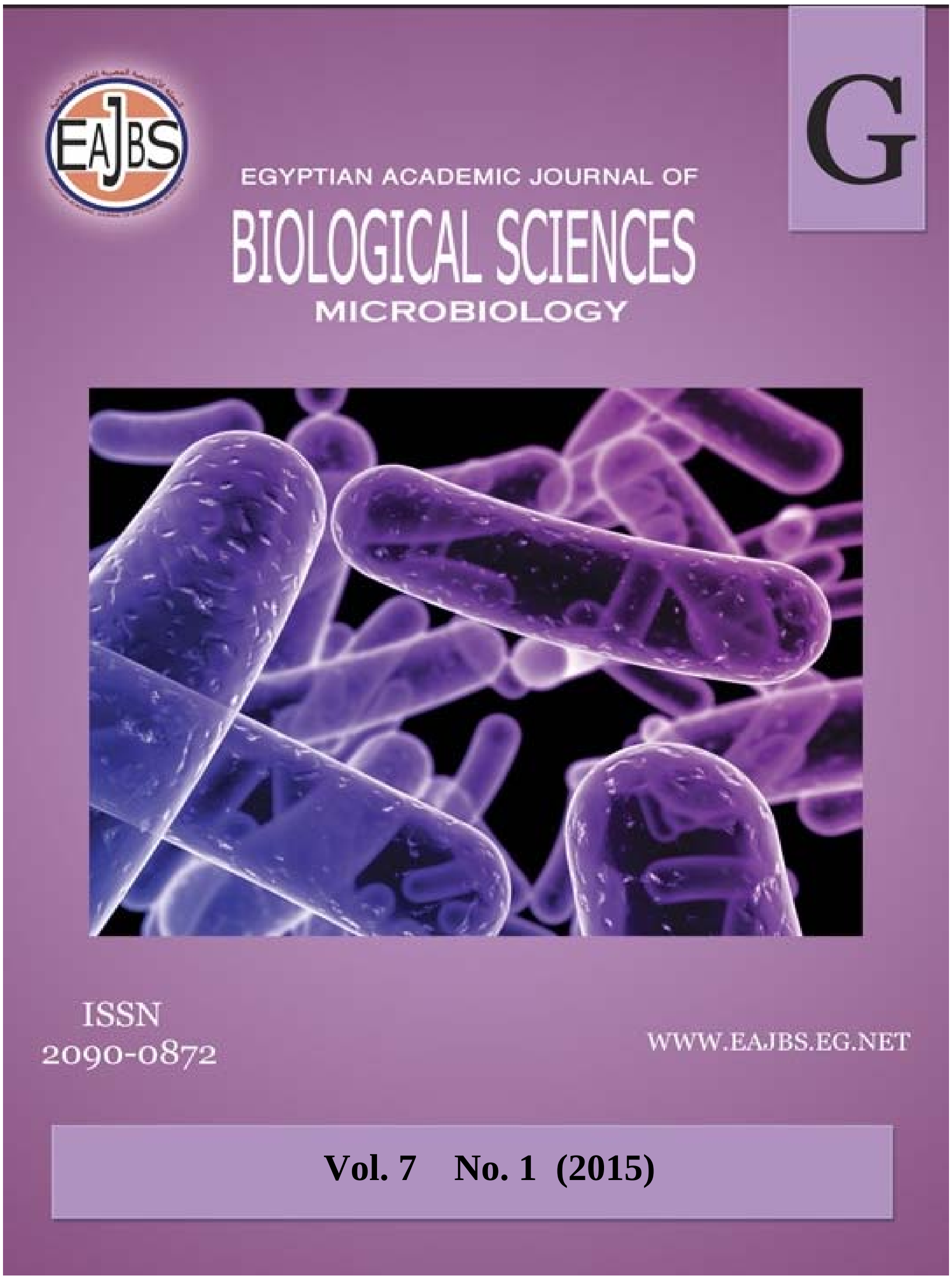

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Microbial Bioremediation of some Heavy Metals in Soils: An updated review

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ABSTRACT

Nowadays, due to industrialization and extraction of natural resources, soil and water pollution is one of the major global concerns. During the recent era of environmental protection, the use of microorganisms for the recovery of heavy metals from soil, sediments and water as well as employment of plants for landfill applications has generated growing attention. The role of microorganisms and plants in biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites. The food and water we consume are often contaminated with a range of chemicals and heavy metals, such as gold, copper, nickel, zinc, lead, cadmium, arsenic, chromium, and mercury that are associated with numerous diseases. Human activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge these metals which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Many studies have demonstrated that microbes have the ability to remove heavy metals from contaminated soils. Among others some of the microorganisms that play great role in bioremediation of heavy metals are *Pseudomonas spp. Alcaligenes spp., Arthrobacter spp., Bacillus spp., Corynebacterium spp., Flavobacterium spp., Azotobacter spp., Rhodococcus spp. Mycobacterium spp., Nocardia spp., Methosinussp., Methanogens, Aspergilusniger, Pleurotusostreatus, Rhizopusarrhizus, Stereumhirsutum, Phormidiumvalderium* and *Ganodermaapplantus.* The encouraging evidence as to the usefulness of microorganisms and their constituents for the remediation of heavy metals from contaminated soils is reviewed in this article.

INTRODUCTION

Nowadays, with growth of industrialization and extraction of natural resources, there has been a considerable increase in the discharge of industrial waste to the environment, mainly soil and water, which has led to the accumulation of heavy metals.

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Consequently, contamination of soils, groundwater, sediments, surface water, and air with hazardous heavy metals and toxic chemicals is one of the major threats facing the world, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. According to recent study by Asha *et al.* (2013), the need to remediate these natural resources has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal because of their potential to enter the food chain.

Scientific report reviled that metals when present in our body are capable of causing serious health problems, by interfering with our normal functions (Suranjana *et al.,* 2009). Some of these metals are useful to the body in low concentration like arsenic, copper, iron, nickel, and the likes but are toxic at high concentration and are not only cytotoxic but also carcinogenic and mutagenic in nature (Salem *et al*., 2000).

Because of human activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge a variety of metals such as silver (Ag), arsenic (As), gold (Au), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn), which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Some metals are required by plants in very small amounts for their growth and optimum performance. However, the increasing concentration of several metals in soil and waters due to industrial revolution has created an alarming situation for human life and aquatic biota.

Conventional methods to remediate heavy metals contaminated site are excavation and solidification/ stabilization, these technologies are suitable to control contamination but not permanently remove heavy metals (Bahi *et al.,* 2012). However, they have some disadvantages, among them cost-effectiveness limitations, generation of hazardous by-products or inefficiency. On the other hand, biological methods potentially solve these drawbacks since they are easy to operate, do not produce secondary pollution. Heavy metals having relatively high density are toxic at low concentration (Iram *et al.,* 2013).

Microorganisms and plants are usually used for the removal of heavy metals. Process of involvement of microorganisms to reduce pollutant concentration is known as bioremediation which is a natural process and its importance of biodiversity (above or below the ground) is increasingly considered for clean-up of metal contaminated and polluted ecosystem. All the metals are toxic, but some of these are useful in low concentration. These metal toxicity cause serious morbidity and mortality (Surajana *et al.,* 2009). Furthermore, Jin *et al.* (2011) reported that the bioavailability can be improved by addition of organic nutrients to the soil such as manure, compost, biosolids, which condition the soil and increases the fertility of soil.

In order to make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements. There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment.

Moreover, most heavy metal salts are water-soluble and get dissolved in wastewater, which means they cannot be separated by physical separation methods ((Hussein *et al*., 2004).Additionally, physico-chemical methods are ineffective or expensive when the concentration of heavy metals is very low. Alternately, biological methods like biosorption and/or bioaccumulation for removal of heavy metals may be an attractive alternative to physico-chemical methods (Kapoor *et al.,* 1995). Use of microorganisms and plants for remediation purposes is thus a possible solution for heavy metal pollution since it includes sustainable remediation technologies to rectify and re-establish the natural condition of soil. However, introduction of heavy metals into the soil causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms at relatively low concentrations (Doelman *et al.*, 1994). Moreover, According to report by Wood and Wang (1983) and Li *et al.* (1994), the modification of the microbial make up is mainly brought about by exerting an inhibitory action through blockage of essential functional groups, displacement of essential metal ions or modification of active conformations of biological molecules. Moreover, the response of microbial communities to heavy metals depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the medium and microbial species (Goblenz*et al.,* 1994).

The Concept of Bioremediation

The quality of life on Earth is linked to the overall quality of the environment. The problems associated with contaminated sites now assume increasing prominence in many countries. Enormous quantities of organic and inorganic compounds are released into the environment each year as a result of human activities. Contaminated lands generally result from industrial activities, use and disposal of hazardous substances, and the like. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of the sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use (Caimey, 1993, Damodaran and Suresh, 2011).

Bioremediation is an innovative and promising technology available for removal of heavy metals and recovery of the heavy metals in polluted water and lands. Since microorganisms have developed various strategies for their survival in heavy metal-polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization.

Bioremediation is a general concept that includes all those processes and actions that take place in order to biotransform an environment, already altered by contaminants, to its original status. Adhikari *et al.* (2004) also defined as bioremediation is the process of cleaning up hazardous wastes with microorganisms or plants and is the safest method of clearing soil of pollutants. Bioremediation uses primarily microorganisms or microbial processes to degrade and transform environmental contaminants into harmless or less toxic forms (Garbisu and Alkorta, 2003).

Microorganisms uptake heavy metals actively (bioaccumulation) and/or passively (adsorption) (Hussein *et al.*, 2001). The microbial cell walls, which mainly consist of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups (Scott and Karanjkar, 1992). Among various microbe-mediated methods, the biosorption process seems to be more feasible for large scale application compared to the bioaccumulation process, because microbes will require addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen demand or chemical oxygen demand in the waste. Further, it is very difficult to maintain a healthy population of microorganisms due to heavy metal toxicity and other environmental factors (Ajmal *et al.,* 1996, Dilek *et al.,* 1998).

Some microorganisms that live in soil and groundwater naturally use certain chemicals that are harmful to people and the environment. The microorganisms are able to change these chemicals into water and harmless gases, such as carbon dioxide. Many algae and bacteria produce secretions that attract metals that are toxic in high levels. The metals are in effect removed from the food chain by being bound to the secretions. Degradation of dyes is also brought about by some anaerobic bacteria and fungi (Colberg 1995). To boost the world's food production rate to compensate for the increasing population, pesticides are being used.

The extensive use of these artificial boosters has led to the accumulation of artificial complex compounds called xenobiotics. By introducing genetically altered microbes, it is possible to degrade these compounds. Plants can also be used to clean up soil, water or air; this is called *phytoremediation*. Thus, Bioremediation has been proposed as a cost effective, environmental–friendly alternative modern emerging technology which can be applied to a number of contaminants and site conditions.

The release of contaminants into the environment by human activities has increased enormously over the past several decades. In fact, although a few decades ago, man's greatest challenge resided in speeding up the industrialization process, today man attempts to find ways to deal with the growing industrialization and the associated problems (Thassitou and Arvanitoyannis, 2001). The relatively sudden introduction of pollutants into the environment has clearly overwhelmed their self-cleaning capacity and, as a consequence, resulted in the accumulation of pollutants. Soil pollution has recently been attracting considerable public attention since the magnitude of the problem in our environment calls for immediate action. Thus, it is essential to minimize poisonous effects of pollutants from soil and water, through the use of bioremediation technique (EPA, 2003).

Types of bioremediation

According to EPA (2001 and 2002) on the basis of removal and transportation of wastes for treatment there are basically two methods. These are *in-situ* bioremediation*ex-situ* bioremediation.

In-situ Bioremediation

In-situ bioremediation is no need to excavate or remove soils or water in order to accomplish remediation. The pollution is eliminated directly at the place where it occurs or at the site of contamination so may be less expensive, create less dust, and it is possible to treat a large volume of soil and cause less release of contaminants. In-situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater (Vidali 2001, Evans and Furlong 2003).

Most often, in-situ bioremediation is applied to the degradation of contaminants in saturated soils and groundwater. It is a superior method to cleaning contaminated environments since it is cheaper and uses harmless microbial organisms to degrade the chemicals and also a safer method in degrading harmful compounds.

In-situ bioremediation can be two types. These are intrinsic bioremediation and engineered *in- situ* bioremediation. In-situ bioremediation approach deals with stimulation of indigenous or naturally occurring microbial populations by feeding them nutrients and oxygen to increase their metabolic activity where as engineered in-situ bioremediation approach involves the introduction of certain microorganisms to the site of contamination. When site conditions are not suitable, engineered systems have to be introduced to that particular site. Engineered in situ bioremediation accelerates the degradation process by enhancing the physicochemical conditions to encourage the growth of microorganisms. Oxygen, electron acceptors and nutrients (nitrogen and phosphorus) promote microbial growth (Evans and Furlong 2003).

Advantage and Disadvantage of in- situ Bioremediation:

This method have many potential advantages as it does not require excavation of the contaminated soil and hence proves to be cost effective, there is minimal site disruption, so the amount of dust created is less and simultaneous treatment of soil and groundwater is possible. It poses some disadvantages also as the method is time consuming compared to the other remedial methods, seasonal variation of the microbial activity due to direct exposure to changes in environmental factors that cannot be controlled and problematic application of treatment additives (EPA, 2003).

Microorganisms act well only when the waste materials present allow them to produce nutrients and energy for the development of more cells. When these conditions are not favorable then their capacity to degrade is reduced. In such cases genetically engineered microorganisms have to be used, although stimulating indigenous microorganisms is preferred (EPA, 2003).

Ex- situ Bioremediation

This process requires excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. This technique has more disadvantages than advantages. *Ex- situ* bioremediation techniques involve the excavation or removal of contaminated soil from ground. Depending on the state of the contaminant to be removed, ex- situ bioremediation is classified as solid phase system and slurry phase systems.

The Solid phase treatment includes organic wastes such as leaves, animal manures andagricultural wastes and problematic wastes like domestic and industrial wastes, sewagesludge and municipal solid wastes. Solid phase soil treatment processes includelandfarming, soil biopiles, and composting.

Land farming is a simple technique in which contaminated soil is excavated and spreadover a preparedbed and periodically tilled until pollutants are degraded. The goal is tostimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. Since land farming has the potential to reduce monitoring and maintenance costs, as wellas cleanupliabilities, it has received much attention as a disposal alternative (EPA, 2003).

Composting is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevatedtemperature characteristic of composting (Cunningham, 2000).

Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surfacecontamination with petroleum hydrocarbons they are a refined version of land farming thattend to control physical losses of the contaminants by leaching and volatilization. Biopilesprovide a favorable environment for indigenous aerobic and anaerobic microorganisms (EPA, 2003).

Slurryphase bioremediation is a relatively more rapidprocess compared to the other treatment processes. Contaminated soil is combined with water and other additives in a large tank called a bioreactor and mixed to keep the microorganisms, which are already present in the soil, in contact with the contaminants in the soil. Nutrients and oxygen are added and conditions in the bioreactor are controlled to create the optimum environment for the microorganisms to degrade the contaminants. When the treatment is completed, the water is removed from the solids, which are disposed of or treated further if they still contain pollutants (Cunningham, 2000).

Bioreactor is a containment vessel and apparatus used to create a three phase: solid, liquid, and gas, mixing condition to increase the bioremediation rate of soil bound and water soluble pollutants as water slurry of the contaminated soil and biomass capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than in-situ or in solid phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pretreatment or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction before being placed in a bioreactor (von Fahnestock *et al.,* 1998 and EPA, 2003).

Microorganisms used in Bioremediation

The bioremediation processes may be conducted by the autochthonous microorganisms, which naturally inhabit the soil/water environment undergoing purification, or by other microorganisms, that derive from different environments. There are a number of microorganisms that can be used to remove metal from environment, such bacteria, fungi, yeast and algae (White *et al.*, 1997 and Vieira and Volesky, 2000).

Microorganisms can be isolated from almost any environmental conditions. Microbes canadapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. The main requirements are an energy source and a carbon source (Vidali 2001). Because of the adaptability of microbes and other biologicalsystems, these can be used to degrade or remediate environmental hazards. Natural organisms, either indigenous or extraneous (introduced), are the prime agents used for bioremediation (Prescott *et al.,* 2002). The organisms that are utilized vary, depending on the chemical nature of the polluting agents, and are to be selected carefully as they onlysurvive within a limited range of chemical contaminants (Prescott *et al.,* 2002; Dubey, 2004). Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be required for effective mediation (Watanabe *et al.,* 2001). The first patent for a biological remediation agent was registered in 1974, being a strain of *Pseudomonas putida*that was able to degrade petroleum (Prescott *et al.*, 2002; Glazer and Nikaido, 2007).

These microorganisms can be subdivided into the following groups:

Aerobic: *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium.* These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Anaerobic: There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

Ligninolytic fungi: Fungi such as the white rot fungus *Phanaerochaetechrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corncobs.

Methylotrophs: Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1, 2-dichloroethane (EPA, 2003).

Bioremediation is not effective only for the degradation of pollutants but it can also be used to clean unwanted substances from air, soil, water and raw materials form industrial waste. With this in view, though many engineered processes for applying bioremediation have been developed but the inexpensive treatment of such sites has remained an elusive goal (Zeyaullah *et al.,* 2009).

Table 1: Microorganisms having biodegradation potential for xenobiotics (Source, Vidali 2001).

Microorganisms	Toxic chemicals	Reference
Pseudomonas spp.	Benzene, anthracene, hydrocarbons, PCBs	Cybulski *et al,* 2003
Alcaligenes spp.	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	Kapley *et al.,* 1999
Arthrobacter spp.	Benzene,hydrocarbons,pentachlorophenol, phenoxyacetate, polycyclic aromatic Aromatics, long chain alkanes, phenol, cresol	Jogdand, 1995
Bacillus spp.	Halogenated hydrocarbons, phenoxyacetates	Cybulski *et al.,*2003
Corynebacterium spp.	Aromatics	Jogdand, 1995
Flavobacterium spp.	Aromatics Naphthalene, biphenyl	Jogdand, 1995
Azotobacter spp.	Aromatics, branched hydrocarbons benzene,cycloparaffins	Jogdand, 1995 DeanRoss *et al.,* 2002
Rhodococcus spp.	Hydrocarbons Aromatics	DeanRoss *et al.,* 2002
Mycobacterium spp.	Aromatics Hydrocarbons, polycyclic hydrocarbons	Park *et al.,* 1998
Nocardia spp.	Phenoxyacetate, halogenated hydrocarbon diazinon	Jogdand, 1995
Methosinus sp.	PCBs, formaldehyde	Ijah, 1998
Methanogens	PCBs, polycyclic aromatics, biphenyl	Jogdand, 1995

Table 2: Microbes utilize the heavy metals (Source: Vidali 2001).

Microorganism	Elements	References
Bacillus spp.	Cu, Zn	Philip *et al.,* 2000; Gunasekaran *et al.,* 2003
Pseudomonas aeruginosa		
Zooglea spp.	U, Cu, Ni	Sar and D'Souza, 2001
Citrobacter spp.	Co, Ni, Cd	
Citrobacter spp.	Cd, U, Pb	Gunasekaran *et al.,* 2003
Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg,Zn	
Aspergilusniger	Cd, Zn Zn, Ag, Th, U	Gunasekaran *et al.,* 2003
Pleurotusostreatus	Cd, Cu, Zn	Gunasekaran *et al.,* 2003
Rhizopusarrhizus	Ag, Hg, P, Cd, Pb, Ca	Favero *et al.,* 1991, Gunasekaran *et al.,* 2003
Stereumhirsutum	Cd, Co, Cu, Ni	Gabriel *et al.,* 1994 and1 996
Phormidiumvalderium	Cd, Pb	Gabriel *et al.,* 1994 and 1996
Ganodermaapplantus	Cu, Hg, Pb	Gabriel *et al.,* 1994 and 1996

Phytoremediation

Plants can also be used to clean up soil, water or air; this is called *phytoremediation*. For instance, plants like locoweed remove large amounts of the toxic element Selenium. The Selenium is stored in plant tissues where it poses no harm until and unless the plant is eaten. Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (Glick, 2003). This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Phytoremediation applications can also be classified based on the mechanisms involved. Such mechanisms include extraction of contaminants from soil or groundwater; concentration of contaminants in plant tissue; degradation of contaminants by various biotic or abiotic processes; volatilization or transpiration of volatile contaminants from plants to the air; immobilization of contaminants in the root zone; hydraulic control of contaminated groundwater (plume control); and control of runoff, erosion, and infiltration by vegetative covers (Macek *et al.,* 2000).

Degradation

Plants may enhance degradation in the rhizosphere (root zone of influence). Microbial counts in rhizosphere soils can be one or two orders of magnitude greater than in non-rhizosphere soils. It is due to microbial or fungal symbiosis with the plant, plant exudates including enzymes, or other physical or chemical effects in the root zone. Contaminants like trinitrotoluene (TNT), petroleum hydrocarbons (PH), pentachlorophenol (PCP), and polynuclear aromatic hydrocarbons (PAH) are degraded in the root zone of planted areas.

Another possible mechanism for contaminant degradation is metabolism within the plant. Some plants may be able to take in toxic compounds and in the process of metabolizing the available nutrients, detoxify them. Trichloroethylene (TCE) is possibly degraded in poplar trees and the carbon used for tissue growth while the chloride is expelled through the roots (EPA, 2003).

Extraction

Phytoextraction, or phytomining, is the process of planting a crop of a species that is known to accumulate contaminants in the shoots and leaves of the plants, and then harvesting the crop and removing the contaminant from the site. Unlike the destructive degradation mechanisms, this technique yields a mass of plant and contaminant (typically metals) that must be transported for disposal or recycling. This is a concentration technology that leaves a much smaller mass to be disposed of when compared to excavation and land filling (EPA, 2003).

Volatilization or transpiration through plants into the atmosphere is another possible mechanism for removing a contaminant from the soil or water of a site. Mercury (Hg) has been shown to move through a plant and into the air in a plant that was genetically altered to allow it to do so. The thought behind this media switching is that elemental Hg in the air poses less risk than other Hg forms in the soil (EPA, 2003).

Containment and Immobilization

A containment using plant binds the contaminants to the soil, renders them non-bioavailable, or immobilizes them by removing the means of transport. Physical containment of contaminants by plants can take the form of binding the contaminants within a humic molecule (humification), physical sequestration of metals as occurs in some wetlands, or by root accumulation in nonharvestable plants. Certain trees sequester large concentrations of metals in their roots, and although harvesting and removal is difficult or impractical, the contaminants present a reduced human or environmental risk while they are bound in the roots. Risk reduction may also be achieved by transforming the contaminant into a form that is not hazardous, or by render ing the contaminant nonbioavailable (EPA, 2003).

Hydraulic control is another form of containment. Groundwater contaminant plume control may be achieved by water consumption, using plants to increase the evaporation and transpiration from a site. Some species of plants use tremendous quantities of water, and can extend roots to draw from the saturated zone (EPA, 2003).

Vegetative cover (evapotranspiration or water-balance cover) systems are another remediation application utilizing the natural mechanisms of plants for minimizing infiltrating water. Originally proposed in arid and semi-arid regions, vegetative covers are currently being evaluated for all geographic regions (EPA, 2003).

Advantages and disadvantage of bioremediation

Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies (Vidali, 2001 and Zeyaullah *et al.,* 2009).

Like other technologies, bioremediation also has its own limitations. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded (EPA, 2003). There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration (Vidali, 2001 and Zeyaullah *et al.,* 2009).

Treatment of wastes

Aerobic treatment of wastes

Aerobic microorganisms require oxygen as a terminal acceptor of electrons donated organic or inorganic substances. The transfer of electrons from donor to acceptor is a source of biologically available energy. Xenobiotics such as aliphatic hydrocarbons and derivatives, chlorinated aliphatic compounds (methyl-, ethyl, methylene and ethylene chlorides), aromatichydrocarbons and derivatives (benzene, toluene, phthalate, ethylbenzene, xylenes and phenol),polycyclic aromatic hydrocarbons, halogenated aromatic compounds (chlorophenols, polychlorinatedbiphenyls, dioxins and relatives, DDT and relatives), AZO dyes, compounds with nitro groups (explosive-contaminated waste and herbicides), and organophosphate wastes canbe treated effectively by aerobic microorganisms (EPA, 2003).

Aerobic Treatment of Solid Wastes

Composting is the simplest way to treat solid waste aerobically. Composting convertsbiologically unstable organic matter into a more stable humus-like product that can be usedas a soil conditioner or organic fertilizer. Additional benefits of composting of organic wastesinclude the prevention of odors from rotting wastes, destruction of pathogens and parasites(especially in thermophilic composting), and the retention of nutrients in the end products (EPA, 2003).

Aerobic Treatment of Liquid Wastes

Wastewater can be treated aerobically in suspended biomass stirred-tank bioreactors. Secondary wastes include polluted air and sediments produced in the bioreactor. Microbial biofilms can be concentrated on the surface of bioreactors and can biodegradehazardous substances with higher rates compared to situations when both substrate andmicrobial biomass are suspended in the wastewater (EPA, 2003).

Aerobic Treatment of Gaseous Wastes

The main applications of biotechnology for the treatment of gaseous wastes includethe bioremoval of biodegradable organic solvents, odors, and toxic gases, such as hydrogen sulfide and other sulfur-containing gases from the exhaust ventilation air in industry and farming. Industrial ventilated air containing formaldehyde, ammonia, and other low molecular weight substances can also be effectively treated (EPA, 2003).

Gaseous xenobiotics, which can be treated biotechnologically, include the following: chloroform, trichloroethylene, 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, carbontetrachloride, xylenes, dibromochloropropane, toluene, methane, methylene chloride, 1,1-dichloroethene, bis (2-chloroethyl) ether, 1,2-dichloroethane, chlorine, 1,1-trichloroethane, ethylbenzene, 1,1,2,2-tetrachloroethane, bromine, methylmercury, trichlorofluoroethane, 1,1

dichloroethane, 1,1,2-trichloroethane, ammonia, trichloroethane, 1,2-dichloroethene,carbon disulfide, chloroethane, p-xylene, hydrogen sulfide, chloromethane, 2-butanone,bromoform, acrolein, bromodichloroethane, nitrogen dioxide, ozone, formaldehyde, chlorodibromomethane,
ethyl ether, and 1,2-dichloropropane (EPA, 2003).

Anaerobic treatment of wastes

The advantageof anaerobic treatment is that there is no need to supply oxygen in the treatment system. This is useful in cases such as bioremediation of clay soil or high-strength organic waste. However, anaerobic treatment may be slower than aerobic treatment, and there may be sign ificantoutputs of dissolved organic products of fermentation or anaerobic respiration.

Anaerobicbiodegradation of organic matter and detoxication of hazardous wastes can be significantly enhanced as a result of precipitation of toxic organics, acids, phenols, or cyanideby Fe (II). Nitrate-respiring bacteria can be used in denitrification, i.e., reduction of nitrate to gaseous N_2. Nitrate can be added to the hazardous waste to initiate the biodegradation of different types of organic substances, for example polycyclic aromatic hydrocarbons (Eriksson *et al.,* 2003). Nitro-groups of hazardous substances can be reduced by similar pathway to relatedamines. Many hazardous substances, for example chlorinated solvents, phthalates, phenols, ethyleneglycol, and polyethylene glycols can be degraded by anaerobic microorganisms (Evans and Furlong, 2003; Borch *et al.,* 2003; Marttinen *et al*., 2003).

Bioagumentation

Many sites have been completely remediated by in-situtechniques that inject a variety of materials eitheras a liquid or slurry to degrade contamination. Underthe umbrella of bioremediation, additional microbes can be added to boost the resident populations of nutrients. In addition, chemicals designed to stimulate the natural degradation processes can be added, such asmolasses, permanganate, vegetable oil or oxygen. Eachapproach has its own unique advantages and limitations. Introducing specialized microbes at a site to essentially “eat” the contamination is called bioaugmentation or it frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. It has proven to be a fast, effective and affordable remediation alternative, and it is findingfavor among site managers and remediation experts (EPA, 2003).

To stimulate and enhance microbial activity, microorganisms (bioaugmentation**)** or amendments **(**biostimulation**),** such as air, organic substrates or other electron donors/acceptors, nutrients, and other compounds that affect and can limit treatment in their absence can be added. Biostimulation can be used where the bacteria necessary to degrade the contaminants are present but conditions do not favor their growth (e.g., anaerobic bacteria in an aerobic aquifer, aerobic bacteria in an anaerobic aquifer, lack of appropriate nutrients or electron donors/acceptors). Bioaugumentation can be used when the bacteria necessary to degrade the contaminants do not occur naturally at a site or occur at too low of a population to be effective. Biostimulation and bioaugmentation can be used to treat soil and other solids, groundwater, or surface water (EPA 2006).

Treatment of heavy metals

According to Sharm and Rehman (2009) heavy metals are normally regarded as metals with an atomic number 22 to 92 in all groups from period 3 to 7 in the periodic table. Some of the metals such as Cu, Zn, Cd, Pb, Fe, Cr, Co, Ni, Mn, Mo, V, Se are essential in trace quantities for the general wellbeing of living organism but an excess of these can be lethal. Costa and Duta (2001) reported that heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants. Their presence in soil and water, even in traces, can cause serious problems to all organisms. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality (safety and marketability), crop growth (due to phytotoxicity) and environmental health. Adhikari *et al.,* (2004) also added heavy metals at higher concentrations are toxic in nature to higher life forms because lead to biomagnifications and their pollution deteriorates the quality of soil and crops produced. Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes namely inhalation and ingestion, and with ingestion being the main route of exposure to these elements in human population. Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries (Costa and Duta, 2001).

In recent years, contamination of large areas of land by heavy metals has become a major concern. It originates mainly from municipal waste incinerators, car exhausts, residues from metalliferous mining and the melting industry, and the use of urban compost, pesticides, fertilizers or sludge and sewage. The sewage is characterized by a complex heterogeneous multifaceted matrix depending upon its origin and production. Urban sewage containing industrial effluent was found to carry relatively high amounts of heavy metals. Damodaran and Suresh (2011) identified heavy metals like Cu, Pb, Fe, Zn, Cd, Mn, Ni, Cr and Co which are responsible for soil and ground water.

Treatment of heavy metals-containing wastes

Liquid and solid wastes containing heavy metals may be successfully treated by biotechnologicalmethods. Some metals can be reduced or oxidized by specific enzymes of microorganisms. Microbial metabolism generates products such as hydrogen, oxygen, H_2O_2, which can be used for oxidation/reduction of metals. Reduction or oxidation of metals is usually accompanied by metal solubilization or precipitation. Solubilization or precipitation of metals may also be mediated by microbial metabolites. Microbial production of organic acids infermentation or inorganic acids (nitric and sulfuric acids) in aerobic oxidation will promote formation of dissolved chelates of metals (EPA 2006).

Microbial production of phosphate, H_2S, and CO_2will stimulate precipitation of non-dissolved phosphates, carbonates, and sulfides of heavy metals such as arsenic, cadmium, chromium, copper, lead, mercury, nickel; production of $H_2S$by sulfate-reducing bacteria is especially useful to remove heavy metals and radionuclides from sulfate-containing mining drainage waters, liquid waste of nuclear facilities, drainage from tailing pond of hydrometallurgical plants; wood straw or saw dust. Organic acids, produced during the anaerobic fermentation of cellulose, may be used as a source of reduced carbon for sulfate reduction and further precipitation of metals (EPA 2006).

The surface of microbial cells is covered by negatively charged carboxylic and phosphate groups, and positively charged amino groups. Therefore, depending on pH, there may besignificant adsorption of heavy metals onto the microbial surface (Moo-Young and Anderson, 1996). Biosorption, for example by fungal fermentation residues, is used to accumulate uranium and other radionuclides from waste streams.

Metal-containing minerals such as sulfides can be oxidized and metals can be solubilized. This approach is used for the bioleaching of heavy metals from sewage sludge beforelandfilling or biotransformation (Xiang *et al.,* 2000 and Ito *et al*., 2001). Some metals, arsenic and mercury for example, may bevolatilized by methylation due to the activity of anaerobic microorganisms. Arsenic can bemethylated by methanogenic Archaea and fungi to volatile toxic dimethylarsine and trimethylarsineor can be converted to less toxic non-volatile methanearsonic and dimethylarsinic acidsby algae. Hydrophobic organotins are toxic to organisms because of their solubility in cellmembranes. However, many microorganisms are resistant to organotins and can detoxicatethem by degrading the organic part of organotins (Gadd, 2000).

In some cases, the different biotechnological methods may be combined. Examples wouldinclude the biotechnological precipitation of chromium from Cr (VI)-containing wastes fromelectroplating factories by sulfate reduction to precipitate chromium sulfide. Sulfate reductioncan use fatty acids as organic substrates with no accumulation of sulfide. In the absence of fatty acids but with straw as an organic substrate, the direct reduction of chromium has been observed without sulfate reduction (Vainshtein *et al.,* 2003).

Enhancement of biotechnological treatment of wastes

The goal of bioremediation is the neutralization of the pollution to achieve undetectable concentrations. Several key factors are critical for the successful application of biotechnology for the treatment of hazardous wastes. Bioremediation is utilized for cleanup of grounds and ground waters as well as sewage and sludge. Kołwzan, *et al.*(2006) elaborated that during the utilization of bioremediation for the purpose of pollution neutralization the following conditions must be met:

The environment undergoing bioremediation should contain microorganisms characterized by the specific catabolic processes,

1. microorganisms utilized within the bioremediation process should be capable of efficiently converting chemical compounds and reducing their concentration down to the level allowed by the regulations,
2. metabolites produced during the biodegradation cannot have toxic, mutagenic or carcinogenic properties,
3. the conditions in the immediate area where the process is being conducted should be favorable to the growth and activity of the microorganisms (adequate nutrients, acceptable pH, oxygen or other electron acceptor, acceptable redox level, favorable moisture). The rate of biodegradation may be limited by temperature, the toxicity of concentrated contaminants; or mass transfer limitations.

Biomonitoringof Bioremediation: Biosensors

An important application of environmental biotechnology is biomonitoring, including-monitoring of biodegradability, toxicity, mutagenicity, concentration of hazardous substances, and monitoring of concentration and pathogenicity of microorganisms in wastes and in the environment. Simple or automated off-line or on-line biodegradability tests can be performed by measuring CO_2 or CH_4 gas production or O_2 consumption (Reuschenbach *et al.,* 2003). Biosensors are analytical tools, which use the biological specificity in sensing the target molecule.

Biosensors may utilizeeither whole bacterial cells or enzyme to detect specific molecules of hazardous substances. Toxicity can be monitored specifically by whole cell sensors whose bioluminescence may beinhibited by the presence of hazardous substance.

The most popular approach uses cells with an introduced luminescent reporter geneto determine changes in the metabolic status of the cells following intoxication (Bentley *et al.,* 2003).Nitrifying bacteria have multiple-folded cell membranes, which are sensitive to all membrane disintegrating substances: organic solvents, surfactants, heavy metals, and oxidants. Therefore, respirometric sensors measuring the respiration rates of these bacteria can be used for toxicity monitoring in wastewater treatment (Inui *et al.,* 2002).

Biosensors measuring concentrations of hazardous substances are often based on the measurement of bioluminescence (Lajoie *et al.,* 2002). This toxicity sensor is a bioluminescent toxicity bioreporter for hazardous wastewater treatment. It is constructed by incorporating bioluminescence genes into a microorganism. These whole cell toxicity sensors are very sensitive and may be used on-line to monitor and optimize the biodegradation of hazardous soluble substances.

Similar sensors can be used for the measurement of the concentration of specific pollutants.

A gene for bioluminescence has been fused to the bacterial genes coding for enzymes that metabolize the pollutant. When this pollutant is degraded, the bacterial cells will produce light. The intensity of biodegradation and bioluminescence depend on the concentration of pollutant and can be quantified using fiber-optics on-line. Combinations of biosensors in array can be used to measure concentration or toxicity of a set of hazardous substances (Lajoie *et al.,* 2002).

The mutagenic activity of chemicals is usually correlated with their carcinogenic properties. Mutant bacterial strains have been used to determine the potential mutagenicity of manufactured or natural chemicals. The most common test, proposed by Ames in 1971, utilizes back mutation in auxotrophic bacterial strains that are incapable of synthesizingcertain nutrients. When auxotrophic cells are spread on a medium that lacks the essentialnutrients (minimal medium), no growth will occur. However, cells that are treated with a testedchemical that causes a reversion mutation can grow in a minimal medium. The frequency of mutation detected in the test is proportional to the potential mutagenicity and carcinogenicity of the tested chemical. Microbial mutagenicity tests are used widely in modern research (Czyz *et al.,* 2000; Hwang *et al.,* 2001 and Yamamoto *et al.,* 2002).

Cell components or metabolites capable of recognizing individual and specific molecules can be used as the sensory elements in molecular sensors (Loy *et al.,* 2002). Sensors may be enzymes, sequences of nucleic acids (RNA or DNA), antibodies, polysaccharides or other “reporter” molecules. Antibodies, specific for a microorganism used in the biotreatment, can be coupled with fluorochromes to increase sensitivity of detection. Such antibodies are useful in monitoring the fate of bacteria released into the environment for the treatment of a polluted site.

Fluorescent or enzyme-linked immunoassays have been derived and can be used for a variety

Of contaminants, including pesticides and chlorinated polycyclic hydrocarbons. Enzymesspecific for pollutants and attached to matrices detecting interactions between enzymes and pollutants are used in on-line biosensors of water and gas biotreatment (Nielsen *et al.,* 2002).

A useful approach tomonitor microbial population in the biotreatment of hazardous wastesinvolves the detection of specific sequences of nucleic acids by hybridization with complementary oligonucleotide probes. Radioactive labels, fluorescent labels, and other kinds of the labels are attached to the probes to increase sensitivity and simplicity of the hybridization detection. Nucleic acids which are detectable by the probes include chromosomal DNA, extra chromosomal DNA such as plasmids, synthetic recombinant DNA such as cloning vectors, phage or virus DNA, rRNA, tRNA and mRNA transcribed from chromosomal or extrachromosomal DNA. These molecular approaches may involve the hybridization of wholeintact cells, or extraction and treatment of targeted nucleic acids prior to probe hybridization (Hatsu *et al.,* 2002). Microarrays for simultaneous semi-quantitative detection of different microorganismsor specific genes in the environmental sample have also been developed (Fredrickson *et al.,* 2001; Koizumi *et al.,* 2002 and Loy *et al.,* 2002).

CONCLUSION

Bioremediation provides a technique for cleaning up pollution by enhancing the natural biodegradation processes. So by developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting field trials of new bioremediation techniques which are cost effective, and dedicating sites which are set aside for long term research purpose, these opportunities offer potential for significant advances. There is no doubt that bioremediation is in the process of paving a way to greener pastures. Regardless of which aspect of bioremediation that is used, this technology offers an efficient and cost effective way to treat contaminated ground water and soil.

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