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REVIEW ON BIOREMEDIATION – POTENTIAL TOOL FOR REMOVING ENVIRONMENTAL POLLUTION

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ABSTRACT

Rapid industrialization, Intensive agriculture and other anthropogenic activities have caused land degradation, environmental pollution and a decline in the crop productivity and sustainability and increased pressure on the natural resources and contributing to their adulteration. The use of microorganisms to destroy, or reduce the concentration of, hazardous wastes on a contaminated site are called bioremediation. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. Use of microbial resources, coupled to other modern techniques is one of the most promising and economical strategies for removing environmental pollutants. Bioremediation, both in situ and ex situ have also enjoyed strong scientific growth, in part due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Bioremediation technology, which leads to degradation of pollutants, may be a lucrative and environmentally beneficial alternative. This article presents the important bioremediation technologies like Phytoremediation, Bioventing, Land farming, Bioreactor, Composting, bioaugmentation and Rhizofiltration and their application in potential areas.

Key Words: *Phytoremediation, Bioventing, Land Farming, Bioreactor, Composting, Bioaugmentation and Rhizofiltration*

INTRODUCTION

Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems facing the industrialized world today. Indiscriminate and uncontrolled discharge of industrial and urban wastes into the environmental sink has become an issue of major global concern (Hernandez *et al.*, 1998; Gupta and Mahapatra, 2003; Strong and Burgess, 2008). Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil this limiting crop production (Kamaludeen *et al.*, 2003).

The need to remediate these natural resources (soil, water and air) has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal. Bioremediation is the use of biological interventions of biodiversity for mitigation (and wherever possible, complete elimination) of the noxious effects caused by environmental pollutants in a given site. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment.

Bioremediation uses biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). Microorganisms used to perform the function of bioremediation are known as bioremediators. Bioremediation can be used at the site of contamination (in situ) or on contamination removed from the original site (ex situ). In situ bioremediation involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and sorbed contaminants and use them as substrates for transformation (Bouwer and Zehnder, 1993). Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water (Eccles and Hunt, 1986; Kamaludeen *et al.*, 2003). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources

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(Hess *et al.*, 1997; Agarwal, 1998; Tang *et al.*, 2007). Bioremediation/Phytoremediation and Rhizoremediation, Microflora associated with plants; endophytic bacteria, rhizosphere bacteria and mycorrhizae have the potential to degrade organic compounds in association with plants (Dowling and Doty, 2009; Ma *et al.*, 2011; Weyens *et al.*, 2009) and this process is termed rhizoremediation. It has been unequivocally demonstrated that a number of xenobiotics including nitro-glycerine (explosive) can be cleaned up through bioremediation.

Transgenic plants engineered for the transformation of explosives and metabolic pathway engineering for degradation of xenobiotics are in progress (Abhilash *et al.*, 2009; Van Aken, 2009).

Thus bioremediation, phytoremediation and rhizoremediation contribute significantly to the fate of hazardous waste and can be used to remove these unwanted compounds from the biosphere (Ma *et al.*, 2011; Schroeder and Schwitzgubel, 2004). Bioremediation processes can also be assessed through a multifaceted approach such as: Natural attenuation, sensing environmental pollution, metabolic pathway engineering, applying phyto and microbial diversity to problematic sites, plant-endophyte partnerships and systems biology (Prasad *et al.*, 2010).

Principle of Bioremediation

Since the late 1980s, after the chemical and mechanical treatments of lands and water bodies and thermal treatment (incineration) of hazardous wastes proved economically and environmentally unsustainable, focus shifted towards the biological methods which are cost-effective as well as environmentally sustainable and also socially acceptable.

The rapid expansion and increasing sophistication of the chemical industries in the last century has meant that there has been increasing levels of complex toxic effluents being released into the environment and contaminating the natural resources and it is bioremediation that can remove pollutants from the environment thus restoring the original natural surroundings and preventing further pollution. Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds (Lal *et al.*, 1996).

It 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, 1996). Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products (Vidali, 2001). Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Colberg and Young, 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules. Most important parameters for bioremediation are i) the nature of pollutants, ii) the soil structure, pH, Moisture contents and hydrogeology, iii) the nutritional state, microbial diversity of the site and iv) Temperature and oxidation-reduction (redox-Potential) (Dua *et al.*, 2002).

In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Tang *et al.*, 2007). Bioremediation activity through microbe is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities (Ma *et al.*, 2007; Baldwin *et al.*, 2008).

Agents of Bioremediation

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). 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. Bioremediation can occur naturally or through intervention processes (Agarwal, 1998).

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Microorganisms having biodegradation potential for xenobiotics

Organism	Toxic chemicals	Reference
<i>Pseudomonas</i> spp	Benzene, anthracene, hydrocarbons, PCBs	Kapley <i>et al.</i> , 1999; Cybulski <i>et al.</i> , 2003
<i>Alcaligenes</i> spp	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	Lal and Khanna, 1996
<i>Arthrobacter</i> spp	Benzene, hydrocarbons, pentachlorophenol, phenoxyacetate, polycyclic aromatic	Jogdand, 1995
<i>Bacillus</i> spp	Aromatics, long chain alkanes, phenol, cresol	Cybulski <i>et al.</i> , 2003
<i>Corynebacterium</i> spp	Halogenated hydrocarbons, phenoxyacetates	Jogdand, 1995
<i>Flavobacterium</i> spp	Aromatics	Jogdand, 1995
<i>Azotobacter</i> spp	Aromatics	Jogdand, 1995
<i>Rhodococcus</i> spp	Naphthalene, biphenyl	Dean-Ross <i>et al.</i> , 2002
<i>Mycobacterium</i> spp	Aromatics, branched hydrocarbons benzene, Cycloparaffins	Sunggyu, 1995
<i>Nocardia</i> spp	Hydrocarbons	Park <i>et al.</i> , 1998
<i>Methosinus</i> sp	Aromatics	Jogdand, 1995
Methanogens	Aromatics	Jogdand, 1995
<i>Xanthomonas</i> spp	Hydrocarbons, polycyclic hydrocarbons	Jogdand, 1995; Ijah, 1998
<i>Streptomyces</i> spp	Phenoxyacetate, halogenated hydrocarbon Diazinon	Jogdand, 1995
<i>Candida tropicalis</i>	PCBs, formaldehyde	Ijah, 1998
<i>Cunniughamela elegans</i>	PCBs, polycyclic aromatics, biphenyls	Jogdand, 1995

Classification of Microbes

1. Aerobic: Examples of aerobic bacteria recognized for their degradative abilities are *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.

2. Anaerobic: Anaerobic bacteria are not as frequently used as aerobic bacteria. 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.

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

4. Methylophils: 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

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active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1, 2-dichloroethane.

Microbial Bioremediation of Heavy Metals

Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions. Other metals have no biological role e.g. cadmium (Cd), lead (Pb), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Some of them e.g. Cd²⁺, Ag²⁺, Hg²⁺ tend to bind the SH groups of enzymes and inhibit their activity (Turpeinen, 2002). Soil micro-organisms have been shown to bio-accumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil.

Oscillatoria spp. (a blue-green algae), *Chlorella vulgaris* & *Chlamydomonas* spp. (green algae), *Arthrobacter*, *Agrobacter*, *Enterobacter* & *Pseudomonas aeruginosa* are some metal reducing microbes (Ramasamy *et al.*, 2006). Degradation of dyes is also brought about by some anaerobic bacteria and fungi (Colberg, 1995).

Microbial Remediation of Toxic Metals Occurs in Two Ways –

1). Direct reduction by the activity of the bacterial enzyme ‘metal reductase’. It is applied for groundwater decontamination, using bioreactors (pump & treat) and also for soils after excavation (pulping or heaping and inoculation with appropriate microbial consortium). These techniques are *ex-situ* methods, and very expensive and has low metal extraction efficiencies.

2). Indirect reduction by biologically produced hydrogen sulfide (H₂S) by sulfate reducing bacteria to reduce and precipitate the metals. This is an *in-situ* method, and an environmentally sound & inexpensive alternative to pump & treat (for contaminated groundwater) or excavate & treat (for contaminated soils). Microbial growth is induced in sub-surface zones by injecting substrates. The migrating metals are intercepted and immobilized by precipitation with biologically produced H₂S. Toxic metals readily binds to sulfhydryl group of proteins (Nies, 1999; Sandrin and Hoffman, 2006). In-situ bioremediation of uranium contaminated sites have been conducted successfully with *Desulfosporosinus* spp. and *Closteridium* spp. (Bruschi and Goulhen, 2006).

Microorganisms that utilize heavy metals

Microorganism	Elements	References
Bacillus spp.	Cu, Zn	Philip <i>et al.</i> , 2000; Gunasekaran <i>et al.</i> , 2003
Pseudomonas aeruginosa	U, Cu, Ni	Sar <i>et al.</i> , 1999; Sar and D'Souza, 2001
Zooglea spp.	Co, Ni, Cd	Gunasekaran <i>et al.</i> , 2003
Citrobacter spp.	Cd, U, Pb	Yan and Viraraghavan, 2001; Gunasekaran <i>et al.</i> , 2003
Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg, Zn	Pearson, 1969; Gunasekaran <i>et al.</i> , 2003
Aspergillus niger	Cd, Zn, Ag, Th, U	Guibal <i>et al.</i> , 1995; Gunasekaran <i>et al.</i> , 2003
Pleurotus ostreatus	Cd, Cu, Zn	Favero <i>et al.</i> , 1991
Rhizopus arrhizus	Ag, Hg, P	Gunasekaran <i>et al.</i> , 2003
Stereum hirsutum	Cd, Pb, Ca	Gabriel <i>et al.</i> , 1994, 1996
Phormidium valderium	Cd, Co, Cu, Ni	Gabriel <i>et al.</i> , 1994, 1996
Ganoderma applanus	Cd, Pb	Gabriel <i>et al.</i> , 1994, 1996
Volvariella volvacea	Cu, Hg, Pb	Purkayastha and Mitra, 1992; Jagadevan and Mukherji, 2004
Volvariella volvacea	Zn, Pb, Cu	Sanglimsuwan <i>et al.</i> , 1993; Gabriel <i>et al.</i> , 1994, 1996

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Naturally Occurring Bacteria Capable of Destroying Some Hazardous Wastes and Chemicals by Biodegradation

Organisms	Chemicals Degraded
<i>Flavobacterium</i> spp.	Organophosphates
<i>Cunniughamela elegans</i> & <i>Candida tropicalis</i>	PCBs (Polychlorinated Biphenyls) & PAHs (Polycyclic Aromatic Hydrocarbons);
<i>Alcaligenes</i> spp. & <i>Pseudomonas</i> spp.	PCBs, halogenated hydrocarbons and alkylbenzene sulphonates, PCBs, organophosphates, benzene, anthracene, phenolic compounds, 2,4 D, DDT and 2,4,5- trichlorophenoxyacetic acid etc.
<i>Actinomycetes</i>	Raw rubber
<i>Nocardia tartaricans</i>	Chemical Detergents (Ethylbenzene)
<i>Closteridium</i>	Lindane
<i>Arthrobacter</i> & <i>Bacillus</i>	Endrin
<i>Trichoderma</i> & <i>Pseudomonas</i>	Malathion

Sources: Various Publications of UNEP, WWF and WHO (1992-2002)

Naturally Occurring Fungus Capable of Destroying Some Hazardous Wastes and Chemicals by Biodegradation

Organisms	Chemicals Degraded
<i>Phanerochaete chrysosporium</i> ,	Halocarbons such as lindane, pentachlorophenol,
<i>P. sordida</i> & <i>Trametes hirsute</i>	DDT, DDE, PCBs, 4,5,6-trichlorophenol, 2,4,6- trichlorophenol, dichlorophenol, and chlordane
<i>Zylerion xylestrix</i>	Pesticides / Herbicides (Aldrin, dieldrin, parathion and malathion)
<i>Mucor</i>	Dieldrin
Yeast (<i>Saccharomyces</i>)	DDT

Sources: Various Publications of UNEP, WWF and WHO (1992-2002)

Types of Bioremediation

There are two approaches to bioremediation: (1) in situ bioremediation involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and sorbed contaminants and use them as substrates for transformation (Bouwer and Zehnder, 1993). Since the in situ process is slow, it is not the best approach when immediate site cleanup is desired (Iwamoto and Nasu, 2001). (2) Ex situ bioremediation is a different approach that utilizes specially constructed treatment facility. It is more expensive than in situ bioremediation.

In-Situ Bioremediation

In situ bioremediation is the application of biological treatment to the cleanup of hazardous chemicals present in the subsurface. 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

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as the infiltration of water containing nutrients and oxygen or other electron acceptors for groundwater treatment (Vidali, 2001). The optimization and control of microbial transformations of organic contaminants require the integration of many scientific and engineering disciplines.

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.

Bioventing

Bioventing is a promising new technology that stimulates the natural *in-situ* biodegradation of any aerobically degradable compounds in NAPL within the soil by providing oxygen to existing soil microorganisms. In contrast to soil-vapor extraction (SVE), Bioventing uses low air-flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil (Lee, *et al.*, 2006)

Bioaugmentation

Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. It is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms. At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentation is used to ensure that the *in situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic (Niu *et al.*, 2009). Monitoring of this system is difficult. Since the *in situ* process is slow, it is not the best approach when immediate site cleanup is desired (Iwamoto and Nasu, 2001).

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)

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

1. Land Farming: Land farming, also known as land treatment, is a bioremediation technique that involves the excavation of the contaminated soil and spreading it on a thin surface. Biodegradation of pollutants is stimulated aerobically by tilling or plowing the soil. Nutrients and minerals are also added to promote the growth of the indigenous species. According to The United States Environmental Protection Agency (EPA) report on underground storage tanks (EPA, 2003), before the remediation takes place the site must be prepared by clearing and grading the soil, by installing leachate collection and treatment systems, and also building vapor treatment facilities. Also, the report states that if a contaminated soil is less than three feet then there is no need for excavation. Soil moisture is controlled by periodically sprinkling soil with water, building barriers or terraces around the contaminated soil controls erosion. Sprinkling with water also minimizes the dust created while tilling the soil to promote aeration (Rubinos *et al.*, 2007).

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2. Composting: It involves mixing the contaminated soil with a bulking agent such as straw, hay, or corncobs to make it easier to deliver the optimum levels of air and water to the microorganisms. The most common designs are static pile composting, mechanically agitated composting, and window composting. In static pile composting the contaminated soil is placed into piles and aerated with blowers or vacuum pumps. Mechanically agitated composting involves the placement of the contaminated soil in treatment vessels where it is mixed to achieve aeration. In window composting, the soil is placed in long piles known as windows and periodically mixed by tractors (Cunningham, 2000). As stated before, the contaminated soil is mixed with a bulking agent or compost to enhance bacterial growth. A typical ratio of soil to compost is 75% contaminated soil to 25% compost. This ratio is changeable depending on the soil type, contaminants level and characteristics. After mixing, the soil is covered to protect it from erosion and to maintain the proper moisture and temperature necessary for bacterial growth. Compost remediation is known to have faster cleanup results since cleanup can be estimated in terms of weeks instead of months.

3. Biopiles: 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).

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.

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).

Phytoremediation

Phytoremediation is the use of higher plants to bio remediate contamination in soil, water, or sediments. Variations of phytoremediation that have been used in the past include wetlands to treat municipal sewage or neutralize acidic mine drainage.

Types

Phytodegradation

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- Specifically, phytodegradation, also called “phytotransformation,” refers to the uptake of contaminants with the subsequent breakdown, mineralization, or metabolization by the plant itself through various internal enzymatic reactions and metabolic processes.
- Depending on factors such as the concentration and composition, plant species, and soil conditions, contaminants may be able to pass through the rhizosphere only partially or negligibly impeded by phytosequestration and/or rhizodegradation.
- In this case, the contaminant may then be subject to biological processes occurring within the plant itself, assuming it is dissolved in the transpiration stream and can be phytoextracted.

Phytovolatilization

- Phytovolatilization is the volatilization of contaminants from the plant either from the leaf stomata or from plant stems (Anonymous, 2009).
- Chemical characteristics such as the Henry’s constant, and vapor pressure dictate the ability of organic contaminants to volatilize.
- In some cases, a breakdown product derived from the rhizodegradation and/or phytodegradation of the parent contaminant along the transpiration pathway may be the phytovolatilized constituent.
- This effect was studied for the uptake and phytovolatilization of trichloroethene (TCE) or its breakdown products in poplars (Anonymous, 2009).
- Similarly, certain inorganic constituents such as mercury may be volatilized as well. Specifically, tobacco plants have been modified to be able to take up the highly toxic methyl-mercury, alter the chemical speciation, and phytovolatilize relatively safe levels of the less toxic elemental mercury into the atmosphere (Anonymous, 2009).
- Once volatilized, many chemicals that are recalcitrant in the subsurface environment react rapidly in the atmosphere with hydroxyl radicals, an oxidant formed during the photochemical cycle.

Phytostabilization

- Phytostabilization refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils.
- Establishment of rooted vegetation prevents windblown dust, an important pathway for human exposure at hazardous waste sites.
- Hydraulic control is possible, in some cases, due to the large volume of water that is transpired through plants which prevents migration of leachate towards groundwater or receiving waters.
- Phytostabilization is especially applicable for metal contaminants at waste sites where the best alternative is often to hold contaminants in place.
- Metals do not ultimately degrade, so capturing them *in situ* is the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas where a large-scale removal action or other *in situ* remediation is not feasible.

Phytoextraction

- Phytoextraction refers to the ability of plants to take up contaminants into the roots and translocate them to the aboveground shoots or leaves.
- For contaminants to be extracted by plants, the constituent must be dissolved in the soil water and come into contact with the plant roots through the transpiration stream.

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- Alternatively, the uptake may occur through vapor adsorption onto the organic root membrane in the vadose zone.
- Once adsorbed, the contaminant may dissolve into the transpiration water or be actively taken up through plant transport mechanisms.

Rhizofiltration

- Rhizofiltration can be defined as the use of plant roots to absorb, concentrate, and/or precipitate hazardous compounds, particularly heavy metals or radionuclides, from aqueous solutions.

Process	Function	Pollutant	Plants	References
Phytoextraction	Remove metals pollutants that accumulate in plants. Remove organics from soil by concentrating them in plant parts.	Cd, Pb, Zn, As	Viola baoshanensis Sedum alfredii Rumex crispus Helianthus annuus	Macek <i>et al.</i> , 2000; Prescott <i>et al.</i> , 2002; Zhuang <i>et al.</i> , 2007
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT	Elodea canadensis Pueraria thunbergiana	Garrison <i>et al.</i> , 2000; Prescott <i>et al.</i> , 2002; Newman and Reynolds, 2004
Rhizofiltration	Roots absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams	Zn, Pb, Cd, As	Brassica juncea Helianthus annuus	Dushenkov <i>et al.</i> , 1995; Prescott <i>et al.</i> , 2002; Verma <i>et al.</i> , 2006
Phytostabilization	Use of plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn	Anthyllis vulneraria Festuca arvernensis Koeleria vallesiana Armeria arenaria Lupinus albus	Prescott <i>et al.</i> , 2002; Frerot <i>et al.</i> , 2006; Vazquez <i>et al.</i> , 2006
Phytovolatilization	Use of plants to volatilize pollutants	Se, CCl ₄ , EDB, TCE	Stanleya pinnata Zea mays Brassica sp.	Prescott <i>et al.</i> , 2002, Ayotamuno and Kogbara, 2007

- Rhizofiltration is effective in cases where wetlands can be created and all of the contaminated water is allowed to come in contact with roots.
- Contaminants should be those that sorb strongly to roots, such as lead, chromium (III), uranium, and arsenic (V).
- Roots of plants are capable of sorbing large quantities of lead and chromium from soil water or from water that is passed through the root zone of densely growing vegetation.

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Conclusion

Soil is the soul of infinite life. It is being polluted by various organic and inorganic pollutants due to rapid industrialization and use of agrochemicals in imbalanced proportions. Restrictive and clean up measures to avert hazards from contaminated soil belong to the curative soil protection. Bioremediation is one such a technique for cleaning up pollution by enhancing the natural biodegradation processes. So developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes helps to increase capabilities to degrade pollutants and recovery of land and ground water.

REFERENCES

- Abhilash PC, Jamil S and Singh N (2009)**. Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. *Biotechnology Advances* **27** 474–488.
- Agarwal SK (1998)**. Environmental Biotechnology (1st edition), APH Publishing Corporation, New Delhi, India 267289.
- Anonymous (2009)**. ITRC (Interstate Technology & Regulatory Council). Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised, PHYTO-3, Washington, D.C.
- Ayotamuno JM and Kogbara RB (2007)**. Determining the tolerance level of *Zea mays* (maize) to a crude oil polluted agricultural soil. *African Journal of Biotechnology* **6** 1332-1337.
- Baldwin BR, Peacock AD, Park M, Ogles DM, Istok JD, McKinley JP, Resch CT and White DC (2008)**. Multilevel samplers as microcosms to assess microbial response to biostimulation. *Ground Water* **46** 295-304.
- Bouwer EJ and Zehnder AJB (1993)**. Bioremediation of organic compounds putting microbial metabolism to work. *Trends in Biotechnology* **11** 287-318.
- Bruschi, Mireille and Florence Goulhen (2006)**. New Bioremediation Technologies to Remove Heavy Metals and Radionuclides Using Fe (III)-Sulfate and Sulfur Reducing Bacteria. In: *Environmental Bioremediation Technologies*, edited by Singh SN and Tripathi RD (Springer Publication, NY) 35-55.
- Colberg PJS and Young LY (1995)**. Anaerobic Degradation of Non-halogenated Homocyclic Aromatic Compounds Coupled with Nitrate, Iron, or Sulfate Reduction. In: *Microbial Transformation and Degradation of Toxic Organic Chemicals*, Wiley Liss, New York 307–330.
- Cunningham CJ and Philip JC (2000)**. *Comparison of Bioaugmentation and Biostimulation in ex situ Treatment of Diesel Contaminated Soil*, Land Contamination and Reclamation, University of Edinburgh, Scotland.
- Cybulski Z, Dzurla E, Kaczorek E and Olszanowski A (2003)**. The influence of emulsifiers on hydrocarbon biodegradation by *Pseudomonadacea* and *Bacillaceae* strains. *Spill Science and Technology Bulletin* **8** 503 – 507.
- Dean Ross D, Moody J and Cerniglia CE (2002)**. Utilization of mixtures of polycyclic aromatic hydrocarbons by bacteria isolated from contaminated sediment. *FEMS Microbiology Ecology* **41** 1- 7.
- Dowling DN and Doty SL (2009)**. Improving phytoremediation through biotechnology. *Current Opinion in Biotechnology* **20** 204–206.
- Dua M, Sethunathan N and Johri AK (2002)**. Biotechnology bioremediation success and limitations. *Applied Microbiology and Biotechnology* **59** 143-152.
- Dubey RC (2004)**. A text book of Biotechnology 3rd edition, S Chand and Company Ltd. New Delhi, India 365-375.
- Dushenkov V, Nanda Kumar PBA, Motto H and Raskin I (1995)**. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental Science and Technology* **29** 1239-1245.
- Eccles H and Hunt S (1986)**. In: Immobilization of ions by biosorption, John Wiley and Sons, New York 1646.
- EPA (2003)**. *Underground Storage Tanks*. Available: www.epa.gov/swerust1/ustsystem/erpdoc.pdf.

Review Article

Favero N, Costa P and Massimino ML (1991). In vitro uptake of cadmium by basidiomycete *Pleurotus ostreatus*. *Biotechnology Letters* **10** 701-704.

Frerot H, Lefebvre C, Gruber W, Collin C, Dos Santos A and Escarre J (2006). Specific interactions between local metalicolous plants improve the phytostabilization of mine soils. *Plant and Soil* **282** 53-65.

Gabriel J, Kofronova O, Rychlovsky P and Krenzelok M (1996). Accumulation and effect of cadmium in the wood rotting basidiomycete, *Daedalea quercina*. *Bulletin of Environmental Contamination and Toxicology* **57** 383-390.

Gabriel J, Mokrejs M, Bily J and Rychlovsky P (1994). Accumulation of heavy metal by some Wood-rooting fungi. *Folia Microbiologica* **39** 115-118.

Garrison AW, Nzengung VA, Avants JK, Ellington JJ, Jones EW, Rennels D and Wolfet NL (2000). Phytodegradation of p, p' - DDT and the enantiomers of o, p' - DDT. *Environmental Science and Technology* **34** 1663-1670.

Gui-Lan Niu, Jun-Jie Zhang, Shuo Zhao, Hong Liu, Nico Boon and Ning-Yi Zhou (2009). Bioaugmentation of a 4-chloronitrobenzene contaminated soil with *Pseudomonas putida* ZWL73. *Environmental Pollution* **57** 763-771.

Guibal E, Roulph C and Le Clouire P (1995). Infrared spectroscopic study of uranyl biosorption by fungal biomass and materials of biological origin. *Environmental Science and Technology* **29** 2496-2503.

Gunasekaran P, Muthukrishnan J and Rajendran P (2003). Microbes in Heavy Metal Remediation. *Indian Journal of Experimental Biology* **41** 935-944.

Gupta R and Mahapatra H (2003). Microbial biomass: An economical alternative for removal of heavy metals from waste water. *Indian Journal of Experimental Biology* **41** 945-966.

Hess A, Zarda B, Hahn D, Hanner A and Stax D (1997). In situ analysis of denitrifying toluene and m-xylene degrading bacteria in a diesel fuel contaminated laboratory aquifer column. *Journal of Applied and Environmental Microbiology* **63** 2136-2141.

Hernandez A, Mellado RP and Martinez JL (1998). Metal accumulation and vanadium-induced multidrug resistance by environmental isolates of *Escherichia herdmann* and *Enterobacter cloacae*. *Applied and Environmental Microbiology* **64** 4317-4320.

Iranzo M, Sainz-Padro I, Boluda R, Sanchez J and Mormeneo S (2001). The Use of Microorganisms in Environmental Engineering. *Annals of Microbiology* **51** 135-143.

Iwamoto T and Nasu M (2001). Current bioremediation practice and perspective. *Journal of Bioscience and Bioengineering* **92** 1-8.

Ijah UJJ (1998). Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. *Waste Management* **18** 293 – 299.

Jagadevan S and Mukherji S (2004). Successful in-situ oil bioremediation programmes – key parameters. *Indian Journal of Biotechnology* **3** 495 – 501.

Jogdand SN (1995). Environmental biotechnology, 1st edition, Himalaya Publishing House, Bombay, India 104-120.

Kapley A, Purohit HJ, Chhatre S, Shanker R and Chakrabarti T (1999). Osmotolerance and hydrocarbon degradation by a genetically engineered microbial consortium. *Bioresource Technology* **67** 241-245.

Lal B and Khanna S (1996). Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*. *Journal of Applied Bacteriology* **81** 355-362.

Lee TH, Byun IG, Kim YO, Hwang IS and Park TJ (2006). Monitoring biodegradation of diesel fuel in Bioventing processes using in situ respiration rate. *Water Science and Technology* **53**(4-5) 263-72.

Macek T, Mackova M and Kas J (2000). Exploitation of plants for the removal of organics in environmental remediation. *Biotechnology Advances* **18** 23-34.

Ma Y, Prasad MNV, Rajkumar M and Freitas H (2011). Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnology Advances* **29** 248-258.

Review Article

Ma X, Novak PJ, Ferguson J, Sadowsky M, LaPara TM, Semmens MJ and Hozalski RM (2007). The impact of H₂ addition on dechlorinating microbial communities. *Bioremediation Journal* **11** 45-55.

Newman LA and Reynolds CM (2004). Phytodegradation of organic compounds. *Current Opinion in Biotechnology* **15** 225-230.

Nies DH (1999). Microbial Heavy-Metal Resistance. *Journal of Applied Microbiology & Biotechnology* **51** 730-750.

Park AJ, Cha DK and Holsen TM (1998). Enhancing solubilization of sparingly soluble organic compounds by biosurfactants produced by *Nocardia erythropolis*. *Water Environment Research* **70** 351–355

Pearson RG (1969). Hard and soft acids and bases. *Survey of Progress in Chemistry* **5** 1-52.

Philip L, Iyengar L and Venkobacher L (2000). Site of interaction of copper on *Bacillus polymyxa*. *Water Air Soil Pollution* **119** 11-21.

Prescott LM, Harley JP and Klein DA (2002). Microbiology, 5th edition, McGraw- Hill, New York 1014.

Purkayastha RP and Mitra AK (1992). Metal uptake by mycelia during submerged growth and by sporocarp of an edible fungus, *Volvariella volvacea*. *Indian Journal of Experimental Biology* **30** 1184-1187.

Ramasamy K, Kamaludeen and Sara Parwin Banu (2006). Bioremediation of Metals: Microbial Processes and Techniques. In: *Environmental Bioremediation Technologies*, edited by Singh SN and Tripathi RD (Springer Publication, NY) 173-187.

Rubinos D, Villasuso R, Muniategui S, Barral M and Díaz- Fierros F (2007). Using the land farming technique to remediate soils contaminated with hexachlorocyclohexane isomers, *Water, Air and Soil Pollution* **181** 385-399.

Sar P and D'Souza SF (2001). Biosorptive uranium uptake by *Pseudomonas* strain: Characterization and equilibrium studies. *Journal of Chemical Technology and Biotechnology* **76** 1286-1294.

Sar P, Kazy SK, Asthana RK and Singh SP (1999). Metal adsorption and desorption by lyophilized *Pseudomonas aeruginosa*. *International Biodeterioration and Biodegradation* **44** 101-110.

Sandrin Todd R and Douglas R Hoffman (2006). Bioremediation of Organic and Metal Contaminated Environments: Effects of Metal Toxicity, Speciation and Bioavailability on Biodegradation. In: *Environmental Bioremediation Technologies*, edited by Singh SN and Tripathi RD (Springer Publication, NY) 1-34.

Sanglimsuwan S, Yoshida N, Morinaga T and Murooka Y (1993). Resistance to and uptake of heavy metals in mushrooms. *Journal of Fermentation and Bioengineering* **75** 112-114.

Schroeder P and Schwitzguebel JP (2004). New cost action launched: Phytotechnologies to promoted sustainable land use and improve food safety. *Journal of Soils and Sediments* **4**(3) 205.

Sunggyu L (1995). Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil. *Journal of Cleaner Production* **3** 255.

Strong PJ and Burgess JE (2008). Treatment methods for wine related ad distillery wastewaters: a review. *Bioremediation Journal* **12** 7087.

Tang CY, Criddle QS Fu CS and Leckie JO (2007). Effect of flux (transmembrane pressure) and membranes properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing waste water. *Journal of Environmental Science and Technology* **41** 20082014.

Van Aken B (2009). Transgenic plants for enhanced phytoremediation of toxic explosives. *Current Opinion in Biotechnology* **20** 231–236.

Vazquez S, Agha A, Granado A, Sarro M, Esteban E, Penalosa J and Carpena R (2006). Use of white Lupin plant for phytostabilization of cd and as polluted acid soil. *Water, Air and Soil Pollution* **177** 349- 365.

Review Article

Verma P, George K, Singh H, Singh S, Juwarkar A and Singh R (2006). Modeling Rhizofiltration: heavy metal uptake by plant roots. *Environmental Modeling and Assessment* **11** 387-394.

Vidali M (2001). Bioremediation An overview. *Pure and Applied Chemistry* **73**(7) 1163–1172.

Weyens N, Van der Lelie D, Taghavi S and Vangronsveld J (2009). Phytoremediation: plant–endophyte partnerships take the challenge. *Current Opinion in Biotechnology* **20** 248–254.

Yan G and Viraraghavan T (2001). Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass. *Bioresource Technology* **78** 243- 249.

Zhuang P, Yang QW, Wang HB and Shu WS (2007). Phytoextraction of heavy metals by eight plant species in the field. *Water, Air and Soil Pollution* **184** 235-242.