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Review Article

Bioremediation of some frequently encountered non essential heavy metals: a review

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Abstract
Industrialization, urbanization and other anthropogenic activities have led to a rapid increase in metal pollution. Some microorganisms are capable of developing strategies to resist the elevated levels of various toxic metals. Therefore, the metal resistant microorganisms especially bacteria can be applied as bioremediation agents. This review deals with cadmium, mercury, lead and chromium resistant bacteria to describe their roles in bioremediation of the pollutants and highlights the potential of different bacterial species for treatment of the polluted environment.
Key words: Bacterial remediation, metal resistant bacteria, heavy metals

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INTRODUCTION

Aquatic pollution is growing continually in relation to an unrestricted rise in population growth, urbanization, industrialization and other diverse anthropogenic activities in developing countries (Hanif *at al.,* 2005; Khan *et al.,* 2009). Industrial operations such as mining, electroplating, smelting, manufacturing of paints and alkaline storage batteries, fossil fuel combustion and paint manufacturing are responsible for release of various pollutants including heavy metals into soil and water (Ansari *et al*., 2004; Benson, 2006; Rajaganapathy *et al.*, 2011).

Heavy metals are non-biodegradable and accumulated in microorganisms, plants, animals and human and may lead to many physiological and metabolic disorders (Hashem and Abed, 2002; Ozer and Pirincci, 2006; Subroto *at al.,* 2007; Bhattacharya *et al.,* 2008; Rani and Goel, 2009; Ogbo and Okhuoya, 2011). The waste water polluted with heavy metals is responsible for reducing microbial activity and thus badly affects biological treatment processes of industrial waste water (Braam and Klapwijk, 1981; Waara, 1992; Ajmal *et al.,* 1996; Madoni *et al.,* 1996).

Heavy metals are a group of metals whose atomic density is larger than 5 g/cm^3 (Nies, 1999). Heavy metals can be grouped as essential and nonessential. Essential heavy metals including zinc, cobalt, copper, nickel, and manganese are those needed in trace amounts, and are vital for development of organisms but their high concentrations have lethal effects on various organisms (Filali *et al.,* 2000).

On the other hand, some other heavy metals that are non-essential (lead, mercury, chromium and cadmium *etc.*) have no biological roles and even at a very low concentration cause damaging effects on the organisms (Huang *et al.,* 2004; Roane and Pepper, 2000). Toxicity of non essential metals comes about through the dislocation of essential metals from their binding sites (Nies, 1999; Bruins *et al.*, 2000). In addition, both essential and nonessential metals at high levels, may cause harm to plasma membranes, disturb enzyme functions and may lead to destruction of DNA's structure (Bruins *et al*., 2000).

Trace of some heavy metals, such as cadmium (Cd^{2+}), chromium (Cr^{6+}), copper (Cu^{2+}), lead (Pb^{2+}) may affect growth of plants and microbes negatively by increasing iron deficiency symptoms (Yoshihara *et al*., 2006; Christian *et al*., 2008). Furthermore, they can restrain the activity of enzymes by combining with proteins. Thus the permanent existences of copper (Cu^{2+}), cadmium (Cd^{2+}), chromium (Cr^{6+}) and lead (Pb^{2+}) in polluted ecosystems is threaten the health of entire human beings all

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the time (Nogaw and Kido, 1996). Therefore, heavy metals must be removed from industrial waste before discharge.

A great number of physicochemical strategies, such as filtration, ion exchange, evaporation recovery electrochemical treatment, chemical precipitation, reverse osmosis, oxidation/ reduction and membrane technology, have been established for eliminating heavy metals from the industrial polluted/waste water (Xiao *et al*., 2010). However, most of them are low efficient, expensive, labor-demanding operational or scarcity of selectivity in the treating procedure (Chen *et al*., 2008; Tang *et al*., 2008).

Due to these constrains, an alternative treating techniques, such as those using microbial biomass are gaining more serious considerations (Volesky, 1999; Volesky, 1999a; Slaveykova *et al*., 2003; Nanda *et al.,* 2011). Bioremediation is a process that utilizes bacteria, fungi and plants to break down/reduce pollutants during the course of activities these organisms perform their routine life functions. In other words, bacterial bioremediation is the process in which metal resistant bacteria detoxify the contaminants (White *et al.,* 2006). Metabolic activities of these organisms are able to render the pollutants innocuous or less toxic forms in most cases by using contaminants as an energy source. Some metal resistant bacteria are able to reduce very toxic soluble forms into less toxic forms of metal.

The capability of bacterial stains to grow in the presence of toxic metals would be helpful in the waste water management (Prasenjit and Sumathi, 2005; Munoz *et al.,* 2006). The tolerance of heavy metals by *P. aeruginosa* AT18 was investigated by Silva *et al.* (2009). Microorganisms can only alter the speciation of metal contaminants.

Generally, the higher concentration of metals above threshold levels has harmful effect on the life processes of microbial communities in environment. But microorganisms exposed to the varying concentrations of toxic heavy metals may develop resistance against the toxic materials continually coming into their surroundings. Such metal resistant microorganisms may be used as effective bioremediation agents (Khan *et al.,* 2009; Ahemad, 2012).

This review highlights overview of persistence of cadmium, mercury, chromium and lead contaminant in environment, their toxicity and bacterial role in bioremediation.

Persistence of chromium in environment

Chromium presents a number of oxidation states from Cr^{2+} to Cr^{6+}. Usually, chromium exists as Cr^{3+} or Cr^{6+}. It naturally occurs in earth crust predominately in Cr^{3+} form and is ever-present in water, air and soil (EVM, 2003). The Cr^{3+} is considered as an essential element and has significant role in the maintenance of normal carbohydrate, protein and lipid metabolism (EVM, 2003). Most of the Cr^{6+} compounds are produced associated with man-made activities and do not occur in nature. Large amounts are produced through a range of activities, such as stainless steel welding, chromium plating, tanning, coal and oil combustion, cement and waste ignition (EVM, 2003; ECB, 2005).

Chromium Toxicity

Chromium toxicity is associated with its oxidation state. The Cr^{6+} presents more toxicity and is readily soluble than Cr^{3+}. The later form is thus considered to a certain extent benign (Kotas and Stasicka, 2000). A slight increase in the concentration of Cr^{6+} brings outs environmental problems due to its high toxicity, carcinogenicity and mutagenicity nature (Venitt and Levy, 1974; Nishioka, 1975; Sharma *et al.,* 1995). The Cr^{6+} has been listed among toxic chemicals posing serious health hazards by different regulatory agencies (Tchobanoglous and Burton, 1991; Hedgecott, 1994; Sawyer *et al.*, 1994; Marsh and McInerney, 2001; LaGrega *et al.,* 2001). In humans, a number of sicknesses associated with Cr^{6+} exposure, including ulceration, skin and nasal irritation and lung carcinoma have been documented (Gibb *et al.,* 2000a, b). Persistent exposure of Cr^{6+} compounds to animals causes similar effects to those observed in humans' like respiratory and immunological disturbance (ECB, 2005; IPCS, 2006).

Bacterial bioremediation of chromium

The toxicity of Cr^{6+} by bacterial biosorption can significantly decrease the hazards to human health (Kamaludeen *et al.,* 2003). Many microorganisms (*Acinetobacter* and *Ochrobactrum Arthrobacter*, *Pseudomonas* sp., *Bacillus megaterium, Serratia marcescens*, *Bacillus megaterium, Ochrobactrum* sp., *Bacillus* sp., *Bacillus licheniformis*, *Desulfovibrio vulgaris Cellulomonas* spp. *Aeromonas caviae, Bacillus coagulans, Zoogloea ramigera, Bacillus thuringiensis, Chryseomonas luteola, Pseudomonas sp.* and *Staphylococcus xylosus,*) have showed potential to convert highly soluble and toxic Cr^{6+} to less soluble and less toxic Cr^{3+}. (Sağ and Kutsal, 1989; Srinath *et al.,* 2002; Megharaj *et al.,* 2003; Loukidou *et al*., 2004a; Ozdemir and Baysal, 2004; Şahin and Öztürk, 2005; Campos *et al*., 2005; Thacker and Madamwar, 2005; Goulhen *et al.,* 2006; Viamajala *et al.,* 2007; Ziagova *et al.,* 2007; Zhou *et al.,* 2007). Some species of extremophiles (*Deinococcus radiodurans* R1; *Thermoanaerobacter ethanolicus; Pyrobaculum islandicum)* have also been found to reduce Cr^{6+} (Fredrickson *et al*., 2000; Kashefi and Lovley, 2000; Roh *et al.,* 2002). Similarly, Zahoor and Rehman (2009) investigated the role of *Staphylococcus capitis* and *Bacillus sp.* in bioremediation of chromium and found that both the bacterial species not only revealed the aptitude to stay alive in polluted wastewater but were also able to reduce Cr(VI) (100 µg/mL) upto 81% and 85% from the medium after 96 h, respectively. The *S. capitis* and *Bacillus* sp. were also able to decrease 89% and 86% of Cr^{6+} after 144 hours of incubation directly. The MIC values of Cr^{6+} up to of 4800 µg/mL for *Bacillus* sp. and 2800 µg/mL for *S. capitis* indicate the potential of both isolates to change high toxic form of chromium into less toxic form. The *Arthrobacter* sp. and *Bacillus* sp., isolated from tannery waste contaminated soil showed the ability to reduce 50 µg/ml and 20 µg/ml of Cr^{6+}, resptectively (Megharaj *et al*., 2003). In a study conducted by Srinath *et al. (*2002) *B. circulans* and *B. megaterium* isolated from treated tannery contaminants were found capable of bioaccumulating Cr(VI) up to 34.5 mg Cr/g and 32 mgCr/g (on dry weight basis), respectively.

Persistence of Mercury in the environment

Mercury exists in liquid form at room temperature. Its Hg^0 (zero oxidation state) exists as vapor or liquid form, its Hg^{1+} (mercurous state) exists as inorganic salts and Hg^{2+} (mercuric state) may form either inorganic salts or organomercury compounds; the three groups vary in effects. Due to its toxicity, it is included in the list of most toxic chemicals (Dechwar *et al.,* 2004; White *et al.,* 2005). It is a common toxicant associated with industrial effluents. The paints, pharmaceuticals, disinfectants and pulp and paper industries are the examples of mercury sources. In addition to the man associated activities, natural developments like soil erosion, volcanic eruptions, wild forest fires, geothermal activities and hydrological cycle add in universal mercury load. Moreover, urban sewage treatment plants also cause both inorganic and organic mercury contaminations (Wang *et al*., 2004). Mercury being major pollutants is enlisted among environmental toxic chemicals due to its persistent nature for long periods. It changes its chemical form gradually. The residence time was estimated about 0.5 to 2 years (Siudek *et al.,* 2011). Release of mercury to environment is increasing day by day. Recent world investigations account that anthropogenic activities had discharged 2480000 Kg of mercury in 2006 as compared to 2190000 Kg in 2000 (Streets *et al*., 2009).

Mercury Toxicity

Mercury is toxic for all living beings. Mercury binds with enzymes and proteins (sulfhydryl groups) and thus inactivates various cell functions (Dobler *et al.,* 2000b). All forms of mercury can produce toxic effects. Toxic effects include damage to the kidneys, brain, and respiratory system (Clifton, 2007).

Bacterial bioremediation of mercury

Mercury elimination from polluted locations is a big challenge for environmental management. The most promising conventional techniques for remediating mercury involve coagulation, precipitation, ion exchange and adsorption by natural materials, adsorption by activated carbons and reverse osmosis with some limitation (Siudek *et al.,* 2011) Bacterial bioremediation for mercury from polluted sites is very important practice. For this purpose, the bacteria should have mercury tolerant mechanisms. The bacteria have developed resistance to mercury present at mercury contaminated sites (De *et al.,* 2003). The genetic modification helps in remediation (Cairns *et al*., 1988). Bacterial bioremediation of mercury comprising primarily on microbe-mediated enzymatic reduction of both inorganic and organic mercury to volatile elemental mercury is a bi-phasic process. The *mer* operon is generally distributed amongst mercury resistant bacteria. Presence of mer operon facilitates the production of mercuric reductase (an oxidoreductase) and organomercury lyase. Mercury bioremediation process involves Hg^{2+} transportation into the cell, Hg^{2+} delivery to reductase and export of reduced mercury (Dash and Das, 2012). In mercuric ions resistance mechanism, the permeable membrane limits the mercury access to cell or thiols bind with mercury compounds and reduce the mercury toxicity in cell (Barkay *et al.,* 2003; Huang *et al.,* 2010). In different studies, *Deinococcus geothermalis, Cupriavidus metallidurans, Pseudomonas putida; Streptococcus canis*, *Pragia fontium, Enterococcus saccharolyticus, Pseudomonas aeruginosa* CH07, *Alkaligenes faecalis and member of* family Enterobactericeae shown capable of remediating the mercury (De *et al.,* 2006; Adelaja and Keenan, 2012).

Persistence of lead in environment

Lead (Pb) is found in water, air and soil. It is distributed naturally in biologically inactive form. The use of lead in bearing metals, batteries, gasoline additives, cable covering, explosives, antifouling paints are major source of pollution (Johnson, 1998).

Lead Toxicity

This metal is hazardous and highly poisonous to microbes, plants, animals and human (Low *et al.,* 2000). Bio-availability of lead can be hazardous for children and causes mental retardation. Lead may cause lethal effects on muscular, gastrointestinal, neurological and reproductive systems. It may enter into our body through ingestion of food and blood stream. Lead disturbs the communication between cells and neuronal set up (Klassen and Watkins, 2003).

Bacterial bioremediation of Lead

Many bacterial species are capable of bioremediating lead by biosoprtion *e.g. Streptoverticillium cinnamoneum*, *Streptomyces rimosus*, *Pseudomonas putida*, *Pseudomonas aeruginosa* PU21, *Enterobacter* sp. J1, *Corynebacterium glutamicum* and *Bacillus* sp. (ATS-1) (Chang *et al.,* 1997; Puranik and Paknikar, 1997; Pardo *et al*., 2003; Choi and Yun, 2004; Selatnia *et al.,* 2004b; Lin and Lai, 2006; Lu *et al.,* 2006; Tunali *et al.,* 2006; Uslu and Tanyol, 2006). The quantity of lead may be decreased by bacteria to producing complexes between acidic sites of cell wall and lead (Cabuk *et al.,* 2006). The possible means comprise elimination by forming intra-and extracellular sequestration, extracellular metal precipitation, a permeable barrier, active transport, dissolution of lead by acid production, enzymatic detoxification, precipitation of lead through the production of organic bases, and biotransformation reactions like volatilization, methylation, oxidation and reduction. Researchers have also found that *Bacillus* sp. L14 and *Solanum nigrum* L. could reduce the toxicity of metal by specifically uptaking up to 80.48% of Pb (II) within 24 h of incubation (Guo et al., 2010)

Persistence of Cadmium in environment

Cadmium enters the environment from natural resources as well, soil, rock, fossil fuels and volcanic activity have some cadmium levels too (Jin *et al*., 2002; Jain *et al.,* 2005).

Cadmium Toxicity

Cadmium is a heavy metal and is enlisted among the top ten in the black list of agreements on environmental protection and the World Health Organization (Sharma *et al.,* 2000). Cadmium is a toxic pollutant that enters water and sediments in different ways and has a high potential to precipitate in plant and animal tissues. Cadmium binds to two cellular antioxidants, glutathione and metallothionins. This binding is likely a natural defensive mechanism that helps the cells rid themselves of low concentrations of cadmium and some other heavy metals. Problems occur at higher heavy metal concentration. The cell can only make a limited amount of these antioxidants, and chelating heavy metals is not their only job. The main job of these antioxidants is to protect cells from reactive oxygen species (ROS); small toxic chemicals produced by reactions involving oxygen. When all the antioxidants are used up in chelating heavy metals, there are none left to help protect the cells from ROS. Exessive cadmium in the presence of environmental and biological cycles leads to human diseases such as the spongy bone disease, kidney disorders, lung disorders, autoimmune diseases, destruction of red blood cells and some categories of cancers (Titus and Pfister, 1984; Koplan, 1999; Satarug and Moore, 2004). Cadmium can cause contamination of crops and livestock and the pollutant is transmitted to humans and animals (Jin *et al.,* 2002; Tripathi *et al.,* 2005). Permissible presence of metal is 0.006 ppb (standard) for aquatic life, but, unfortunately, in all the stations tested in a study cadmium contents exceed the limit significantly (Kafilzadeh *et al*., 2010).

Bacterial bioremediation of Cadmium

Bacteria in contaminated areas get resistant to cadmium by many ways and become able to remove cadmium. Bacteria isolated from polluted areas have high ability in removing cadmium. Therefore, their use is recommended for wastewater bioremediation and this can lead to more successful cleanup of heavy metals in the environment and this is crucial in order to achieve higher efficiency and more economical feasibility (Kafilzadeh *et al*., 2010). Cadmium is harmful to most organisms, but some living beings like *Burkholderia cenocepaci* can tolerate higher cadmium concentrations by pumping out the ions (Siudek *et al.,* 2011). Alternatively, *Thalassiosira wessflogii* can use cadmium as a nutrient (Lee *et al.,* 1995). Finding of Siudek *et al.* (2011) confirmed that this species is a good candidate for elimination of cadmium and has the ability to remove 75 to 89 percent of the metal. Shamim and Rehman (2012) studied biological removal of heavy metals by *Klebsiella pneumoniae* CBL-1 and the results showed that this species is a good candidate for the removal of metals, especially cadmium, at a concentration of 1500 mg/ml. Titus and Pfister (1984), Loukidou *et al.* (2004), Vullo *et al.* (2005) and Singh *et al.* (2010) were able to isolate the bacterium *Pseudomonas* from sediment and various sewages and examined. Rajbanshi (2008) studied the tolerance of isolated bacteria to various heavy metals and found that the amount of cadmium resistance in bacteria *Citrobacter, Flavobacterium* and *Acinetobacte*r reported after 10 days of incubation in terms of MIC were 300¸ 220 and 150 mM, respectively. Chovanová *et al.* (2004) revealed the effect of cadmium resistant bacterial isolates; *Comamonas testosteroni, Klebsiella planticola, Alcaligenes xylosoxidans, Pseudomonas fluorescens, Pseudomonas putida* and *Serratia liquefaciens*, isolated from a cadmium-contaminated sewage sludge, for bioremediating cadmium and their results showed cadmium reduction upto 40% in an experimental flask, in contrast to 5% reduction recorded in control samples till 74 hrs. In another research, the *Enterobacter agglomerans* SM 38 and *B. subtilis* WD 90 removed Cd upto 32%. and 25%, respectively (Kaewchai and Prasertsan, 2002).

Conclusion

Untreated industrial effluents containing heavy metals pose the detrimental health affects to human and other organisms. In this regard, bacterial bioremediation practices provides efficient modern procedures for treatment of a broad range of pollutants. The Cd, Hg, Cr and Pb are among the most toxic heavy metals. Use of bacteria in bioremediation of these metals for developing most cost effective and efficient method appears promising. In the light of literature review *Pseudomonas spp., Aeromonas spp., Bacillus spp. Enterobacter spp. Streptomyces spp.* and *Pseudomonas spp.* have been widely studied for their resistance and bioremediation capabilities against Cd, Hg, Cr and Pb. Analysis of the results of various studies allowed to conclude that the different bacterial species are good candidates for treatment and elimination of the cadmium, mercury, chromium and lead present in contaminated wastes. However, besides isolation and preservation of such useful bugs, search for their low-cost cultivation is the need of day.

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