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# A Bird's Eye View on Bioremediation Approaches of Heavy Metals Contaminated Soil Regimes

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**Abstract:**
**The pollution of environment, particularly that caused by various industrial activities, have been responsible for the organic and inorganic matter in the ecosphere. Heavy metals and other compounds even low concentrations can be toxic to humans and other forms of life. Many of the remediation technologies currently being used for contaminated soil and water involve not only physical and chemical treatment, but also biological processes, where microbial activity is the responsible for pollutant removal and/or recovery. Fungi are present in aquatic sediments; water surfaces and soil play a significant role in natural remediation of metal compounds. The bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. The utilization of organisms, primarily microbes, to clean up contaminated soils, aquifers, sludges, residues, and air known as 'Bioremediation', is a rapidly changing and expanding area of environmental cleanup technique than conventional physico-chemical methods. Bioremediation has been used at a number of sites worldwide with varying degrees of success. Microbes are very helpful to remediate the contaminated environment. Number of microbes including aerobes, anaerobes and fungi are involved in bioremediation process. Fungi also have advantages over bacteria since fungal hyphae can penetrate contaminated soil, reaching heavy metals. This review provides basic understanding of the bioremediation technique and the possible mechanisms.**

**Keywords: Heavy metals, Bioremediation, Fungi, Contaminated soil, Toxicity**

## 1.0 Introduction:

A major environmental concern due to the human activities is the contamination of soil. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. A wide range of inorganic and organic compounds cause contamination, these include heavy metals, combustible and putriscible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminates are heavy metals **(Adriano, 1986; Alloway, 1990),** they present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, while metals need immobilization or physical removal. Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function **(Henry, 2000).** Thus, metals render the land unsuitable for plant growth and destroy the biodiversity **(Ghosh *et al.,* 2005).** Industrialization is accelerating the deposition of heavy metals in soil and water bodies. In some ecosystems these metals can be easily incorporated by organic and inorganic fractions of the soil and by sediments. The extent of this incorporation depends on the concentration of metals and on characteristic biotic and abiotic factors. However, in water bodies or soil, metals can be remobilized, acting as toxic elements. This way, it is essential to minimize deleterious effects of dispersion in natural waters, through the use of suitable technology-based techniques.

**Bioremediation** is very useful method for wider application in the area of environmental protection. Bioremediation approach is currently applied to contain contaminants in soil, ground water, surface water and sediments including air. These technologies have become attractive alternatives to conventional cleanup technologies due to relatively low capital costs and their essentially aesthetic nature **(Subrahmanyam, & Prasad, 2011).** Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds **(Lal *et al.,* 1996).** The success of bioremediation depends on having the appropriate microorganisms in place under suitable environmental conditions. Its operational use can be limited by the composition of the contaminant **(Mandal *et al.,* 2011).** Bioremediation is the use of micro-organism metabolism to remove pollutants. Technologies can be generally classified as *in situ* or *ex situ*. *In-situ* bioremediation involves treating the contaminated material at the site, while ex-situ involves the removal of the contaminated material to be treated elsewhere. Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators **(Agarwal, 1998).** Several microorganisms (*Pseudomonas, Burkholderia, Sphingomonas, Ralstonia, Comamonas, Achromobacter, Alcaligenes, Rhodococcus, Dehalococcoides*) are known to degrade xenobiotics, or to accumulate or detoxify heavy metal pollutants such as Cd, Hg, Pb, Zn, U, etc., **(Daly, 2000; Lloyd *et al.,* 2003).** An important difference between bioremediation of toxic metals and bioremediation of xenobiotics is the existence of heavy metals under different elemental state (e.g., conversion of Hg2+ to the volatile Hg0, thus moving the metal from the soil to the atmosphere). In contrast, the bioremediation of xenobiotics results in complete mineralization of the toxic substances. In situ bioremediation uses naturally occurring non engineered microorganisms and is often enhanced (biostimulation) by the addition of nutrients, such as N and P, surfactants and oxygen during the treatment **(Watanabe, 2001).**

Bioremediation uses biological agents, mainly microorganisms i.e. yeast, fungi or bacteria to clean up contaminated soil and water **(Strong & Burgess, 2008).**This technology relies on promoting the growth of specific micro flora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities **(Agarwal, 1998).** Establishment of such microbial consortia can be done in several ways e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions **(Hess *et al.,* 1997; Agarwal, 1998; Smith *et al.,* 1998).** In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources **(Hess *et al.,* 1997; Agarwal, 1998; Tang *et al.,* 2007).** In nature there are various fungi, bacteria and microorganisms that are constantly at work to break down organic compounds but the question arises when pollution occurs, who will do this clean up job? Since the quality of life is inextricably linked to the overall quality of the environment, global attention has been focused on ways to sustain and preserve the environment. This endeavor is possible by involving biotechnology. The types of contaminants that Environmental Biotechnology investigators have expertise with include chlorinated solvents, petroleum hydrocarbons, polynuclear aromatic hydrocarbons, ketones, TNT, inorganic nitrogen (NO3, NH4), Tt, , Pu, Np, Cr, U and other heavy metals. Bioremediation is the term used to describe biological strategies applicable to repair of damaged environment using biological factors. In the case of oil spills, the process exploits the catabolic ability of microorganism feeding on oil. Several workers **(Odu, 1978; Solan, 1987; Ijah and Antai, 1988; Okpokwasili and Okorie, 1988; Barnhart and Meyers, 1989; Anon, 1990; Pritchard, 1991; Pritchard and Costa, 1991; Hoyle, 1992; Ijah, 2002 and Ijah, 2003)** have described various application of microorganism in the bioremediation of oil pollution with encouraging results. Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Not all contaminants are readily treated through the use of bioremediation; Heavy metals such as cadmium and lead are not readily absorbed or captured by organisms **(Vidali 2001).** The integration of metals such as mercury into the food chain may make things worse as organism bioaccumulate these metals. However, there are a number of advantages to bioremediation, which may be employed in areas which cannot be reached easily without excavation. The foundation of bioremediation has been the natural ability of microorganisms to degrade organic compounds. The conventional techniques used for remediation have been to dig up contaminated site and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability. A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high temperature incineration and various types of chemical decomposition. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity **(Gupta 2003).**. Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Alternatively, the contaminants may be enzymatically transformed to metabolites that are less toxic or harmless. It should be noted that in some instances, the metabolites formed are more toxic than the parent compound. Whether bioremediation is the appropriate cleanup remedy for a site is dependent on whether the rate and extent of contaminant degradation is sufficient to maintain low risks to human or environmental receptors.

**Sources of Heavy Metal Contamination in Soils:**
Heavy metals such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury are discharged from industrial operations such as smelting, mining, metal forging, manufacturing of alkaline storage batteries, and combustion of fossil fuel. Moreover, the agricultural activities like application of agrochemicals, and long-term usage of sewage sludge in agricultural fields also add a significant amount of metals in the soils **(Giller *et al.*, 1998; Malik & Ahmad, 1995).** Various anthropogenic sources of metal contamination of soils have been shown in Figure– 1. **(Munees, 2012).**

Inorganic pollutants which contaminate land and water bodies include heavy metals, metalloids, fluorides and cyanides etc. Heavy metals can occur in different valence states, so that one element may be more or less toxic in different states. Normally heavy metals in the environment are in low concentrations but may be elevated because of human activities, fossil fuel combustion, mining, smelting, and sludge amendment to soil, fertilizer application and agrochemical application. At low concentrations some trace elements eg: Cu, Cr, Mo, Ni, Se, and Zn, etc. are essential for healthy functioning of biota. However, higher concentrations of all essential elements can also cause toxicity. Some trace elements are also non-essential eg: As, Cd, Hg and Pb etc. are extremely toxic to biota even at very low concentrations **(Subrahmanyam& Prasad, 2011).** A number of pollutants and waste materials containing heavy metals are released into the environment due to rapid industrialization **(Shukla *et al.*, 2010).** Recent advances in the field of molecular biology have enabled us to understand the metal-microbe interaction and their application for bioremediation of metal in the environment **(Rajendran *et al.,* 2003).** Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water **(Eccles & Hunt, 1986; Kamaludeen *et al.,* 2003).** Bioremediation uses biological agents, mainly microorganisms like fungi or bacteria, yeast to clean up contaminated soil and water **(Strong & Burgess, 2008).** This technology relies on promoting the growth of specific micro flora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities **(Agarwal, 1998).** Establishment of such microbial consortia can be done in several ways, eg: by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions, among others **(Hess *et al.,* 1997; Agarwal, 1998; Smith *et al.,* 1998).** In Bioremediation processes, microorganisms use the contaminants as nutrient or energy sources **(Hess *et al.,* 1997; Agarwal, 1998; Tang *et al.,* 2007).**

**Principles of Bioremediation**
Recent studies in molecular biology and ecology offers numerous opportunities for more efficient biological processes. Important activities of these studies include the cleanup of polluted water and land areas. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities **(Mueller *et al.,* 1996).** By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the

environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products **(Vidali, 2001).** As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions **(Colberg & Young, 1995)** may permit microbial organisms to degrade otherwise recalcitrant molecules.

**Types of Bioremediation**

On the basis of removal and transportation of wastes for treatment there are basically two methods: a. *In situ* bioremediation, b. *Ex. situ* bioremediation

**a. *In Situ* Bioremediation**

*In situ* bioremediation means there is no need to excavate or remove soils or water in order to accomplish remediation. *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. Generally, this technique includes conditions such as the infiltration of water containing nutrients and oxygen or other electron acceptors for groundwater treatment **(Vidali, 2001).** 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. Chemo taxis is important to the study of *in situ* bioremediation because microbial organisms with chemotactic abilities can move into an area containing contaminants. So by enhancing the cells' chemotactic abilities, *in situ* bioremediation will become a safer method in degrading harmful compounds.

**Types of *In Situ* Bioremediation**

**i. Intrinsic bioremediation**

This approach deals with stimulation of indigenous or naturally occurring microbial populations by feeding them nutrients and oxygen to increase their metabolic activity.

**ii. Engineered *in situ* bioremediation**

The second 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. 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. 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.

**b. *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:

1. Solid phase system (including land treatment and soil piles)
2. Slurry phase systems (including solid liquid suspensions in bioreactors)

**i. Solid phase treatment:** It includes organic wastes (leaves, animal manures and agricultural wastes) and problematic wastes e.g. domestic and industrial wastes, sewage sludge and municipal solid wastes. Solid phase soil treatment processes include land farming, soil biopiles, and composting.

**1. Land farming**: It is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since land farming has the potential to reduce monitoring and maintenance costs, as well as cleanup liabilities, it has received much attention as a disposal alternative. It also helps to contain any evaporated contaminants.

**2. Biopiling:** This is exactly what you would expect from its name. Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms (U.S.EPA handbook). The contaminated soil is excavated and put into piles. These piles are usually 2-3 meters in height. These piles are placed over an aeration system. This system pulls air through pile of contaminated soil by means of a vacuum pump. This movement of air not only provides oxygen to the microorganisms, by it also pulls some of the contaminants out of the soil as it passes through soil. A collection system similar to the one used in land farming is also used with soil biopiles. Optimal bioremediation conditions are maintained by the control of the moisture and nutrient levels. Another form of control is the placement of the piles into enclosures. This prevents and unwanted weather changes and helps to control any temperature changes. Volitatile contaminants (evaporated contaminants) are minimal because the vacuum pump pulls any evaporated contaminants through the pile, keeping them from escaping into the atmosphere. These piles do require quite a bit of space, but they do not need as much space as land farming does. It is a short term technology that usually only operates for a few weeks or a few months.

**3. Composting:** 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 elevated temperature characteristic of composting. It involves first the excavation of the contaminated soil. A bulking agent of some sort is added to the contaminated soil, which is then known as compost material. Bulking agents include things like: hay, straw and corn cobs. These things make it much easier for the cleanup crews to maintain the maximum rate of degradation of the contaminants. The bulking agents allow the cleanup crews to easily control the amounts of water and air that are available to the microorganisms involved in the degradation reaction. There are three methods of composting that are used. The first is called static pile composting. This involves the formation of piles and aerating them by means of a blower or a vacuum pump. The second is called mechanically agitated in-vessel composting, which involves the compost material being placed in a treatment vessel. Here, it undergoes mixing and aeration. The third is called windrow composting. This method involves placing the compost material into windrows (long piles as in a farmer's field). These windrows are then mixed up thoroughly by tractors and other such equipment. Windrow composting is the most common method, mainly because it is the most cost-effective method. One interesting thing about composting is that it not only works in soil but it also can be applied to contaminated lagoons and swampy areas. Another good thing about composting is that all of the necessary equipment can be commercially obtained.

**ii. Slurry Phase Bioremediation**: Slurry phase bioremediation is a relatively more rapid process 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.

**1. Bioreactors**: Slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material e.g. soil, sediment, sludge or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three phases e.g. solid, liquid, and gas, mixing condition to increase the bioremediation rate of soil bound and water soluble pollutants as a 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 pre treatment or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction before being placed in a bioreactor (U.S. EPA Handbook).

**2. Bioventing**: It is the most common in situ treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

**3. Biosparging**: Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small diameter air injection points allows considerable flexibility in the design and construction of the system.

**4. Bioaugmentation**: Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit: no indigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and most soils with long term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

**Advantages of Bioremediation**

1. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.
2. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
3. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
4. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
5. Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

**Disadvantages of Bioremediation**

Bioremediation, although considered a boon in the midst of present day environmental situations, can also be considered problematic because, while additives are added to enhance the functioning of one particular bacterium, fungi or any other microorganisms, it may be disruptive to other organisms inhabiting that same environment when done in situ **(Vidali, 2001).** Even if genetically modified microorganisms are released into the environment after a certain point of time it becomes

difficult to remove them. Bioremediation is generally very costly, is labor intensive, and can take several months for the remediation to achieve acceptable levels. Another problem regarding the use of in situ and ex situ processes is that it is capable of causing far more damage than the actual pollution itself.
1. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
2. There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
3. 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.
4. It is difficult to extrapolate from bench and pilot scale studies to full scale field operations.
5. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment.
6. Contaminants may be present as solids, liquids and gases.
7. Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration **(Vidali, 2001 & Kumar *et al.,* 2011).**

**Other Types of Bioremediation**

Bioremediation can be broken down into four categories: bacterial remediation, mycoremediation, phytoremediation, and compost bioremediation.

**(a) Bacterial remediation** is the process of using bacteria to break down molecular contaminants like hydrocarbons into simpler, safer components. It can be accomplished by culturing (breeding) bacteria in high numbers and then introducing them into a contaminated area, and/or by turning the affected soil into an ideal habitat for bacterial growth. Large numbers of beneficial bacteria can be introduced into soil by brewing something called compost tea or through use of a product called Effective Microorganisms.

**(b)Phytoremediation** is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal **(Alloway, 1990).** The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. The use of genetic engineering to create organisms specifically designed for bioremediation has great potential (Henry, 2000). The bacterium Deinococcus radiodurans (the most radio resistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste (Baker & Walker P, 1990).

Phytoremediation is a form of bioremediation and applies to all chemical or physical processes that involve plants for degrading or immobilizing contaminants in soil and ground water. While the technology is not new, current trends suggest its popularity is growing. The following is a list of six different types of phytoremediation with explanations describing how they work.

1. **Phytosequestration** also called **phytostabilization**. Many different processes fall under this category which can involve absorption by roots, adsorption to the surface of roots or the production of biochemicals by the plant that are released into the soil or ground water in the immediate vicinity of the roots, and can sequester, precipitate, or otherwise immobilize nearby contaminants. It is mostly used for the remediation of soil, sediment and sludges **(USPAR, 2000 & Muller *et al*., 1999)** and depends on roots ability to limit contaminant mobility and bioavailability in the soil. Phytostabilisation can occur through the sorption, precipitation, complex action, or metal valence reduction. The plants primary purpose is to decrease the amount of water percolating through the soil matrix, which may result in the formation of hazardous leachate and prevent soil erosion and distribution of the toxic metal to other areas. A dense root system stabilizes the soil and prevents erosion **(Berti, & Cunningham, 2000).** It is very effective when rapid immobilization is needed to preserve ground and surface water and disposal of biomass is not required. However the major disadvantage is that, the contaminant remains in soil as it is, and therefore requires regular monitoring.

**2. Rhizodegradation**

This takes place in the soil or ground water immediately surrounding the plant roots. Exudates from plants stimulate rhizosphere bacteria to enhance biodegradation of soil contaminants.

**3. Rhizofiltration:**
It is defined as the use of plants, both terrestrial and aquatic; to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots. Rhizofiltration can partially treat industrial discharge, agricultural runoff, or acid mine drainage. It can be used for lead, cadmium, copper, nickel, zinc and chromium, which are primarily retained within the roots **(Chaudhry *et al.,* 1998 & USPAR, 2000).** The advantages of rhizofiltration include it ability to be used as in-situ or ex-situ applications and species other than hyper accumulators can also be used. Plants like sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability. Indian mustard has proven to be effective in removing a wide concentration range of lead (4 –500 mg/L) **(Raskin *et al.,* 2000).** The technology has been tested in the field with uranium (U) contaminated water at concentrations of 21-874 µg/L; the treated U concentration reported by Dushenkov was < 20 µg/L before discharge into the environment **(Dushenkov *et al*., 1997).**

**4. Phytohydraulics**
Use of deep-rooted plants (usually trees) to contain appropriate or degrade ground water contaminants that come into contact with their roots. In one example of this, poplar trees were used to contain a ground water plume of methyl-tert-butyl-ether (MTBE) **(Hong *et al*., 2001).**

**5.Phytoextraction** also known as **phytoaccumulation**. Plants take up or hyper accumulate contaminants through their roots and store them in the tissues of the stem or leaves. The contaminants are not necessarily degraded but are removed from the environment when the plants are harvested. This is particularly useful for removing metals from soil and, in some cases; the metals can be recovered for reuse, by incinerating the plants, in a process called phytomining.
It is the best approach to remove the contamination primarily from soil and isolate it, without destroying the soil structure and fertility. It is also referred as phytoaccumulation **(USPAR, 2000).** As the plant absorb, concentrate and precipitate toxic metals and radionuclide from contaminated soils into the biomass, it is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and superficially **(Rulkens *et al*., 1998).** Several approaches have been used but the two basic strategies of phytoextraction, which have finally developed are; i) Chelate assisted phytoextraction or induced phytoextraction, in which artificial chelates are added to increase the mobility and uptake of metal contaminant. ii) Continuous phytoextraction in this the removal of metal depends on the natural ability of the plant to remediate; only the number of plant growth repetitions are controlled **(*Salt et al.,* 1995; Salt *et al*., 1997).** Discovery of hyper accumulator species has further boosted this technology. In order to make this technology feasible, the plants must, extract large concentrations of heavy metals into their roots, translocate the heavy metals to surface biomass, and produce a large quantity of plant biomass. The removed heavy metal can be recycled from the contaminated plant biomass **(Brooks *et al.*, 1998).** Factors such as growth rate, element selectivity, resistance to disease, method of harvesting, are also important **(Cunningham, and Ow, 1996; Baker *et al*., 1994).** However slow growth, shallow root system, small biomass production, final disposal limit the use of hyper accumulator species **(Brooks, 1994).**

**6. Phytovolatilization**
Plants take up volatile compounds through their roots, and transpire the same compounds, or their metabolites, through the leaves, thereby releasing them into the atmosphere. Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere. Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations **(Muller, *et al*., 1999).** Phytovolatilization has been primarily used for the removal of mercury; the mercuric ion is transformed into less toxic elemental mercury. The disadvantage is, mercury released into the atmosphere is likely to be recycled by precipitation and then redeposit back into ecosystem **(Henry, 2000).** Gary Banuelos of USDS's Agricultural Research Service have found that some plants grow in high Selenium media produce volatile selenium in the form of dimethylselenide and dimethyldiselenide **(Branuelos, 2000).** Phytovolatilization has been successful in tritium (3H), a radioactive isotope of hydrogen; it is decayed to stable helium with a half-life of about12 years reported **Dushenkov, 2003).**

**7. Phytodegradation**

Contaminants are taken up into the plant tissues where they are metabolized, or biotransformed. Where the transformation takes place depends on the type of plant, and can occur in roots, stem or leaves. In phytoremediation of organics, plant metabolism contributes to the contaminant reduction by transformation, break down, stabilization or volatilizing contaminant compounds from soil and groundwater. Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues **(Chaudhry *et al*., 1998).** Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases **(Black, 1995).** Rhizodegradation is the breakdown of organics in the soil through microbial activity of the root zone (rhizosphere) and is a much slower process than phytodegradation. Yeast, fungi, bacteria and other microorganisms consume and digest organic substances like fuels and solvents. All phytoremediation technologies are not exclusive and may be used simultaneously, but the metal extraction depends on its bio available fraction in soil. The advantages and disadvantages have been discussed in Table 2 **(Ghosh & Singh., 2005).**

**Table1. Advantages and disadvantages of phytoremediation (recollected from Ghosh & Singh., 2005)**

| No | Advantages | Disadvantages / Limitations |
|---|---|---|
| 1 | Amendable to a variety of organic and inorganic compounds | Restricted to sites with shallow contamination within rooting zone of remediative plants. |
| 2 | *In Situ* / *Ex Situ* Application possible with effluent/soil substrate respectively. | May take up to several years to remediate a contaminated site. |
| 3 | *In Situ* applications decrease the amount of soil disturbance compared to conventional methods. | Restricted to sites with low contaminant concentrations. |
| 4 | Reduces the amount of waste to be land filled (up to 95%), can be further utilized as bio-ore of heavy metals. | Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence disposal should be proper. |
| 5 | *In Situ* applications decrease spread of contaminant via air and water. | Climatic conditions are a limiting factor |
| 6 | Does not require expensive equipment or highly specialized personnel. | Introduction of nonnative species may affect biodiversity |
| 7 | In large scale applications the potential energy stored can be utilized to generate thermal energy. | Consumption/utilization of contaminated plant biomass is a cause of concern. |

**(c)Mycoremediation** is a form of bioremediation in which fungi are used to decontaminate the area. The term *mycoremediation* refers specifically to the use of fungal mycelia in bioremediation. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. **Mycofiltration** is a similar process, using fungal mycelia to filter toxic waste and microorganisms from water in soil. The process of bioremediation can be monitored indirectly by measuring the *Oxidation Reduction Potential* or redox in soil and groundwater, together with pH, temperature, oxygen content, electron acceptor/donor concentrations, and concentration of breakdown products (e.g. carbon dioxide).

**Microbial Populations for Bioremediation Processes**

Microorganisms can be isolated from almost any environmental conditions. Microbes can adapt 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. The main requirements are an energy source and a carbon source **(Vidali, 2001).** Because of the adaptability of microbes and other biological systems, 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 only survive 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 (Table1; **Watanabe *et al.*, 2001).** The first patent for a biological remediation agent was registered in 1974, being a strain of *Pseudomonas putida* **(Prescott *et al.,* 2002)** that was able to degrade petroleum. In 1991, about 70 microbial genera were reported to degrade petroleum compounds **(U.S Congress, 1991)** and almost an equal number has been added to the list in the successive two decades **(Glazer and Nikaido, 2007).** Bioremediation can occur naturally or through intervention processes **(Agarwal, 1998).** Natural degradation of pollutants relies on indigenous micro flora that is effective against specific contaminants and it usually occurs at a slow rate. With intervention processes, the rate of biodegradation is aided by encouraging growth of microorganisms, under optimized physic-chemical conditions **(Blackburn & Hafker, 1993; Bouwer *et al.,* 1998; Smith *et al.,* 1998).** Fungi grow in a filamentous form toward the contaminant. Many different types of organisms such as plants can be used for bioremediation but microorganisms show the greatest potential. Microorganisms primarily bacteria and fungi are nature's original recyclers. Their capability to transform natural and synthetic chemicals into sources of energy and raw materials for their own growth suggests that expensive chemical or physical remediation processes might be replaced with biological processes that are lower in cost and more environmentally friendly. Therefore, microorganisms represent a promising, largely untapped resource for new environmental biotechnologies. Research continues to verify the bioremediation potential of microorganisms. For instance, a recent addition to the growing list of bacteria that can reduce metals is *Geobacter metallireducens,* which removes uranium, a radioactive waste from drainage waters in mining operations and from contaminated groundwater. Even dead microbial cells can be useful in bioremediation technologies. These discoveries suggest that further exploration of microbial diversity is likely to lead to the discovery of many more organisms with unique properties useful in bioremediation **(U.S. EPA Seminars, 1996).** The microorganisms capable of degrading petroleum include pseudomonads, various corynebacteria, mycobacteria and some yeast **(Mueller, 1996).** Apart from degrading hydrocarbons, microbes also have the ability to remove industrial wastes, reduce the toxic cations of heavy metals to a much less toxic soluble form. 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).**

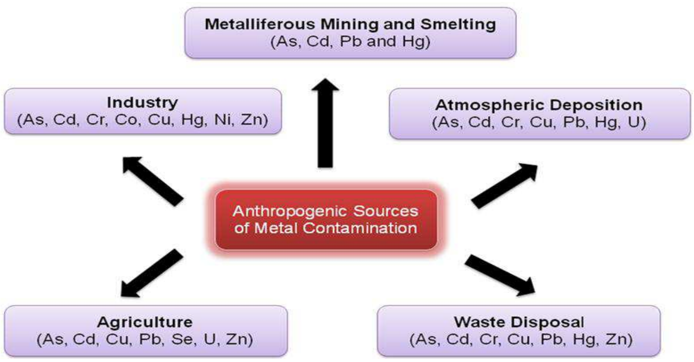


**Fig: 1. Anthropogenic activities leading to the contamination of soils with heavy metals (recollected from Munees, 2012).**

**Table 2: Microbes utilize he heavy metals**

| Microorganism | Elements | References |
|---|---|---|
| *Bacillus spp.* | Cu, Zn | Philip *et al.,* 2000; Gunasekaran *et al.,*2003 |
| *Pseudomonas* | U, Cu, Ni | Sar *et al.,* 1999; Sar and D'Souza, 2001 |
| *aeruginosa* | Co, Ni, Cd | Gunasekaran *et al.,* 2003 |
| *Zooglea spp.* | Cd, U, Pb | Yan and Viraraghavan, 2001; |
| *Citrobacter spp.* | Au, Cu, Ni, U, Pb, Hg, Zn | Gunasekaran *et al.,* 2003 |
| *Chlorella vulgaris* | Cd, Zn Zn, Ag, Th, U | Pearson, 1969; Gunasekaran *et al.,* 2003 |
| *Aspergilus niger* | Cd, Cu, Zn | Guibal *et al.,* 1995; Gunasekaran *et al.,* 2003 |
| *Pleurotus ostreatus* | Ag, Hg, P | Favero *et al.,* 1991 |
| *Rhizopus arrhizus* | Cd, Pb, Ca | Gunasekaran *et al.,* 2003 |
| *Stereum hirsutum* | Cd, Co, Cu, Ni | Gabriel *et al.,* 1994 and1 996 |
| *Phormidium valderium* | Cd, Pb | Gabriel *et al.,* 1994 and 1996 |
| *Ganoderma applantus* | Cu, Hg, Pb | Gabriel *et al.,* 1994 and 1996 |

**8. Microbe Assisted Bioremediation**

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as Bioremediators (bioaugmentation). Metallic pollutants are not degraded during composting but may be converted into organic combinations that have less bioavailability than mineral combinations of the metals **(Barker and Bryson, 2002).**

Many micro-organisms can produce iron-complexing molecules, named siderophores. These molecules are synthesized in case of iron deficiency. Some of these siderophores also have high affinities for heavy metals, and in case of *Pseudomonas aeruginosa* and *Alcallgenes eutrophus* siderophore (pyoverdin and alcaligme E, respectively), synthesis was also induced by heavy metals even in the presence of high iron concentrations. A comparison between negative and constitutive siderophore mutants leads to the conclusion that siderophores or, more generally metallophores, can play a role in metal solubilization.

The metal solubilization and biocrystallization capacity of *A. eutrophus* CH34 was used to treat sandy soils contaminated with heavy metals. The bacterium can solubilize the metals (or increase their bioavailability) via the production of siderophores and adsorb the metals in their biomass, on metal-induced outer membrane proteins, and by bioprecipitation. The difficult point is to find an easy way to separate the biomass, loaded with metals, from the soil matrix. In case of *A. eutrophus* CH34, a special phenomenon was observed. The bacterium was able to improve the settling of the soil by production of some extracellular polymers. In that way, biomass and soil could be separated more easily, e.g., by settling or flotation. The heavy metal resistance, bioprecipitation capacity, and improved soil flocculation lead to the development of a bioremediation method for heavy metal contaminated soils (**Diels *et al.,* 1999**).

Only limited studies have been conducted in our country to systematically screen filamentous fungi from metal polluted sites for their metal tolerance and their biosorption potential **(Bai and Abraham, 2003).** Therefore, they studied filamentous fungi from a polluted environment to evaluate their metal tolerance and metal removal potential from aqueous solution. In recent years, the biosorption process has been studied extensively using microbial biomass as a biosorbent for heavy metal removal **(Zafar *et al.,* 2007).**

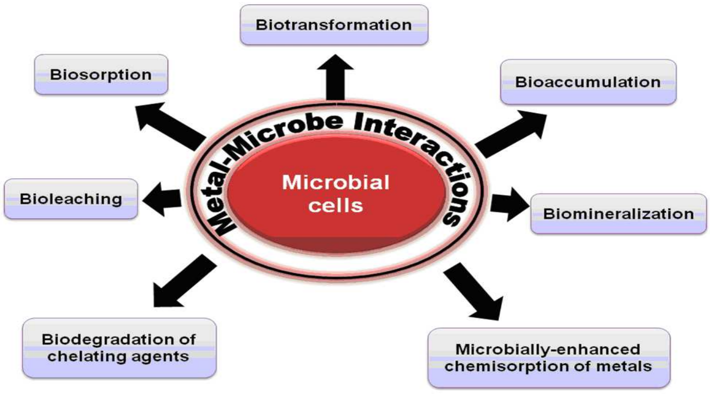


**Fig. 3: Metal-microbe interactions affecting bioremediation (recollected from Munees, 2012)**

Generally, many microbial species with high cell wall chitin contents act as an effective biosorbent in addition to the chitosan and glucans. Furthermore, the walls of fungi, yeasts, and algae, are also efficient metal biosorbents. Moreover, the cell walls of the Gram-positive bacteria attach higher concentrations of metals than that of the Gram-negative bacteria **(Rani & Goel, 2009).** In a recent study, **Mukhrjee *et al.,* (2008)** reported the industrial emissions of mercury from coal combustion, iron and steel industry, non-ferrous metallurgical plants, chloroalkali plants, cement industry, waste disposal and other minor sources **(Subrahmanyam G.V and Prasad MNV., 2011). Dilna Damodaran *et al.,* (2011)** conducted on the biosorption of heavy metals from the synthetic soil contaminated with metal salts of lead and cadmium by *S.cerevisiae* revealed that the organism has high potential of removing heavy metals from the soil through biosorption mechanism.

Chromate resistant determinants in bacteria are carried by plasmids-having potential to detoxify chromate polluted water **(Cervantes, 1991)** such plasmid can be transferred to make biomasses capable of reducing metal toxicity. Similar work was done by **Kao *et al*** using a MerP expressing recombinant *Escherichia coli*, where the MerP originated from Gram-positive (*Bacillus cereus*) and Gram-negative (*Pseudomonas* sp) were used to adsorb Ni, Zn and Cr in aqueous solution (**Kao *et al.,* 2008).** Some scientists in Varanasi used *Eichhornia crassipes* (water hyacinth) to remove heavy meals from contaminated water **(Mishra & Tripathi, 2009).** Others in Chennai showed that *Spirulina fusiform* can remove 93-99% of chromium from tannery effluents. Chromium pollution in the effluent can be detected by alga like *Chlamydomonas reinhardtii* **(Rodriguez MC, 2007)** and water lilies (*Nymphaea spontanea*) **(Choo TP, *et al.,* 2006).** The environmental impact of heavy metal toxicity should be rapidly handled by using the bioremediation processes to reduce the toxic levels of heavy metals **(Pandi *et al.,* 2009).**

**Hyun *et al.,* (1998)** concluded, however, that phytoavailable cadmium did not increase as organic matter decreased in soils after sludge application was terminated. Mobility of metals in compost varies with their speciation. **Sawhney *et al.,* (1996)** noted that leaching of arsenic (more than 20% of the initial arsenic content of compost) was much higher than leaching of cadmium, chromium, nickel, and lead (about 3% of the total initial content).

**Suthar *et al.,* (2008)** have demonstrated higher ranges of bioaccumulation factors for earthworms collected from contaminated substrates; while some earlier studies reported considerable ranges of bioconcentration factors for metals in earthworms (**Dia *et al.,* 2004; Hsu *et al.,* 2006). Suthar *et al.,* (2008)** reported that species-specific metal physiology in earthworms may alter the concentration of metals in their tissues. The amount of organic fractions in ingesting material denotes the availability of soluble forms of metals in a worm's gut. **Lukkari *et al.,* (2006)** stated that binding of metals to organic matter particularly more tightly bound fractions partly reduced the availability of metals to earthworms. The earthworm gut could modify the mobility of metals and favor their assimilation. **Holmstrup *et al.,* (2010)** demonstrated in their study that cadmium, lead and copper accumulated to high concentrations in *Dendrobaena octaedra.*

**Pandey *et al.,* (2013)** concluded that *A. flavus* can be successfully used for bioremediation of Ni from aqueous media. Therefore, bio-removal carried out by this fungus could serve as an economical mean of treating leachate, effluent and the polluted water areas charged with toxic metallic ions. The symbiosis with AM fungi has been proposed as one of the mechanisms of heavy metal plant tolerance **(Hildebrandt *et al.,* 2007)** and water stress avoidance **(Auge, 2004; Ruiz-Lozano and Azcón, 1996; Ruíz-Lozano *et al,* 1995).** Contaminated soils are generally characterized by poor soil structure, low water-holding capacity, organic matter lack and nutrient deficiency. In this respect, the application of organic amendments to the soil, prior to the inoculation of AM fungi, has been recommended **(Medina *et al,* 2004a, b). Vassilev *et al., (*2002)** reported that mycorrhizal plants benefited from Phosphorous solubilized from Rock Phosphate by *A. niger* by the use of isotopic $^{32}$P dilution technique. **Alguacil *et al.,* (2008a)** showed a positive interaction between the amendment *A. niger-treated* Dry Olive Cake and *G. mosseae* in terms of plant growth. Similarly, **Caravaca *et al.,* (2005b, c; 2006c)** reported that the combined treatments, involving mycorrhizal inoculation with *G. intraradices* and the addition of fermented Dry Olive Cake increased the growth of *J. oxycedrus* to a higher extent than each treatment applied separately. **Valls *et al.,* (2000)** have reported on the addition of specially engineered *Ralstonia eutropha*, a natural inhabitant of soil, to sequester metals from polluted soils.

**McGrath *et al.,* (1995)** have shown deleterious effects of the metals on the activity and diversity of soil microbial populations. Soil degradation usually produces changes in the diversity and abundance of AM fungal populations (**Koomen *et al.,* 1990; Jasper *et al.,* 1991; Loth, 1996; Del Val *et al.,* 1998). Gildon & Tinker (1981)** reported that high amounts of heavy metals can delay, reduce or even completely eliminate AMF spore germination and AM colonization at concentrations at which phytotoxic effects were not observed. Similarly, **Boyle & Paul (1988)** reported a negative correlation between Zn concentrations in a soil treated with urban-industrial sludge and AM colonization in barley. A higher tolerance to Cu, Zn, Cd and Pb of indigenous fungi from sludge-polluted sites, in comparison to reference isolates from unpolluted soils, has been reported by **Gildon & Tinker, 1983; Weissenhorn *et al.,* 1993; Diaz *et al.,* (1996).**

**Daniel *et al.,* (2006)** reported the production of Armillaria rhizomorphs in heavy metals contaminated soils and to determine the extent of accumulation of heavy metals into the Rhizomorphs. Removal of toxic heavy metals to environmentally safe level in a cost effective and environment friendly manner assumes great important. Microorganisms have been used as such a low cost method to remove metals from effluent **(Volesky B, 2007)** with fungi known to be more tolerant to metals and to have a higher microorganism surface to volume ratio than bacteria or actinomycetes **(Fomina *et al.*, 2007).** Fungi are not only a major component of the biota in soils and mineral substrates, but also under certain environmental conditions (low pH), they can be efficient biogeochemical agents and bioaccumulators of soluble and particulate forms of metals among them, *Penicillium spp.* are prominent ones **(Niu, 1993). Iqbal *et al.,* (2005)** indicated that *A.niger* and *Penicillium sp.* Have promising bioadsorption capacity of Cr, Ni and Cd from single and multi-metal solutions and highlighted possible exploitation of the filamentous fungi of metal polluted habitat**.**

**Parameswari *et al.,* (2010)** indicated that fungi from soil contaminated with heavy metals have metal biosorption potential and could be exploited for metal removal from aqueous metal solution and also indicated no direct relationship between level of metal resistance and biosorption capacity.

## 9. Conclusion:

Metal contamination of soil due to metal mining, metal plating, agricultural activities and industrial waste disposal has increased considerably in recent years leading to contamination of the environmental reservoirs such as water bodies and soils. Soil usually acts as a sink for harboring metals. These metals being immobile in soil accumulate and influence the physical, chemical and biological properties of soil adversely.

In recent years Bioremediation has proved a novel, efficient and economic technique for the recovery of contaminated soils. Bioremediation is a fast developing field, since last ten years lot of field application were initiated all over the world. But, it is an immature technology and needs to define its boundaries between promise and reality. It frequently addresses multiphasic, heterogenous environments (i.e., soils), and so successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry. The interdisciplinary approach is also required because of the complexity encountered in the type and extent of contamination and the social and legal issues relevant to most contaminated sites. Through improved understanding of the ecology, physiology, evolution, biochemistry, and genetics of microorganisms, the prospect for successfully stimulating and exploiting microbial metabolism for environmental purposes appears very promising. Although its limitations, the future of bioremediation appears bright as the advances in the diverse disciples that shape bioremediation are accelerating. Progress in developing strategies for *in situ* microbial approaches to metals remediation has clearly lagged significantly behind the development of *in situ* bioremediation of organics. However, and since funding opportunities for research on *in situ* bioremediation of metals has increased significantly in recent years, it seems likely that novel advances in this area will be forthcoming.

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