IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 6 Ver. I (Nov – Dec 2018), PP 75-80
www.iosrjournals.org

Bioremediation of hazardous Heavy Metals from solutions or soil using living or dead microbial biomass

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Abstract: *Among the different pollutants found on the earth, heavy metals are of environmental and public health concerns due to their high toxicities and their presence in soil decrease its quality and plant growth and development. Crops or air contaminations with heavy metal influenced human health and caused many health problems due to their accumulated in the human body and in the food chains. Removal of heavy metal from the soil or the environment is of great concern. Plants and microbial removal of heavy metals from soil or solutions is effective, eco-friendly and low coast treatment. Dead or alive microbes have various mechanisms for metal removal. This review is about heavy metals importance, sources, remediation technologies and mechanisms of resistance.*

Key words*: Heavy metals, bioremediation, remediation, resistance,*

Date of Submission: 04-11-2018 Date of acceptance: 18-11-2018

I. Introduction

Heavy metals are a unique group of naturally occurring inorganic micro-pollutants released into the environment by different processes (Camobreco et al. 1996, Thevenon et al. 2011). Although soils are natural source of heavy metals, geologic and anthropogenic activities like metal mining, industry, atmospheric deposition, agriculture, and waste disposal add to their rate in the environment (Ezzouhri et al. 2009, Haferburg and Kothe 2007), thus promote chronic and acute disorder and the clear serious human health problems (Çeribasi and Yetis 2001, Zafar et al. 2007). Generally, accumulation of lead in the human body caused anemia, reproductive malfunction, renal failure or neurodegenerative injury which lead to cell damage or death (Rai et al. 2010) while excess manganese, zinc, and copper cause heart and liver damage (Grzegorczyk et al. 2014).Moreover, heavy metals can substitute essential metals in enzymes, thus disrupting their functioning (Pourrut et al. 2011a, Pourrut et al. 2011b)

Long-term use of heavy metals contaminated vegetables or crops caused continuous build-up of toxic metals in the kidney and liver of human (Järup 2003) and induced oxidative stress that destroyed cell's inherent defence (Shahid et al. 2014). High level of Pb^{++} in soils may decrease soil productivity but low concentration of Pb^{++} inhibited some fundamental processes, like photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short roots (Bhattacharyya et al. 2008). Heavy metals are potentially toxic and phytotoxicity for plants resulting in chlorosis, decrease plant growth, metabolism, yield, and nutrient uptake in addition to nitrogen fixation in leguminous plants (Guala et al. 2010).

Heavy metal importance

Heavy metals considered as a group of elements with both biologically and industrially important and at high concentrations in the environment, they have a toxic effect. They are divided to essential micronutrients (iron, copper, zinc, cobalt, and nickel) that are critical for normal cell growth and development and the physiological functions of living organisms (Broadley et al. 2007, Marschner 1995) and nonessential micronutrients (cadmium, lead, and mercury) that are actually needed for cell growth s in low concentrations (Adriano 2001). Some metals such as vanadium, iron, cobalt, nickel, copper, zinc are required for cellular functions acting as cofactors, for structural and catalytic roles in enzymes and proteins or a component of metallo-enzyme that form about 30% of all cell enzymes (Wackett et al. 2004). Furthermore, they are used in electron transfer or osmotic equilibrium (Bruins et al. 2000). Soil is the direct path way for the contamination of heavy metals in vegetables and crops via root uptake (Pierart et al. 2015) and the widespread contamination of soil with heavy metals represents currently one of the most severe environmental problems that can seriously affect environmental quality and human health.

Sources of soil heavy metal pollutions

Heavy metals are accumulated in soil and water environments through natural and anthropogenic sources and may be introduced to food chains or groundwater (Pečiulytė and Dirginčiutė-Volodkienė 2009). Each heavy metal has its own damaging effects on plant, animal and human health. Heavy metals are found naturally in soils resulting from weathering of underlying bedrock or through mineral processing processes (Shakoor et al. 2015) and their concentrations in soils varies according to the nature of the rock, its location and age. They represent less than 1% of the Earth's crustal composition due to alteration of the rocks and natural incidents.

Anthropogenic sources of soil contamination by heavy metals include: refine and mining of rocks, pesticides, batteries, paper industries, tanneries, fertilizer industries, solid wastes disposal including sewage sludge, wastewater irrigation and vehicular exhaust (Niazi Nabeel K et al. 2011, Niazi Nabeel Khan et al. 2015, Shahid et al. 2015).

Effects of heavy metals on soil microorganisms

Metals without biological function are generally tolerated only in minute concentrations, whereas essential metals with biological functions, are usually tolerated in higher concentrations (Haferburg and Kothe 2007)and facilitate secondary metabolism in bacteria, actinomycetes and fungi (Haferburg and Kothe 2007, Weinberg 1990). However, high concentrations of heavy metals may have inhibitory or toxic effects on living organisms (Bruins et al. 2000) and influence microbial populations and their biological activities in soil which affect soil fertility (Minnikova et al. 2017).

Microorganisms are the first biota that undergoes direct and indirect impacts of heavy metals. For example, chromium is known to have stimulatory effect on both actinorhodin production and growth yield of the model actinomycete *S. coelicolor* (Abbas and Edwards 1990). Some of the general changes in morphology, the disruption of the life cycle and the increase or decrease of pigmentation are easy to observe and evaluate (Haferburg and Kothe 2007). Moreover, heavy metals alter the conformational structures of nucleic acids and proteins, and consequently form complexes with protein molecules which render them inactive. Those effects result in disruption of microbial cell membrane integrity or destruction of entire cell (Bong et al. 2010). Sensitive microorganisms are negatively affected by heavy metals, but it should also be noted that heavy metals lead to the development of tolerant species that can survive and adapt due to their genetic characteristics (Kelly and Tate 1998, MacNaughton et al. 1999) and can also degrade and detoxify a wide variety of hazardous compounds (Wasi et al. 2008).

The effect of heavy metals on humans

A long-term exposure to heavy metals can be carcinogenic, affecting central and peripheral nervous system and circulatory effects. Consumption of food contaminated with heavy metals considered to be the major path way (>90%) of human exposure compared to dermal contact or inhalation (Mombo et al. 2016, Xiong et al. 2016).

High concentrations of heavy metals can cause adverse health effect to human being, only several of them are important to human in low concentration. Heavy metal has ability to accumulate in living tissues (Ngah and Hanafiah 2008). Numerous human health problems are associated with exposure to lead. The effects of lead poisoning occur when it is present in the bloodstream causing symptoms, such as anemia, reproductive failure, impatience, renal failure, and neurodegenerative damage (Rai et al. 2010). Other heavy metals, such as manganese, zinc, and copper, may cause severe cases, including hypophosphatemia, heart disease, liver damage, and sensory disturbance (Grzegorczyk et al. 2014). Excessive human intake of Cupper may lead to severe mucosal irritation and corrosion, widespread capillary damage, hepatic and renal damage and central nervous system irritation followed by depression. Severe gastrointestinal irritation and possible necrotic changes in the liver and kidney can also occur. The effects of Nickel exposure vary from skin irritation to damage to the lungs, nervous system, and mucous membranes (Argun et al. 2007).

Physical and chemical removal of heavy metal

Soil contamination by heavy metals is a worldwide problem; therefore effective remediation approaches are necessary. Remediation method is to find a protective solution for both human health and the environment (Martin and Ruby 2004). For heavy metal-polluted soils, the physical and chemical form of the heavy metal contaminant in soil strongly influences the selection of the appropriate remediation approach. Physical characteristics of polluted soils, type and level of the pollution at the site must be known to enable accurate assessment of the problem severity and adjustment of remedial measures (Wuana and Okieimen 2011). Remediation of heavy metal-polluted sites is very difficult, expensive and required a lot of energy and materials, therefore the best method is to protect and prevent the environment from contamination. The contaminated soil may be remediated by physical and chemical methods like ion exchange, electrochemical treatment, reverse osmosis, and precipitation (Zhang Hao et al. 2017). The bioavailability and chemical characteristic of heavy metals may be changed through oxidative and reductive reactions (Singh and Kalamdhad 2013). However, the elemental properties remain stable because heavy metals are indecomposable under natural environment. In contrast to organics, metals are persistent in the environment and not easily

Bioremediation of heavy metals

Rremediation of heavy metals from contaminated environments using biological methods is known as bioremediation which offers high specificity in the removal of particular heavy metals of interest. Bioremediation could be in-situ or ex-situ. In-situ bioremediation is an onsite clean-up process of contaminated environments which involves supplementing contaminated soils with nutrients to stimulate microorganisms in their ability to degrade contaminants, as well as add new microorganisms to the environment or improve the indigenous microorganisms to degrade specific contaminants using genetic engineering (Mani and Kumar 2014, Rayu et al. 2012). Ex-situ bioremediation involves taking the contaminated media from its original site to a different location for treatment based on the cost of treatment, deepness of contamination, pollutant type and the extent of pollution, geographical locality and geology of the contaminated site (Azubuike et al. 2016).

Bioremediation of heavy metals using plants

Plants are used to remove heavy metals from contaminated areas (phytoremediation) which are efficient, inexpensive, and eco-friendly techniques.Soil physical and chemical properties, metal bioavailability in soil, exudates of microbes or/and plant roots, and the activity of living organisms affect phytoremediation process.

Phytoremediation is generally categorized into phytovolatilization in which heavy metals are taken up from the soil and converted into less toxic vapors, then released into the atmosphere through transpiration process of the plants (Marques et al. 2009) while phytostabilization is the use of plants to decrease the bioavailability and mobility of heavy metals in soils (Sylvain et al. 2016) and phytoextraction involves clean-up of heavy metals like Ni^{++}, Cd^{++}, Pb^{++}, and Zn^{++}.from soil by means of plant uptake (Ali et al. 2013).

Microbial remediation of heavy metal

Microbial remediation means uptake, accumulate, sequester, translocate and detoxify metals which depends on numerous factors. Microbial remediation included several techniques and applications which differ greatly in the mechanism by which microbial cells can immobilize, remove, or degrade metals. Presence of heavy metals in the environment changed microbial communities and activities (Jansen et al. 1994, Matyar et al. 2008). Bioremediation has been developed to immobilize heavy metals by microorganisms. Bioremediation using of microorganisms/plants to detoxify or remove heavy metals from the soil is cost-effective, provides a permanent solution and is less expensive compared to physicochemical methods and has recently become prevalent in treating soils contaminated by heavy metals (Zhang Xi et al. 2010). Microbial remediation by local microbes showed great use for heavy metal removal especially in harsh soil. Bioremediation technologies have been broadly divided into two categories: in situ or ex situ (Blackburn and Hafker 1993). In situ bioremediation involves treating contaminated material at the site, while ex situ involves removing the contaminated material to be treated elsewhere.

Bioremediation using fungi

Fungi are an essential component of the soil food web, providing nourishment for the other biota that live in the soil. They are important part for the decomposition of waste matter (Rhodes 2012). Mycoremediation is meaning using fungi in bioremediation and the long threads hyphae interact with soil particles, roots, and rocks forming a filamentous body which are known to tolerate heavy metals (Baldrian 2003) and can adjust themselves to grow under various extreme conditions of pH, temperature, nutrient accessibility and high metal concentrations (Anand et al. 2006). Zafar et al. (2007) reported promising biosorption for Cd^{++} and Cr^{++} by two filamentous fungal genera, *Aspergillus* and *Rhizopus,* isolated from metal contaminated agricultural soil (Zafar et al. 2007).Species of Ascomycetes and Basidiomycetes are the most commonly reported fungi for heavy metalremoval from contaminated soils (Narendrula-Kotha and Nkongolo 2017). The oxalate crystals produced by mycorrhizal fungi are also known to immobilize and detoxify heavy metal(Gadd Geoffrey Michael et al. 2014). Their filamentous hyphal structure deeply penetrates in to the deeper soil aggregates and chelates or adsorbs heavy metal. Root exudates also play important role in changing metal bioavailability, as release of certain organic compounds not only mobilizes metals by forming metal complexes but also provide nutrient and energy sources to microbial communities which in turn support plant growth and survival.

Bioremediation using bacteria

Different studies are carried out about the tolerance of bacteria to heavy metals through their abilities to adsorb, bioaccumulate and / or transform metals (Fomina et al. 2007, Singhal et al. 2004). Bacterial cells developed numerous strategies to decrease metal poisoning: (1) resistant to the metal by intra- and extracellular mechanisms; (2) metals excretion using transport systems; (3) detoxification of metals by cytosol sequestration compounds (4) formation of extracellular chelators for binding and fixing metals; (5) binding large quantities of metals by sorption o the cell walls (Haferburg and Kothe 2007). Bioremediation techniques using either live or dead, microorganisms are of two categories: (1) bisorption (passive) using non-living cells, (2) bioaccumulation using living cells (Dönmez and Aksu 2001, Li et al. 2004).

Mechanisms of microorganism resistance to heavy metals
Biosorption

Biosorption (Sorption conditions and adsorption) are used when the natural affinity of biological compounds is exploited for metallic elements. This approach relies on components on the cell surface and the spatial structure of the cell wall on one side and on the other, the physico-chemical conditions of the environment where the cell develops (Ledin 2000, Wang and Chen 2009). It is recognized that pH, ionic strength, temperature, and the presence of other metals and organic compounds play an important role in this process (Dönmez and Aksu 2001, Violante et al. 2010, Wang and Chen 2009).

Biomineralization:

Microorganisms can also produce inorganic compounds such as sulfur which reduces the mobility of many metals and even precipitates them. In addition to these processes, microorganisms can influence the mobility of metals since they affect pH, redox potential (Eh), and so on. All these parameters should be kept in mind while studying the influence of microbial metal accumulation on the mobility of metal (Ledin 2000).

Biotransformation:

Enzymes of microorganisms exhibit different mechanisms in metal by oxidation, reduction, methylation and alkylation (Valls and De Lorenzo 2002). Production of pigments, biosurfactants or siderophores may indirectly decrease or augment the mobility of heavy metals. Iron metabolism has also been studied (Chen et al. 1994, So et al. 2001). It was found that ferroxaminesmay chelate Ni^{++} , Cd^{++} and Al^{+++} (Luterotti and Grdinić 1986, Neu et al. 2000), while coelichelin chelate $Ni^{++}$and Cd^{++} (Dimkpa et al. 2008).

Absorption or active bioaccumulation:

The term "absorption" is used when intracellular transport-dependent metabolism is involved during accumulation and many living cell characteristics, such as intracellular sequestration followed by localization within cellular components, binding metallothioneins, and the formation of a complex (Gadd Geoffrey M 2004, Malik 2004). Envelopes of the cell surface of bacteria can absorb various heavy metals, by virtue of ionic bonds to their intrinsic chemical groups (Rho and Kim 2002). Anionic groups such as carboxylate groups and peptidoglycan phosphate and teichoic acid groups are considered the primary metal binding sites (Beveridge and Murray 1980, Rho and Kim 2002).

II. Conclusion

At high concentrations, heavy metal pollution posed a serious threat to the environment metals and are toxic to human, plants and microorganisms. They could be dispersed in soil and consequently in human beings through food chain biomagnifications that could cause serious health hazards. Microorganisms possess inherent biological mechanisms that enable them to survive under heavy metal stress and remove the metals from the environment. Importance of microorganisms, plant and fungi in bioremediation are immense as they perform multiple functions such as improved soil quality, enhanced plant growth, detoxification, and removal of heavy metal from soil.

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Magda M. Aly. " Bioremediation of hazardous Heavy Metals from solutions or soil using living or dead microbial biomass . ""IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.6 (2018): 75-80.