)
Cadmium (Cd)	Welding, pesticide, fertilizer, mining, refining	Cancer, lung insufficiency, hypertension ,Kidney damage, bronchitis, Gastrointestinal disorder	(Singh *et al*., 2016)
Copper (Cu)	Mining, electroplating, smelting operations	Stomach and intestinal irritation, anemia, liver and kidney damage	(Salem *et al*.,2000)
Chromium (Cr)	Metallurgy, paints and pigments, cement, steel fabrication , pulp and paper production	Carcinogenic, kidney and liver damage nausea, vomiting, producing lung tumors, damage too nerve tissue.	(Salem *et al*., 2000)
Lead (Pb)	Used in various industrial processes, lead acid batteries, used in paints, e-waste, smelting operations, used in ceramics, fuelcombustion	Vomiting, thirst, nausea, anemia, kidney damage, miscarriages, disruption of nervous systems, brain damage,	(ATSDR, 2007)
Mercury (Hg)	Mining, smelting, fluorescent lamps, hospital waste (damaged thermometers, barometers), used in electrical appliances.	Tiredness, allergic reactions and headaches, damage to brain functions, birth defects and miscarriages	(ATSDR, 1999)
Nickel (Ni)	Paints,porcelain enameling, Electroplating	Headache, lung and nasalcancer, cardiovascular diseases, kidney diseases, nausea, dermatitis,	(Fashola *et al*., 2016; Chibuike and Obiora, 2014)
Zinc (Zn)	Smelting, electroplating Mining, oil refineries	Kidney and liver failure, depression, gastrointestinal irritation, fever, prostate cancer, vomiting	(Chibuike and Obiora, 2014; Gumpu *et al*., 2015)

Several techniques aiming to either completely destroy the pollutants if possible, or at least to transform them to non toxic forms are used for remediation of these contaminated sites. Although these are effective to some levels, yet these have several drawbacks, principally their technological complexity. Also due to the high solubility of most heavy metal salts in solution these separation techniques are very challenging. Hence due to drawbacks related to these methods a need to evaluate alternative techniques have resulted in harnessing modern-day bioremediation process as a suitable alternative.

Bioremediation is a natural process that offers the possibility to destroy or render harmless various contaminants using the ability of plants and microorganisms and/or their derivatives (enzymes or spent biomass) as these organisms carry out their normal life functions (Kumar *et al*., 2011; Sharma, 2012).

As compared to the other physico-chemical methods this process is considered an economical, eco-friendly, versatile and efficient way of dealing with environmental pollutants (Kumar *et al*., 2011; Sharma, 2012). Although a great range of living organism can perform bioremediation, microbial remediation has corroborated to be the most preferable and efficient aspect as these are highly diverse, readily available, rapidly characterized and they can use noxious elements as their nutrient source.

It is possible to completely destroy target organic pollutants without contaminants being transferred from one form to another or one medium to another as it can be done *in situ* and *ex situ.*

Apart from physicochemical characteristics of the environment, other factors on which the efficiency of bioremediation depends include chemical nature and concentration of pollutants. Also the availability of pollutant to microorganisms affects the bioremediation process (El Fantroussi and Agathos 2005). In determining the survival of microorganisms and composition of the hydrocarbons temperature among all the physical factors is the most important one (Das and Chandran, 2011).

Mechanism of Microbial Detoxification of Heavy Metal

In response to metal resistance in the environment microorganisms have adopted have various mechanisms to interact and survive in their presence. Some of these approaches include accumulation, use of enzymes production of exopolysaccharide (EPS), synthesis of metallothioneins, by reducing their bio-availability or toxicity through biomethylation and transformation.

Microorganism use major mechanical means to resist heavy metals which include methylation, enzymatic decrease, metal-organic complexion, metal efflux pumps, demethylation, intracellular and extracellular metal sequestration exclusion by permeability barrier, and production of metal chelators like metallothioneins and bio surfactants (Ramasamy *et al*., 2006).

Cell surfaces have anionic functional groups present on them that bind with cationic metals some of which include cadmium, lead, zinc and iron. The binding of Pb(II) by *Bacillus* sp. ATS-2 involve the presence of carboxyl and hydroxyl groups, along with nitrogen based bio-ligands including amide and sulfonamide (Cabuk *et al*., 2006). In Gram-positive bacteria, peptidoglycan along with teichoic and teichuronicacids are responsible for binding of lead whereas I n Gram-negative bacteria, a important component of the outer membrane the macromolecule lipopolysaccharide binds with metal, while (Beveridge and Fyfe, 1985).

This phenomenon of Binding of metals with microbial cells is ecologically important as it plays important role in the distribution of metals and practically, this ability of microbes to sorb metals is being implemented for the purpose of bioremediation

Increasing industrialization and Fast economic development throughout the world has caused the release of a wide range of pollutants contributing towards environmental pollution this has created the immense pressure to explore the field microbial metal resistance and remediation. Hence in reducing the level of contamination in the environment are Microorganisms happen to play an important role as they have evolved ingenious mechanisms of metal detoxification and resistance. In microorganisms Metal resistance involves some mechanisms which are plasmid-encoded systems as well as general resistance toward diverse metals.

Hence there are two mechanisms of metal resistance:

- General mechanism of metal resistance
- Metal-dependent mechanism of metal resistance

Present naturally in microorganisms following are general mechanism of metal resistance:

Siderophore

Produced by microorganisms' under condition of iron deficiency which enhance uptake of iron to microorganisms siderophores are small organic molecules. Under iron-deprived condition these are synthesized by both gram-positive and gram-negative bacteria and their prime function is to capture the insoluble ferric iron from different habitats (Tian *et al*., 2009; Saharan and Nehra, 2011). Siderophore tightly binds with iron (Fe^{+3}) first and then using the specific siderophore receptors this siderophore-iron complex through the cell membrane moves into the cell. Although siderophores have a high affinity (Kb'1030) (Garrison and Crumbliss, 1987) for ferric iron but with a lower affinity they also form complexes with metals other than Fe like aluminium, copper etc. These are of 3 types based on the oxygen ligands for Fe (III) coordination namely, hydroxamates, catecholates, and carboxylates.

Even though the prime role of siderophores is to chelate ferric iron, these by binding to wide array of toxic metals, e.g., Cr^{3+}, Al^{3+}, Cu^{2+} and Pb^{2+} also play important part in detoxifying heavy-metal-contaminated.samples. For example *Pseudomonas azotoformans* produced siderophore which was associated with the removal of arsenic from contaminated soil (Nair *et al*., 2007) *Pseudomonas aeruginosa*, produced a siderophore Pyochelin which can chelate a number of metals like Mn^{2+}, Al^{3+}, Cd^{2+}, Ag^{+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} and prevents their entry in to the bacteria (Braud *et al*., 2009b).

Exopolymer Binding

A complex mixture of biopolymers such as polysaccharides, carbohydrates, nucleic acids, and fatty acids, lipids, etc. microbial origin Extracellular polymeric substances (EPS) are common in natural environments. Number of microbial processes such as bacterial secretions, shedding of cell surface materials is responsible in EPS formation. Although its production is increased in the presence of stressful conditions, including due to heavy metals but it mainly depends on factors, like microbial species, presence of nutrients and environmental conditions (Pal and Paul, 2008; Neal *et al*., 2007). For a few metals such as lead, cadmium and uranium microbial exopolymers act as efficient metal binders Some of the microorganisms that have been identified as genera of EPS-producing ones are *Agrobacterium* spp., *Xanthomonas campestris*, *Bacillus* spp., *Alcaligenes faecalis*, *Zygomonas mobilis*, *Leuconostoc*, *Pseudomonas* spp. and *Acetobacter xylinum*, (Donot *et al*., 2012). In addition to heavy metal binding they provide aid in cell-to-cell aggregation, adhesion to substratum and protection from dessication, phagocytosis and parasitism. They form complexes with metal cations as they are polyanionic in nature which results in metal immobilization within the exopolymeric matrix. The formed complexes are the result of electrostatic interactions between the metal ligands and negatively charged components of biopolymers. Expolymer binding of heavy metals have been reported in some Bacterial genera such as *Staphylococcus aureus*, *Micrococcus luteus*, and *Azotobacter* spp. (Maier *et al*., 2009).

Biosurfactant Complexation

These are substances that can either be produced extracellularly or as part of the cell membrane by yeast, bacteria, fungi or marine microorganisms. To overcome pollutant-related stress by microorganisms the production Biosurfactants is considered as an important strategy (Bezza and Chirwa, 2016). Based on the molecular weight biosurfactants can be divided into-low-molecular-weight (LMW) that include macromolecules groups like proteins, lipopeptide, phospholipids and glycolipids and high-molecular-weight (HMW) are generally polyanionic hetero polysaccharides containing proteins and polysaccharides. While the former lower surface and interfacial tensions efficiently and the latter bind tightly to surfaces. These have unique metal (loid) binding capacities and selectivity (Arjoon *et al*., 2013) and they undergo complexation with metals such as cadmium, lead, and zinc was shown by recent research reports (Miller, 1995). Observations have also revealed that complexed metal becomes less toxic to cell through biosurfactant complexation. Some studies showed that from metal-contaminated environments these biosurfactant-producing microorganisms can be isolated in greater diversity than from uncontaminated ones (Miller, 1995).

Precipitation by metal reduction

Common metabolic by-products that result in metal reduction can influence metal bioavailability and the solubility of metals further decreases through such reduction. Precipitation of non-dissolved phosphates, carbonates, and sulfides of heavy metals such as arsenic, cadmium, chromium, etc. is stimulated by microbial production of phosphate, H_2S, and CO. It has been reported in the presence of oxygen that through enzymatic action *Citrobacter spp*. produce phosphates which leads to precipitation of lead and copper while precipitation of metals is caused by *Desulfovibrio spp*. in the absence of oxygen, (Roane and Pepper, 2000). During the anaerobic fermentation of cellulose organic acids is produced, which may be used as a source of reduced carbon for sulfate reduction and further precipitation of metals (EPA, 2006).

Metal-dependent mechanism of metal resistance Biosorption

To remediate industrial effluents and to recover metals contained in other media Biosorption of heavy metals by microbial cells has been recognized as a potential alternative. Biosorption the process defined as sorption of metallic ions from solutions by live or dried biomass as passive uptake through surface complexation onto the cell wall and surface layers provide the base for new biosorption technology for metal removal and recovery (Inoue *et al*., 2017). Biosorption mechanisms according to the dependence on the cell's metabolism, can be classified into, metabolism-independent biosorption, and metabolism-dependent biosorption. Metabolism-dependent biosorption comprises sequestration, redox reaction, and species-transformation methods and is exhibited by living biological material while Metabolism independent biosorption mostly occurs on the cells exterior and in biomass consisting of dead cells (Vijayaraghavan and Yun, 2008; Godlewska-Zy³kiewicz, 2006). Biosorbent dosage, pH value, temperature, initial solute concentration and biosorbent size are some of the factors that influence biosorption also presence of functional groups in the cell wall which include phosphonate, amine and hydroxyl groups play important role in the process (Vijayaraghavan and Yun, 2008; Abdi and Kazemi, 2015).

Extracellular Sequestration

Towards metal contaminants, microorganisms have deployed certain non specific mechanisms of resistance through extracellular metal defense. Some of these extracellular defenses have been helpful as through this the metal is neutralized outside the cell as of which the bacteria may be retrained. Extracellular sequestration is the accumulation of metal ions as insoluble compounds by cellular components in the periplasm or complexation of metal ions. Cell wall and the outer membrane act as sink for metals as through interactions with proteins, carbohydrates metals may bind to its surface. Copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) are produced by Copper-resistant *Pseudomonas syringae* strains which bind copper ions and microbial colonies (Cha and Cooksey, 1991). Metal precipitation and secretion of low molecular weight ligands are another way of extracellular sequestration. Under anaerobic conditions hydrogen sulfide from thiosulfate is generated by *Klebsiella planticola* strain and precipitated cadmium ions as insoluble sulfides (Sharma *et al*., 2000). Precipitation of soluble divalent lead as complex lead phosphate salt was done by *Vibrio harveyi* strain (Mire *et al*., 2004).

Intracellular Sequestration

In order to reduce toxicity of metal the microbial cell exhibit several response to metal sequestration in Intracellular regions as well as periplasmic spaces. This involves accumulation, precipitation, redox transformation, repair etc. By interacting with surface ligands accumulation of metals within microbial cells takes place followed by slow transport into the cell. This feature has been and this ability of bacterial cells is exploited in practices like exploite in mining practices, particularly in management of effluent treatment lagoons. Uranium has been shown to accumulate rapidly in cells of *Saccharomyces cerevisiae* and *P. aeruginosa*. Another way of handling on heavy metals is metal precipitation by compartmentalization which is done by precipitating metals as metal oxide, metal sluphide etc.. Also some metal undergo redox transformations in cell altering the chemical reactivity of metal and converting it into less innocuous form thus altering redox state of metal (Silver and Phung, 1996). Another way of handling heavy metals in microorganisms is using metabolic By-Pass which is a enzyme mediated metal detoxicfication response. With the help of cysteine-rich low molecular weight proteins Cadmium-tolerant *P. putida* strain possessed the ability of intracellular sequestration of copper, cadmium, and zinc ions (Higham *et al*., 1986) whereas in *Rhizobium leguminosarum* cells intracellular sequestration of cadmium ions by glutathione was revealed (Lima *et al*., 2006).

Metallothioneins

Metallothioneins are low molecular weight (6-7 kDa), cysteine rich proteins which play a important role in management of stress in microbial cell and heavy metal metabolism. This feature of having high cysteine content of up to 30% and consisting of up to roughly 90 amino acids allows them to bind large numbers of metal ions with a preferentially d^{10} electron configuration, which are organized in metal-thiolate clusters (Blindauer and Leszczyszyn, 2010; Vašák and Meloni, 2011). These are divided into three classes: Cys-Cys, Cys-X-Cys and Cys-X-X-Cys Motifs (where, X denotes amino acid) based on cysteine content and structure metallothioneins. Involved in metalloregulatory processes which include cell growth and reproduction these through the thiol group of its cysteine also have the capacity to bind both physiological such as zinc, copper and xenobiotic such as mercury, arsenic heavy metals (*Sigel and Sigel, 2009*). Having various biological functions, such as essential metal homeostasis, cellular antioxidative defense, and heavy metal detoxification these also maintains redox status by binding and releasing metals (mainly zinc under physiological conditions). WI-1 bmtA gene that encode metallothionein has been found in *Pseudomonas aeruginosa* which bind with Pb(II) also in *Bacillus megaterium* a metallothionein like protein was reported that binds Pb(II) (Naik *et al*., 2012; Roane, 1999).

Methylation of Metals

This enzymatic mechanism involves the transfer of methyl groups (CH_3) to metals and metalloids and since only some metals are methylated this mechanism is considered a metal dependent mechanism of resistance. The compounds formed as a result of Methylation differ in their solubility, toxicity and volatility (Gadd, 2004). As a result of increased lipophilicity this mechanism generally increases metal toxicity, hence increased permeation across cell membranes however in certain metals like selenium there is decrease toxicity as a result of methylation. According to reports the methylated and inorganic forms of Se and Cd are highly toxic (Tabak *et al*., 2005) and the inorganic forms of As are more toxic than methylated species . In metal remediation Microbial methylation plays a significant function as it is effective in decreasing metal toxicity as it facilitates metal diffusion away from cell. Tested at a very small scale methylation was able to remove arsenic from soils and sediments (Mattison, 1993). Bio methylation lead (Pb) to dimethyl lead and of selenium (Se) to volatile dimethyl selenide was witnessed in polluted top soil (Ramasamy *et al*., 2006).

Ion Exchange

One of the mechanisms that help cell wall components to bind heavy metal ions is ion exchange. It involves the exchange of naturally occurring cellular ions such as K^+, Na^+ that are present in cell wall matrix with metal ions like Cu^{2+}, Zn^{2+}, Pb^{2+}. Resulting in the biosorptive uptake of heavy metals the alginates of marine algae that occur as salts of K^+, Na^+, Ca^{2+} and Mg^{2+} (Rubinelli *et al*., 2002) can exchange with counter ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} (Kuyucak and Volesky, 1988). Also to adsorb Co^{2+} the non-living brown algae (*Ascophyllum nodosum*) exchanges the original cell wall adsorption of K^+, Ca^{2+} and Mg^{2+} (Freitas *et al*., 2006). Ion exchange can occur with complexation as revealed by some studies (Brunetti *et al*., 2012). In the process of adsorbing Cu^{2+} yeast releases approximately 60% of Mg^{2+} slowly and 70% of K^+ rapidly (Brady and Duncan, 1994).

CONCLUSION

Due to a series of anthropogenic activities the problem associated with heavy metal pollution is continuously on rise. As a result of which research dealing with phytotoxicity of these contaminants has intensified. This has lead to development of several removal techniques aiming at lowering metal concentrations ,but the potential of microorganisms using different mechanisms to counter the harmful effects and cleaning-up of the polluted sites has drawn the attention of many researchers. These mechanisms are unique in their specific requirements. Apart from other requirements the success of these mechanisms are chiefly determined by the kind of organisms and the contaminants involved. Notable advancement has been made in the identification and understanding of the key components that ensure heavy metal tolerance to microbes like cell wall and exopolysaccharide, siderophore, metallothionein proteins etc.

Hence the processes mentioned are of considerable current interest as they are involved in reducing toxicity and also help to understand the process by which metal tolerance is manipulated by microorganisms. This would help in better approach for identifying microorganisms for bioremediation purposes. However in large scale the application of this type of bioremediation still remains a challenge encouraging a preventive approach to metal pollution problems and an in depth study for extracting the best out of microorganisms as "heavy-metal contamination reliever".

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