# Chapter 23
# Engineering of Microbes for Heavy Metal Tolerance:
## An Approach for Bio Remediation Technology

**Megha D. Bhatt**
*GSFC AgroTech Ltd., Gujarat State Fertilizers & Chemicals Ltd., India*

**Deepesh Bhatt**
*SRICEAS, M.T.B. College Campus, India*

## ABSTRACT

*Use of microorganisms and their enzymes to degrade heavy metal contaminants from the environment, is termed as bioremediation. This chapter majorly deals with heavy metals, their toxicity and their ill effects upon the environment. It depicts how microbes can help to combat the side effect of heavy metal toxicity by stimulating their natural defensive mechanism. In spite of their natural defensive system against metal pollution, still there is an urgent need of utilizing advanced molecular tools to further exaggerate their resistance ability for bioremediation. Earlier accumulation of heavy metals was done through overproduction of various metal binding proteins located in the cytoplasm. Recently cell surface engineering of microbes appears an attractive technology for removal or recovery of metal ions from the environment. To expedite the degradation of pollutant, a number of different molecular tools have been established for improving the microbial strains at molecular and genetic level. Microbial engineering thus, seems a promising approach which elucidates the effect of biotechnological processes used for decontaminating the polluted environment and in the future, humans and animals might gain from these organisms in remediating environmental contamination. However, these genetic modifications should be stable and harmless towards the nature as well as for the microbes itself and any genetic alterations must always ensure the actual pros and cons behind it.*

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## INTRODUCTION

Environmental pollution caused by organic and inorganic pollutants has constantly increased in parallel with growing population, rapid industrialization and increasing urbanization especially in developing countries (Hettige, Huq, Pargal, & Wheeler,1996). Heavy metals are the main constituent of these toxic pollutants. The rapid depletion in the environmental health provoked scientists to develop technologies and strategies for the removal and sequestration of these hazardous contaminants. A potential resolution of this problem could be bioremediation, which makes use of microbial system for the removal and transformation of the xenobiotic compounds and various heavy metals (Siddique et al., 2005; Hussain, Arshad, Saleem, & Khalid, 2007). Although, bioremediation involves multidisciplinary approaches but it solely depends upon the nature of microbial system (Shukla, Singh, & Sharma, 2010). Xenobiotics degrading microorganisms such as (*Pseudomonas, Burkholderia, Sphingomonas, Ralstonia, Comamonas, Achromobacter, Alcaligenes, Rhodococcus, Dehalococcoides*) have properties to accumulate or detoxify heavy metal pollutants namely Cd, Hg, Pb, Zn, U, etc. (Daly, 2000; Lloyd, Lovley, & Macaskie, 2003). These organisms accumulate heavy metals in a metabolic dependent manner as these metals act as a catalytic component of biochemical and enzymatic reactions occurring inside their system (Holm, Kennepohl, Solomon, 1996; Eide, 1998). This property is because of the net negative charge present in bacteria and the cationic charge present in many toxic metals. Bacterial surface majorly contains carboxyl, phosphoryl, hydroxyl and amino functional groups. Upon pH variation these groups usually deprotonate leading to a net negative charge on these functional groups; this property makes them functionally capable of metal adsorption. Moreover the high surface area to volume ratio of bacteria allows them to accumulate metal ions in an amount greater than their own weight (Beveridge, 1989).

This property instigated the idea that whether the genetic engineering of microorganisms may be possible to over accumulate or adsorb these metal ions on the bacterial cell surface. This may facilitate the cleanup of toxic metal ion from the environmental and industrial effluents. Recently bioremediation using engineered organisms has become a fascinating technique that has gained importance as an eco-friendly and efficient strategy (Shukla et al., 2010; Liu, Zhang, Chen, & Sun, 2011). There are still many more challenges ahead to use these engineered bacteria as a catalyst in bioremediation technology, taking into consideration, a virtually uncontrolled condition. In this regard recombinant DNA technology can be an effective tool for optimization of bioremediation, if we target the anaerobic and unculturable bacteria through metagenomic technology (Cases & De Lorenzo, 2002). We can explore genes for heavy metal removal from the nature irrespective of their host. Recent developments in bioremediation technology, which includes the utilization of metabolic engineering, whole-transcriptome profiling, protein engineering and proteomics are also considered significant for removal of toxic contaminant such as heavy metals (Thomas, 2008).

Another aspect is the recovery of metal ions by microorganisms using cell surface engineering, seems as an effective approach for developing customized bio-adsorbants. The cell surface display of known metal-binding proteins/peptides and the molecular design of novel metal-binding proteins/peptides have been performed using a cell surface engineering approach (Kuroda & Ueda, 2011). The cell surface engineering technology utilizes the property of a metal binding protein or peptide by fusing it with the cell surface protein in order to express the protein on the cell surface. These cell surface proteins have an intrinsic property to attach across the cell surface. Therefore over expression of various metal ions binding proteins, such as metallothioneins or cysteine rich peptides and glutathione, in bacteria may prove a promising strategy for remediation through microbial-based bio-adsorbent.