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HEAVY METALS AND ITS POLLUTION LEADING TO RESISTANCE IN BACTERIA OF SURFACE WATERS: A REVIEW

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ABSTRACT

The pollution caused by indiscriminate discharge of heavy metals into the surface waters from various sources has serious ecological implications. The surface waters also harbor various seemingly harmless microbiological population that turn out to be pollution disaster owing to the exposure to such metals that render them resistant in due course of time. Such resistant strains with altered metabolic capabilities can neither be considered as ecologically viable indicator of a healthy ecosystem nor be controlled easily in case of pandemics. In view of the above, the knowledge of heavy metals and the mechanisms by which bacteria develop resistance towards them especially in context of surface waters has been reviewed helping us conclude the immediate problem in hand as well as the measures needs to be taken to manage it.

KEY WORDS : Bacteria, Industrial effluents, Heavy metal resistance, Water pollution

INTRODUCTION

Heavy metal is the collective term used for those elements (metals, metalloids, lanthanides and actinides) which have a molecular weight greater than 53, atomic density greater than 5 g cm^{-3} with metallic properties like ductility, conductivity, stability as cations, ligands specificity etc. (Elsilk *et al.*, 2014). They are wide spread pollutants of great environmental concern as they are non-degradable, toxic and persistent with serious ecological implications in aquatic ecosystems (Aboud and Nandini, 2009). Rapid urbanization and population growth in fast growing cities leading to industrialization poses a major threat of heavy metal pollution for rivers flowing through these cities as they can enter such rivers from either natural or anthropogenic sources (Akoto *et al.*, 2008). The main anthropogenic sources are disposal of untreated and partially treated industrial effluents and sewage containing toxic metals, as well as metal chelates from different industries and indiscriminate use of heavy metal-containing fertilizers and pesticides in agricultural fields (Reza and Singh, 2010). The long-term exposure of microbial population to heavy metal polluted environment imposes a selection pressure that leads to the emergence of resistant strains (Gadd, 2010). This review article focusses on the discussion of heavy metals as pollutants and their effect on the microbial population in terms of heavy metal resistance in the contaminated sites especially river waters.

Heavy metal sources in surface waters

Heavy metals enter river water through two pathways: (i) *via* identifiable point sources such as municipal and industrial wastewater effluents channels and (ii) *via* diffuse sources such as erosion, surface run-off, accidental spills as well as direct dumping of the wastes/effluents containing heavy metals into the rivers (Venugopal *et al.*, 2009). Therefore, heavy metal load of the rivers is contributed both by point and diffuse sources (Barcelo *et al.*, 2004). According to Srivastava *et al.* (2011) drains are one of the main point source of heavy metal pollution, especially for rivers flowing within the city, that themselves carry industrial effluents, domestic waste, sewage and bio-medical wastes. Several studies have shown that the industries (point source) discharge their effluents into the surrounding rivers without any proper treatment to remove metals (Khadse *et al.*, 2008; Venugopal *et al.*, 2009; Malik and Aleem, 2010; Reza and Singh, 2010). Many studies carried out worldwide have shown heavy metal pollution scenario of surface waters from various natural and anthropogenic sources (Frankowski *et al.*, 2009; Danazumi and Bichi, 2010; Mohiuddin *et al.*, 2010).

Heavy metals and Bacteria

Heavy metals are essentially xenobiotics, are persistent and can't be readily removed from the contaminated environment (Mondragon *et al.*, 2011). Arsenic, Cadmium, Mercury and Lead may accumulate within cells and cause destruction as well as deactivation of various enzymes and cellular activities along with DNA damage and cell death (Belyaeva *et al.*, 2012). They (Cd^{2+} and Hg^{2+}) bind to Sulfhydryl groups of proteins thereby denaturing their enzyme functions. Other heavy-metal cations may interact with physiological ions; like Cd^{2+} with Zn^{2+} or Ca^{2+}, Ni^{2+} and Co^{2+} with Fe^{2+}, Zn^{2+} with Mg^{2+}, thereby inhibiting the functioning of these physiological cations (Nies and Silver, 1995). Nickel is essentially required for metabolic processes in microbes. In general microbes sequester Nickel through permeases or ATP-binding cassette-type transport systems. Afterwards, Ni^{2+} is used as a cofactor for several microbial enzymes like methylenediurease, carbon monoxide dehydrogenase, urease, acetyl CoA decarbonylase/ synthase, Ni-Fe hydrogenase, methyl coenzyme reductase, certain superoxide dismutases, and some glyoxylases. However, at higher concentrations Nickel has toxic effects on bacteria (Mulrooney and Hausinger, 2003). Selenium is required for the functioning of selenoproteins (Gu *et al.*, 2002). Magnesium is a primary element that stabilizes cell membrane, nucleic acids and ribosomes. It is also an important element that helps in hydrolysis of ATP molecule during glycolysis as an energy dependent processes (Deng and Wilson, 2001). Zinc at lower concentration is essential as a component of DNA binding proteins chiefly Zinc-finger proteins but at higher concentrations Zinc can induce cell membrane perturbations, DNA damage, and oxygen stress (Noh *et al.*, 1999). Cadmium is highly toxic to microbes even at very low concentrations as once taken up through calcium channels of the plasma membrane, it accumulates intracellularly to cytoplasmic and nuclear material, thereby inhibiting the biosyntheses of DNA, RNA and proteins. It also induces chromosome aberrations and lipid peroxidation (Beyersmann and Hechtenberg, 1997). Copper is used in Cytochrome-C oxidase and other related enzymes, which are oxygen dependent terminal oxidases of the respiratory chain in various organisms. However, Cu^{2+} easily interacts with radicals like molecular oxygen that makes it very toxic due to the production of hydroperoxide radical (Nies, 1999). Hexavalent chromium (highly soluble) is highly toxic as it can enter bacterial cells readily *via* sulphate transport systems (Ackerley *et al.*, 2004). The toxicity is primarily due to production of reactive oxygen species (ROS) that are formed due to consequent reduction of hexavalent Crby glutathione, thiols and other metabolites leading to DNA damage (Klonowska *et al.*, 2008).

Heavy metal resistance and mechanisms

Higher levels of heavy metals in the environment may cause extreme toxicity to bacteria, thus building a selective pressure for the development of metal resistance. To survive under metal stress conditions, some bacteria have indigenous mechanisms to depollute, tolerate as well as uptake heavy metals ions at higher concentrations and some even use them for respiration (Oyetibo *et al.*, 2010). There are general mechanisms imparting resistance to a variety of metals. In addition to this, some specific plasmid encoded resistance mechanisms conferring resistance to a particular metal can also be adapted by certain microbes.

General mechanisms: Immobilization of metals usually takes place by binding of metals to extra cellular materials. A large number of cationic metals such as Cadmium, Iron, Lead and Zinc are prevented from getting entry into the microbial cells by adhering to anionic cell surfaces like phosphoryl groups and phospholipids of lipopolysaccharides layer present in Gram-negative bacterial cell walls. Such adherence of metals with bacterial cell is important both ecologically as well as practically. Distribution of metals, especially in the aquatic environment is primarily influenced by cell surface binding of metals thereby having ecological significance. Practically, this capacity of microbes to sorb metals have been exploited for the purpose of bioremediation, *i.e.* removal of metal contaminants from nature. There are four methods by which bacteria binds heavy metals on its outer surface. i) exopolymer binding using exopolymeric substances like polysaccharides, carbohydrates, nucleic acids and fatty acids that results in immobilization and prevention of entry of metals in the cell mainly Lead and Cadmium (Maier *et al.*, 2009); ii) complexation by siderophore that are iron-chelating organic molecules that may also interact with other metals chemically similar to Iron *viz*., Aluminium, Gallium and Chromium, thereby, reducing their bio-availability and toxicity (Roane and Pepper, 2000); iii) complexation by biosurfactants that are produced by many bacteria and can bind to metals such as Cadmium, Lead and Zinc resulting in complexes that are nontoxic to bacterial cell (Miller, 1995); iv) precipitation by metal reduction in which soluble metals are reduced to less soluble metal salts, including sulfidic and phosphidic metal salts (Roane and Pepper, 2000).

Metal dependent mechanism of metal resistance: The frequently used mechanism of this type involves metal binding and sequestration by metallothioneins (Andrews, 2000) or similar proteins. These methods are only triggered in the bacterial system when metal contamination is present in the environment. Metallothioneins play pivotal role in heavy metal metabolism when bacteria are exposed to heavy metal pollution stress (Wilfried, 2000). Another metal dependent mechanism of metal resistance is the use of efflux systems that are usually plasmid-encoded and energy-dependent systems to remove metals from the cell. Few of such mechanisms involve ATPases and others active proton pumps to pump back toxic metal ions that have entered the cell out of the cell (Spain and Alm, 2003) like arsenate, chromium and cadmium metal efflux resistance mechanism (Ahemad, 2012). An argument was put forward by Konopka and Zakharova (1999) that in case of elevation in the concentrations of essential elements (like Cu, Zn, Ni and Co) to toxic levels, a microbe is presented with a special situation because of their requirement to accumulate some of these cations at trace levels and at the same time to reduce cytoplasmic concentrations from potential toxic levels. In this regard, Brown *et al.* (1999) stated that resistant bacterial strains solve these problems by a careful regulation that results from the interaction between cation transport systems that are chromosomally determined and metal resistance systems that are plasmid determined. Also, heavy metal resistance that is mostly encoded by genes that are located on plasmids and transposons. Therefore, it is often transferable among bacteria from one genus to another, from one species to another, and also from in-situ microflora to indigenous microflora (Malik and Jaiswal, 2000).

The heavy metal resistant bacteria have been frequently isolated from heavy metal polluted habitats. However, unpolluted or less polluted environments also harbour metal resistant or tolerant organisms that can readily acclimate in high concentrations of metals (Malik *et al.*, 2002). Also, the aquatic habitats impose more harmful effects of heavy metal pollution to microorganism because they are in close and prolonged contact with the soluble heavy metals (Shoeb, 2006) that find their way into water *via* several natural and anthropogenic processes.

Studies on heavy metal resistance in bacteria

There are several studies that have analyzed the heavy metal resistance status of various Gram-positive and Gram-negative bacteria isolated from various habitat like surface water, soil, sediments in terms of minimum inhibitory concentration both in India as well as world-wide. Bacteria are used as an indicator of heavy metal pollution in terms of resistance as they are very sensitive to low concentrations of heavy metal and any increase in the level of heavy metal beyond their tolerance capacity either leads to their death, lowering down of metabolic activities, or resulting resistance against that specific heavy metal (Rajkumar *et al.*, 2012). Smejkalova *et al.* (2003) studied the effects of heavy metal concentrations on the biological activities of soil microorganisms. They suggested that due to heavy metal pollution stress from anthropogenic effects, the developmental and biochemical activities of soil microorganisms alter thereby indicating changes in soil quality. They also deduced that though chemical analysis measure particular amounts of heavy metals, however it does not reflect the environmental consequence on living organisms. Therefore, determination of microbiologically related parameters like MIC values can measure actual impact of heavy metal pollutant on soil organisms. Lima de Silva *et al.* (2012) stated that although the heavy metal tolerant strains can survive and grow in the presence of heavy metals, however, presence of heavy metal can lead to specific metabolic and physiological changes in bacteria. Therefore, the number of live bacteria gradually decreases as with increasing heavy metal concentration while determining MIC values (Abskharon *et al.*, 2008) even if the strains are heavy metal tolerant.

Further, site-wise correlation between heavy metal concentrations and heavy metal-resistant strains shows that the latter might occur in areas polluted with heavy metals (Altug and Balkis, 2009) thereby signifying that resistance is directly influenced by pollution. Researchers have isolated and characterized bacteria resistant to heavy metals from tannery effluents indicating industrial effluents as a source of heavy metal resistant bacteria (Singh *et al.*, 2010b; Marzan *et al.*, 2017). These strains were not only tolerant to Cr but also exhibited high level of tolerance towards other heavy metals such as Ni and Cd. Mustapha and Halimoon (2015) isolated heavy metal tolerant bacteria from industrial effluent from electroplating industry. Likewise, Rajkumar *et al.* (2012) reported the presence of heavy metal resistant bacteria in the waters of Barak river that was contaminated with the effluents of paper and pulp industry whereas, Gupta *et al.* (2015) reported the presence of heavy metal resistant bacteria in paper mill effluents from Erode district, Tamil Nadu. This corroborates the fact that industrial effluents are potential source of heavy metal resistant bacteria in the river.

Heavy metal resistant studies have been conducted for the members of Enterobacteriaceae family like *Pseudomonas* (Altug and Balkis, 2009; Singh *et al.*, 2010a; Santhiya *et al.*, 2011; Gupta *et al.*, 2011) as well as for *E. coli* (Singh *et al.*, 2010b; Santhiya *et al.*, 2011; Gupta *et al.*, 2011). Heavy metal resistant bacteria have been isolated from various aquatic environments such as sediments of Krishna Godavari basin, Bay of Bengal (Gunaseelan and Ruban, 2011), industrial effluents from wastewater treatment plant (Singh *et al.*, 2010a), Palk Bay sediments (Nithya *et al.*, 2011), Chennai beaches, Bay of Bengal (Santhiya *et al.*, 2011), soils of Chambal region (Gupta *et al.*, 2011) indicating long term selective pressure exerted on the bacteria by heavy metal pollution implying serious ecological concerns. Rahman and Singh (2016) have recently isolated a total of 88 Hg-resistant bacteria from surface water of different region in Delhi. The highest density of Hg-resistant bacteria was detected in sample taken from Najafgarh drain.

CONCLUSION

Though the heavy metal resistant strains find a valuable use in bioremediation, however, their application is limited due to constraints in mass scale implementation of the same. More importantly is the problem highlighting the role of heavy metal polluted sites that ultimately leads to more and more resistant strain - a phenomenon that alters the natural ecology of the that system. Irrespective of many policy and legislative measure in place to check the heavy metal pollution, there is not much success and there is an urgent need to manage the situation at hand.

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