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# Bioremediation Activity of Microorganisms in Soil Environment Contaminated by Heavy Metals

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## ABSTRACT

Heavy metals are increasingly found in microbial habitats due to natural and environmental processes. In general aerobic heterotrophic populations were more sensitive to metal groups such as Ni, Cd, Cu, Hg, Mn, and least to Zn. Asymbiotic nitrogen fixers showed higher sensitivity to groups like Cd, Pb, Hg, Cu, Cr, Mn, Ni and least to Zn. (Ahmad, 2005). Most common metal removal methods are physical removal and land filling. These techniques are cost effective and most of them move contamination to the location. And that area becomes polluted. Common remediation methods include soil washing; excavation and reburial for metal contaminated soils were already used in the previous years. Another approach is in situ remediation technique, which removes or stabilizes metal. It is a non-invasive and environmentally harmless process (Maier, 2001). Keywords: Bioremediation, Heavy metals, Microorganism, Soil Environment.

#### INTRODUCTION

Pollution of the biosphere by heavy metals due to industrial activities has created a serious problem for rational utilization of soils (Srivastava, 2005; Ali, 2007). The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants (Igwe et al., 2005). Microorganisms play an important role in the environmental fate of toxic metals and metalloids with a multiplicity of physicochemical and biological mechanisms effecting transformations between soluble and insoluble phases (Gadd, 2004).

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Although some heavy metals are essential trace elements, even, at low concentrations of cadmium, mercury, lead etc. are detrimental to the organisms (Bhat, M. A. 2014). However, at high concentrations, most can be toxic to all branches of life, including, by forming complex compounds within the cell (Adarsh, 2007; Gadd, 2004).

Bioremediation is the branch of biotechnology, which deals with the methods of solving environmental problems. Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition (Vinay Kumar et al., 2013). Bioremediation is the general concept that uses primarily microorganisms or microbial processes to degrade and transform an environment, already altered by contaminants and pollutants to its original condition. Treatment of polluted environment with bioremediators is the most efficient and least costly method. The application of heavy metal solubilising microorganisms is very useful approach to decrease the toxic effect of heavy metal in soil. It is also proven fruitful via the addition of matched microbe strains to the medium to enhance the resident microbe population ability to break down contaminations.

#### Heavy Metal Pollution

Although no clear definition is there of heavy metal, it can be defined as the natural elements of the earth crust. Indiscriminate human activities have drastically altered their physico-chemical balance. A heavy metal is the metallic compound which is toxic and has high density (5 g/cm<sup>3</sup>) and atomic weight (Järup, 2003). These metals have been extensively studied and their effects on human health have been reviewed by many international health organizations such as WHO.

Living organisms require minute amount of heavy metals, which are the nutritional trace elements of human body (Järup, 2003). Iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Md) and zinc (Zn), are essential trace elements, required by human. Poisonous or nonessential metals are lead (Pb), mercury (Hg), cadmium (Cd), aluminium (Al), arsenic (As), chromium (Cr) and nickel (Ni), which affects central nervous system, kidney or liver or skin, bones, or teeth. In addition, nutritional trace elements can be toxic at higher concentrations of their physiological range (Nina Mikirova, 2011). The European Union Council's direction limits for concentrations of heavy metals in arable soils indicate limits as 140 mg/kg for Cu, 300 mg/kg for Zn (Council of the European Communities, 1986).

Heavy metals interact with protein sites by displacing other metals from their natural binding sites. Although this is not a regular process but this action could be processed by metals for malfunctioning of cells and eventually toxicity (Flora, 2008). Oxidative stress is one the major mechanisms behind heavy metal toxicity. A growing amount of data have been provided as evidence that metals are capable of causing oxidative deterioration of biological macromolecules such as DNA and interacts with nuclear protein (Leonard, 2004).

Cadmium is present in phosphate fertilizers as a pollutant. Metallic cadmium has mainly been used as an anticorrosion agent. Cadmium contamination of soils mainly occurs due to natural and anthropogenic sources including industrial emission and their application of fertilizer and sewage sludge to farm land. Cadmium uptake is increasing through crops and vegetables and inhalation of cadmium fumes or particles can be life threatening (Järup, 2003). Lead poisoning occurs when lead builds up in the body, often over a period of months or year. It can also be caused by breathing in dust that contains lead. It is not visible to the naked eye. Children under the age of six are at high risk of lead poisoning, which causes metal and physical problems. Even small amount of lead can cause serious health problems. The common sources of lead poisoning are lead-based paint and lead-contaminated dust in older building and other sources include contaminated air, water and soil. Lead exposure to blood is bound to erythrocytes, and it is slowly eliminated via urine (Järup, 2003).

WHO has worked with other UN-system organizations to produce a state-of-the-art review on arsenic in drinking water. Arsenic in drinking water is a hazard to human health. First arsenic is recognized in the year of 1990s in well-water in Bangladesh and it occurs less extensively in many other countries also. Arsenic contamination in ground water, which causes chronic arsenic toxicity (arsenicosis), is a major environmental health hazard throughout the world including India (Mazumder, 2008). Arsenic-rich rocks through which the water has filtered, is the main source of arsenic in drinking water. Main source of arsenic exposure is natural source and industrial source or administrated i.e. accidental source (Saha, 1988). Most laboratory animals appear to be substantially less susceptible to arsenic than humans.

#### What is Bioremediation?

Bioremediation is the physicochemical process that takes place in order to bio-transform a polluted environment such as soil, already altered by contaminants, to its original condition. It uses naturally occurring microorganisms (fungi, bacteria, or yeast) to degrade hazardous and toxic substances into less toxic or non toxic substances. These organic substances are the nutritional source of microorganisms (Alkorta, C. G. 2003). Some examples of bioremediation technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, biostimulation, and rhizofiltration.

In chemical terms "organic" compounds are those that contain carbon and hydrogen atoms. They detoxify maximum amount of contaminants into harmless product, mainly, carbon dioxide and water. Bioremediation is the treatment of polluted environment that can be aerobic or anaerobic process. In aerobic conditions, microorganisms use available atmospheric oxygen and convert many toxic organic compounds into carbon dioxide and water. In anaerobic bioremediation technique, microorganisms break down hazardous chemical compounds in the soil to release the energy they need in the absence of oxygen (Ganguly and Biswas, 2013).

The application of bioremediation falls into two broad categories: in situ and ex situ. In situ techniques release less contaminant than ex situ. They do not require excavation of the contaminated soil so may be less expensive. Ex situ techniques can be faster, easier to control, and used to treat a wider range of contaminants and soil types than in situ techniques. The conventional ex-situ method is the physicochemical technique that remediates, detoxifies or destructs the contaminated soils relies; as a result the pollutants undergo stabilisation, immobilisation and incineration or destruction (Rajendra Prasad Bharti, 2014).

Bioremediation is cost effective technique, solar driven, faster than natural attenuation. People are giving attention to this process to generate less secondary wastes with fewer air and water emissions. Bioremediation has become an integrated 'toolbox' for environmental cleanup and ecosystem service provider (Ganguly et al., 2010).

#### Bioremediation of Toxic Heavy Metals

Several studies have been reported that metals have harmful affection to the growth and biochemical activities of microorganisms, which results in decreased biomass and diversity (Gadd, 2004; Ahmad, 2005; José L. Morenoa, 2002). Microbial survival in polluted soils depends upon intrinsic biochemical and structural properties, physiological, and genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Loïc Nazaries, 2013). Some microorganisms survive even at high level of heavy metals, and the microbial activity can therefore, help to remove and recover the toxic effect of heavy metals from contaminated soil (Sá-Pereiraa et al., 2009).

Common physicochemical technologies for remediation of toxic metals are expensive and unsuitable in treating large contaminated area effectively P. Sa-Pereira et al has identified the gene clusters in rhizobial strains that are regaled by heavy metals, mainly chromium. T. Venkateshwarlu et al used *Saccharomyces cerevisiae* for the removal of heavy metals like Lead, Chromium and Cadmium from Fly ash. They reported that the tolerance level of *Saccharomyces cerevisiae* against the heavy metals was up to 2mg/ml for Pb<sup>2+</sup>, 0.15mg/ml for Cd<sup>2+</sup> and 0.043 mg/ml for Cr. Umrania screened various 72 acidothemophilic autotrophic microbes, isolated and adopted for metal tolerance and biosorption potentiality. The scientist concluded that isolated bacterial flora possessed potential in respect of solubilisation of copper and bioremediation activity. The selected highly potential isolate (ATh-14) showed maximum adsorption of Ag 73%, followed by Pb 35%, Zn 34%, As 19%, Ni 15% and Cr 9% in chalpopyrite.

Anyanwu et al investigated aerobic heterotrophic soil bacteria community in different concentrations of different metals, namely, Mercury, Zinc and Nickel. The results showed that the bacterial growth exhibited responses which were dependent upon the type of metal and level of concentration. Studies showed that growth inhibition of bacteria occurred on the  $28^{th}$  day for 500 µg/g soil for both Mercury and Nickel. And Mercury showed positive response of growth inhibition of bacteria at the concentration of 300 µg/g soil on  $28^{th}$  day. However, for Zinc, there was no complete inhibition of growth throughout the experimental period at all metal concentrations. Evident showed that microbial population is reduced and microbial diversity is imbalanced as metal concentration increased.

Rhizoshere has a significant role in phytoremediation of contaminated soil affected by heavy metals, in which, microbial populations have positive cleaning effect against toxic heavy metals and its availability to the plant through release of chelating agents, acidification, phosphate solubilisation and redox changes. Specific metallophytes are used for phytoremediation of toxic heavy metals. Green plants are the lungs of nature with unique ability for purifying impure air by photosynthesis and remove or detoxify heavy metals toxicity from soil and water ecosystem by absorption, accumulation and biotransformation process. Bharati et al. reviewed some recent advantages in effect and significance of rhizobacteria in phytoremediation of heavy metals toxicity in polluted soil.

Pavel et al. had presented the toxic effect of two common heavy metals that could be found in soil (Chromium and Cadmium) on two microbial strains, which were isolated from soil: *Azotobacter sp.* and *Pichia sp.* Nwuche et al. had showed that metals equally inhibits the rate of respiration of the soil microbial populations.

The scientist had reported that from an average rate of 2.51-2.56  $\mu$ g of C/g respiration of the soil microbes declined to 0.98, 1.08 and 1.61  $\mu$ g of C/g in the Copper and Zinc, Copper and Zinc treated soils by the end of the experiment. The results suggested additive or synergistic effects of the metals. The most characteristics feature of microbial habitats varies with the changes of environmental parameters, like temperature, nutrient availability etc.

In ecological terms, a number of varying microbial niches can be described as many basic requirements of heterogeneous microorganisms are satisfied by various soil microhabitats. Therefore, the microbial community is composed of diverse taxa with the variation of their nutritional requirement. The application of microbially mediated biochemical processes, such as oxidation/reduction or methylation reactions, are the another solution for soil bioremediation (Boroń, 2014). The unifying factor for determining toxicity and carcinogenicity of the metals (particularly, Arsenic, Lead, Mercury and Cadmium) is the generation of reaction oxygen and nitrogen species. Imbalance between pro-oxidant and antioxidant homeostasis which is termed as oxidative stress is the main reason for the toxic manifestations of these metals. Long term exposure to these heavy metals could lead to apoptosis. Signalling components such as growth factor receptors, G-proteins, MAP kinases and transcription factors are affected by metals (Flora, 2008).

Despite many years of research we are still far away from effective treatment against toxicity caused due to exposure to heavy metals/metalloids. Although a number of technologies and measurements have developed for the treatment to remove the toxic level of contamination, there are many degenerated areas that still cannot be successfully treated now. For those cases currently available remediation procedures would be too much expensive. Studies show that supplementation of antioxidant along-with a chelating agent prove to be better treatment procedure than monotherapy with chelating agents.

## CONCLUSION

Heavy metals show profound effect upon the general soil microbial community, depending upon their concentrations, oxidation states, and the pH of the environment and on the capacity of the organisms to tolerate as well as to adept at high concentrations of these heavy metals. The removal of a wide range of pollutants and wastes from the environment is a complex, time consuming and costly affair. Some microorganisms have the resistance against the heavy metal and even they can grow in the heavy metal rich environment. So identification of the microorganisms will help us to eliminate the excess, poisonous and toxic effect of heavy metals from the polluted environment (soil, water etc.). The technologies for the treatment of the polluted environment with these bioremediators is the most efficient and least costly. So the aim of my work is to determine the heavy metalsolubilising microorganisms and describe their activity against the toxic pollutants.

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