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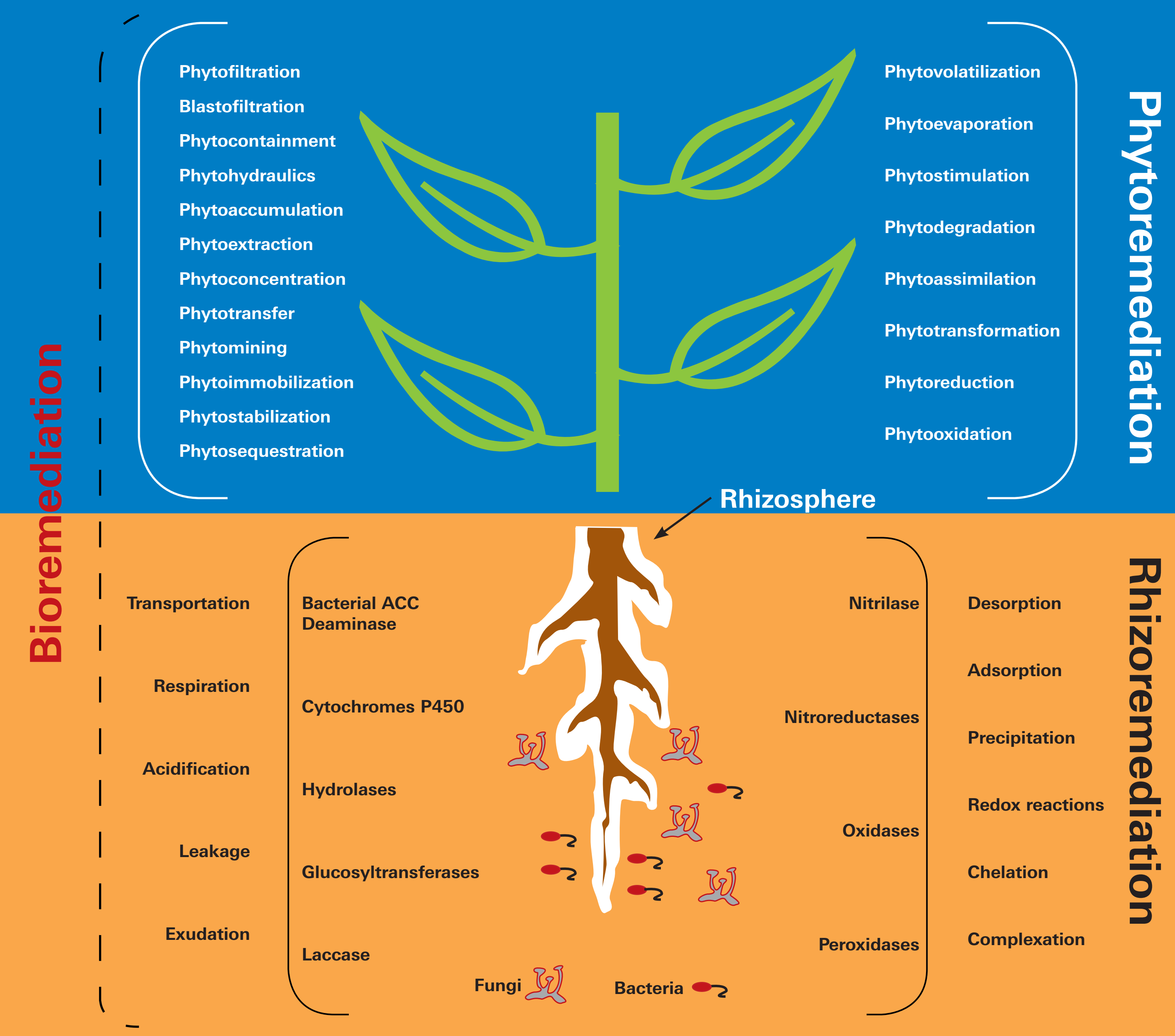

A State-of-the-Art report on

Bioremediation, its Applications to Contaminated Sites in India

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Ministry of Environment & Forests
Government of India

Nelumbo nucifera (Indian Lotus), in constructed wetland for water purification

A State-of-the-Art report on
Bioremediation, its Applications to Contaminated Sites in India

Constructed wetland at NALCO's Angul plant in Orissa for treatment of coal fly ash slurry released from captive power plant

Constructed wetland at NALCO's Angul plant in Orissa with macrophytes for treatment of coal fly ash slurry released from captive power plant

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Loktak lake (Ramsar site) in Manipur showing characteristic Phumdis (floating islands). This water body is serving as receptacle for sewage and agrochemcials. The various aquatic plants are playing a major role in phytosanitation and bioremediaiton.

From the Minister's Desk

The Ministry of Environment and Forests (MoEF) is responsible for protecting the Nation's forests, biodiversity, land, air and water resources. Under its mandate of environment protection, the Ministry formulates and implements various action plans that are compatible and harmonious with human activities and natural systems to support life sustaining activities. It is necessary to curb environmental pollution and understand how to decontaminate the polluted environment. Prevention and control of pollution in air, land and water and remediation of industrial and hazardous waste contaminated sites have been the prioritized agenda for this Ministry. The goal of this State-of-the-Art-Report is to catalyze development through implementation of innovative, cost-effective and environmentally-friendly technologies for bioremediation of contaminated sites in India. The unique features of this report are elaborate illustrations, glossary of terms used in the area of bioremediation and frequently asked questions about bioremediation.

I am delighted to introduce this report to the scientific community, environmentalists and natural resource managers. I congratulate Prof. M.N.V. Prasad for bringing out this **A State-of-the-Art report on Bioremediation, its Applications to Contaminated Sites in India**. I hope that this report would provide basic understanding of the bioremediation mechanisms to the reader and also serve as a reference for researchers, students, teachers, managers and consultants who are interested in application of bioremediation.

Jairam Ramesh
Minister of State for Environment & Forests
(Independent Charge), Government of India

Executive Summary

Increased population, industrialization and urbanization are responsible for environmental contamination. Environmental decontamination is an enigma. However, advances in science and technology enabled us to apply the potential of biological diversity for pollution abatement which is termed as Bioremediation. This is emerging as an effective innovative technology for treatment of a wide variety of contaminants. This technology includes phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Rhizoremediation, which is the most evolved process of bioremediation, involves the removal of specific contaminants from contaminated sites by mutual interaction of plant roots and suitable microbial flora.

Bioremediation is an invaluable tool box for wider application in the realm of environmental protection. Bioremediation approach is currently applied to contain contaminants in soil, groundwater, surface water, and sediments including air. These technologies have become attractive alternatives to conventional cleanup technologies due to relatively low capital costs and their inherently aesthetic nature. This document provides a state-of-the-art report on existing knowledge for the benefit of regulators, who evaluate the quality of environment and for practitioners, who have to implement and evaluate remediation alternatives at a given contaminated site.

This report is expected to provide basic understanding of the bioremediation mechanisms to the reader. The technical descriptions provided in this document concentrate on the functioning mechanisms: phytosequestration, rhizodegradation, phytohydraulics, phytoextraction, phytodegradation, and phytovolatilization.

The scope of environmental bioremediation extends to: Inorganics viz., Arsenic, Mercury, Chromium, Fluoride, Cyanide, abandoned mines, fly ash disposed sites, engineered phytotreatment technologies, biological permeable barriers; and Organics viz., petroleum hydrocarbons, pesticides and explosives.

Mining industries release a variety of waste including abandoned mines and drill cuttings and fluids of fossil fuel exploration. All these constitute as hazardous waste and pose potential public health or environmental risk. Environmental rehabilitation of abandoned mines in India can be carried out in 4 phases- i) Inventory and local surveys, hierarchization of process, definition of characteristic types and Planning, ii) Master /action plans, iii) Rehabilitation works and monitoring effluent treatment systems and iv) Legislative framework for environmental rehabilitation of abandoned mines and maintenance and long-term monitoring.

Quite a variety of plants, natural, transgenic, and/or associated with rhizosphere micro-organisms are extraordinarily active in these biological interventions and in cleaning up pollutants by removing or immobilizing them. While diverse microbes are the most active agents, fungi and their strong oxidative enzymes are key players in degrading/decontaminating recalcitrant polymers and xenobiotic chemicals as well. Constructed wetlands are the result of human skill and technology integrating geology, hydrology and biology. People have built and operated constructed wetlands to treat wastewater since ancient times.

The proactive role of MoEF and industries for implementing bioremediation and envisaged action plan are also discussed. Institutions involved in bioremediation research, frequently asked questions and glossary of terms used in bioremediation are also presented in annexures.

Chapter 1
Introduction

Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. Large amounts of toxic waste have been dispersed in thousands of contaminated sites spread across our nation. Thus every one of us is being exposed to contamination from past and present industrial practices, emissions in natural resources (air, water and soil) even in the most remote regions. The risk to human and environmental health is rising and there is evidence that this cocktail of pollutants is a contributor to the global epidemic of cancer, and other degenerative diseases (Figure 1). These pollutants belong to two main classes: inorganic and organic. The challenge is to develop innovative and cost-effective solutions to decontaminate polluted environments, to make them safe for human habitation and consumption, and to protect the functioning of the ecosystems which support life. Much progress has been made in developed countries like UK, USA, Canada, Australia, Japan and European countries. However, in India there is an urgent need to evaluate the exciting developments coming out of various laboratories.

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. It operates through the principles of biogeochemical cycling (Figures 2 and 3). If the process occurs in the same place affected by pollution then it is called *in-situ* bioremediation. In contrast, deliberate relocation of the contaminated material (soil and water) to a different place to accelerate biocatalysis is referred to as *ex-situ* bioremediation. Bioremediation has been successfully applied for clean up of soil, surface water, groundwater, sediments and ecosystem restoration. It has been unequivocally demonstrated that a number of xenobiotics including nitro-glycerine (explosive) can be cleaned up through bioremediation. Bioremediation is generally considered to include natural attenuation (little or no human action), bio-stimulation or bio-augmentation, the deliberate addition of natural or engineered micro-organisms to accelerate the desired catalytic capabilities 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 Schwitzguebel 2004) (Figure 4).

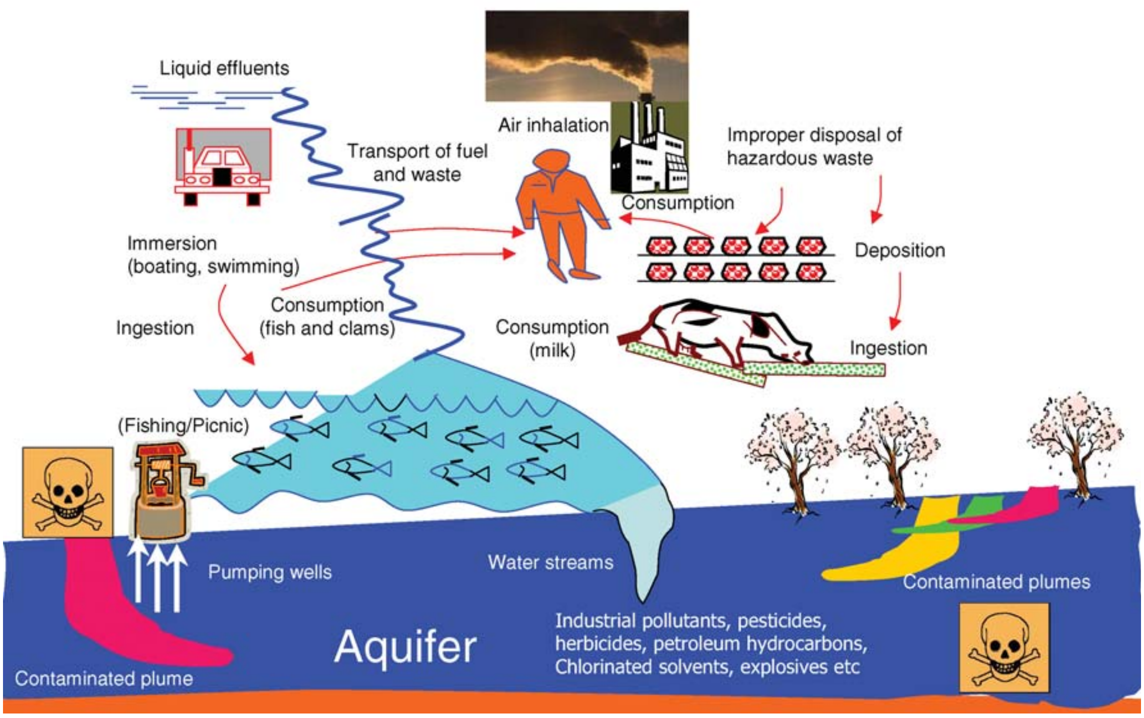


Figure 1: Fate and transport of organic/inorganic contaminants/pollutants and their harmful effects

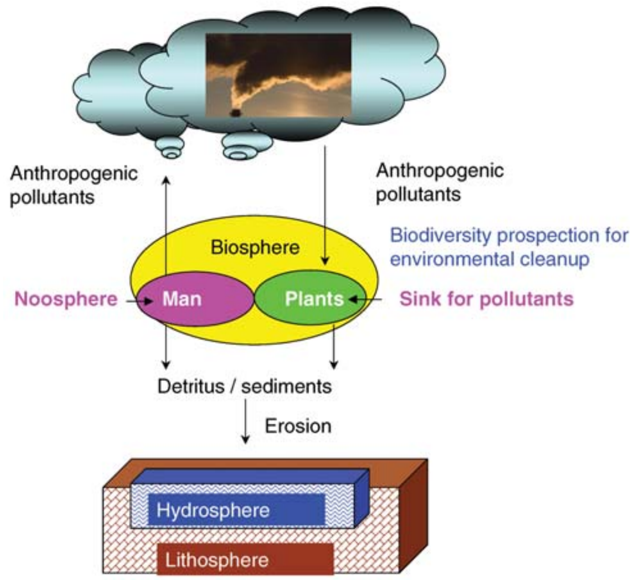


Figure 2: Biogeochemical cycle and its connection to bioremediation (Prasad 2004)

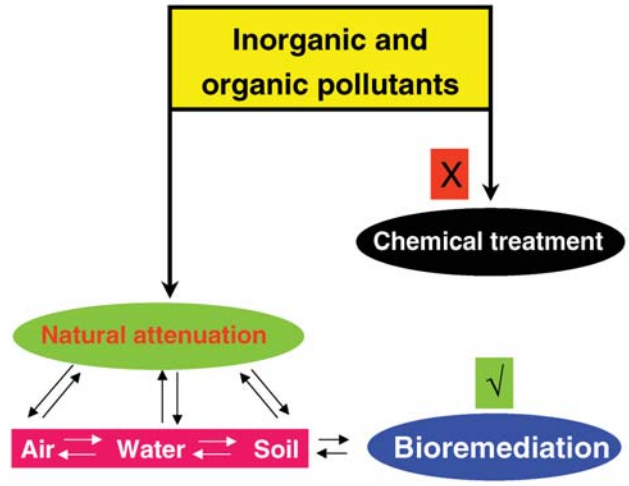


Figure 3: Natural attenuation and bioremediation are widely accepted for environmental cleanup.

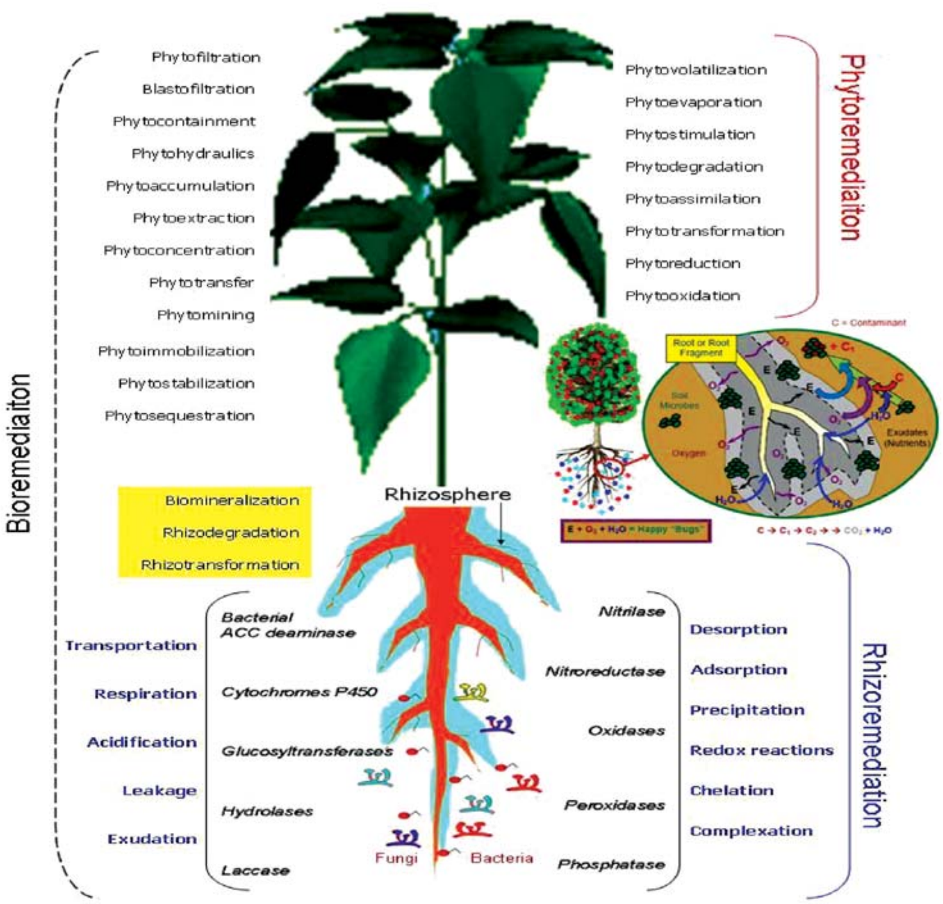


Figure 4: Bioremediation/Phytoremediation and Rhizoremediation .The techniques and strategies involved include the application of appropriate plants for *in-situ* risk reduction in contaminated soil, sediments, and groundwater for contaminant removal, degradation, or containment. This technique can be used along with or, in some cases, in place of, mechanical cleanup methods. Cleanup can be accomplished to certain depths below ground level, within the reach of plants' roots. Such sites need to be maintained (watered, fertilized, and monitored). 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.

Traditionally, the efficacy of bioremediation could be determined by measuring changes in total pollutant concentrations by analytical tools (chromatographic and spectroscopic techniques etc). Recently, attempts have been made to use biosensors, especially microbial whole-cell biosensors, to monitor the rate of pollutant elimination. 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 (Figure 5) (Prasad *et al* 2010). The hierarchy of complexity of bioremediation, limitations and scope in the contemporary science is shown in Figure 6. (Abhilash *et al*., 2009; Ruiz and Daniell, 2009; Scow and Hicks, 2005; Singh *et al*., 2008; Wood, 2008). There has been a steep rise in scientific investigations and publication in the field of bioremediation (Figure 7).

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). Herbicide phytoremediation using transgenics is one of the most successful examples.

Plant physiology, agronomy, microbiology, hydrogeology, and engineering are combined to select the proper plant and conditions for a specific site. The specific mechanisms that are emphasized in an application depend on the mobility, solubility, degradability, and bioavailability of the contaminant(s) of concern. Phytoremediation involves the use of certain plants to cleanup soil and water contaminated with inorganics and/or organics. The use and transformation of over thousands of individual compounds whose current locations are largely unknown have resulted in the establishment of new fields of research, which have one thing in common: they link ecological, physiological, and chemical/analytical lines. This complex system of interactions and interrelations requires intensified efforts to provide integrated information on the status and development of environmental quality. Bioindicators and biomonitors have proven to be excellent tools in many of these cases and could provide information which cannot be derived from technical measurements alone (Markert *et al*., 2003; Prasad, 2008).

Biotechnology and systems biology approaches are also implicated in bioremediation and are gaining

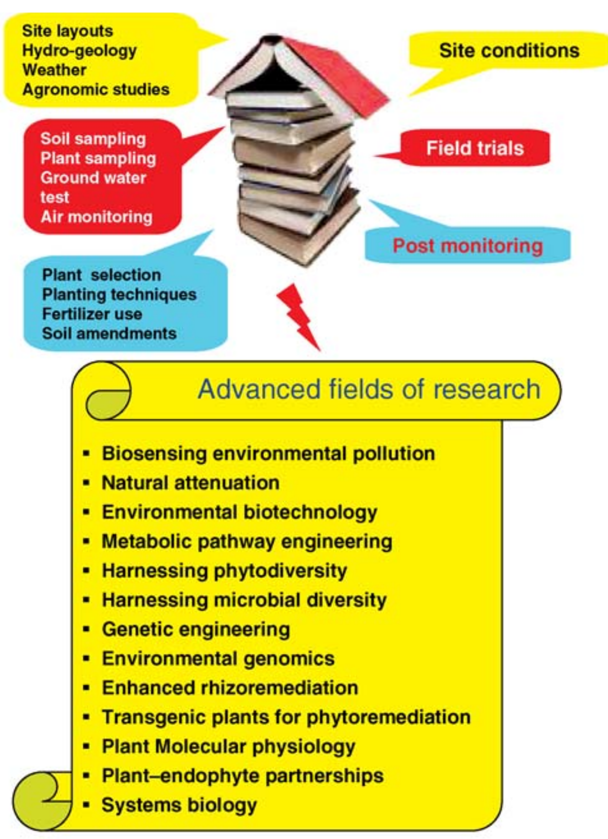


Figure 5: Knowledge explosion in bioremediation

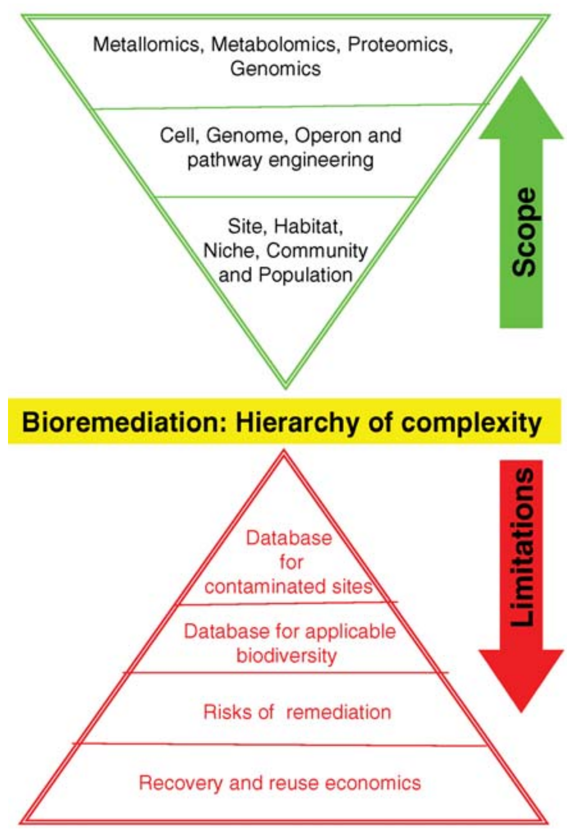


Figure 6: Scope and limitations of bioremediation application

considerable importance in fostering bioremediation (De Lorenzo, 2008; Van Aken, 2009). It is strongly believed that there are three dimensions for the effectiveness of vital bioremediation process, i.e., chemical landscape (nutrients-to-be, electron donors/ acceptors and stressors), abiotic landscape and catabolic landscape of which only the catabolic landscape is ''genuinely'' biological. The chemical landscape has a dynamic interplay with the biological interventions on the abiotic background of the site at stake. This includes humidity, conductivity, temperature, matrix conditions, redox status, etc. (De Lorenzo, 2008).

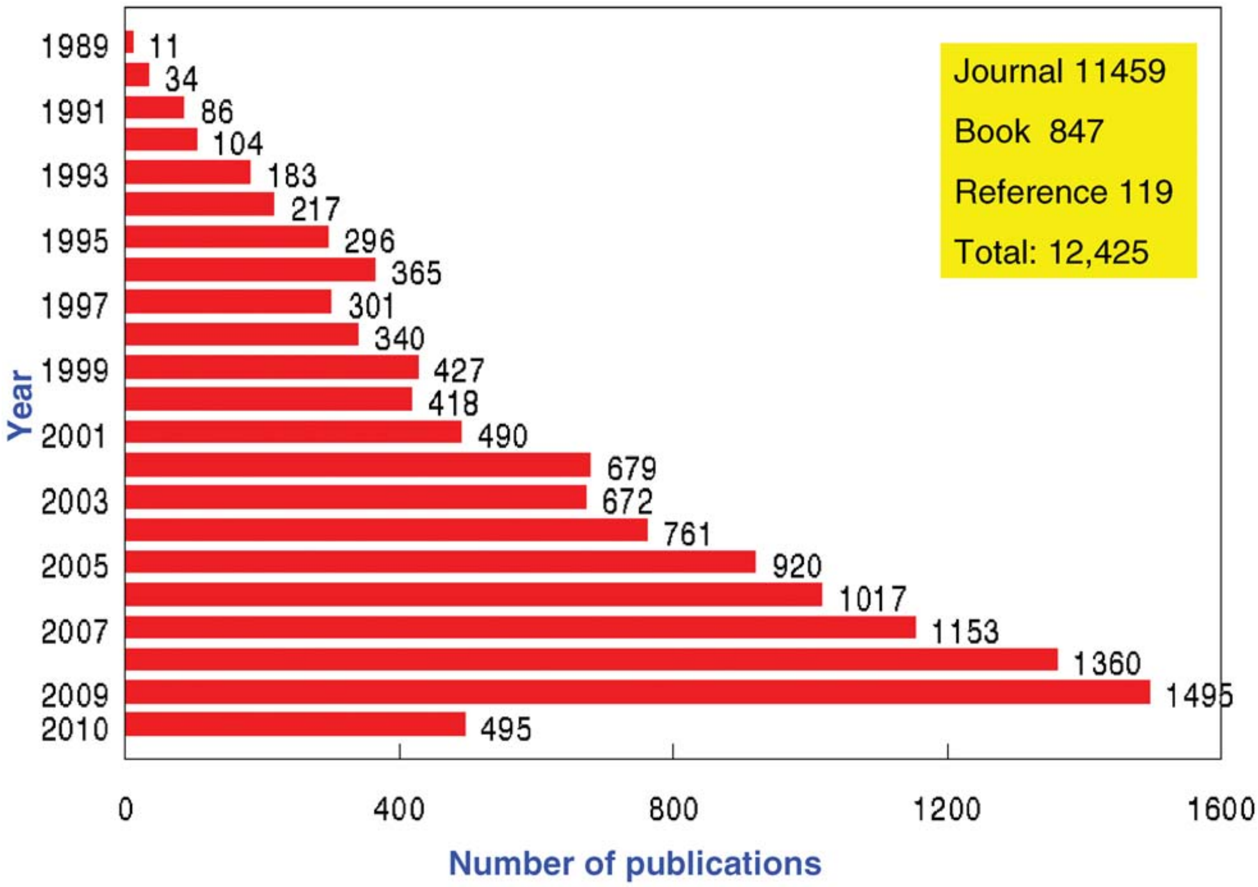


Figure 7: Articles published on bioremediation (Global) Source: www.sciencedirect.com

Chapter 2
Description of mechanisms involved in bioremediation

The role of bioremediation for cleanup of organics and inorganics including miscellaneous uses are described in Figures 8-10. These mechanisms are dealt in an orderly fashion as the sequence of how contaminants come into contact with the plant system, rhizosphere and transportation processes. These mechanisms are interrelated and dependent upon plant physiological processes driven by solar energy, rhizospheric processes and other available precursors. Therefore, in bioremediation application, multiple mechanisms are involved depending on the designed application.

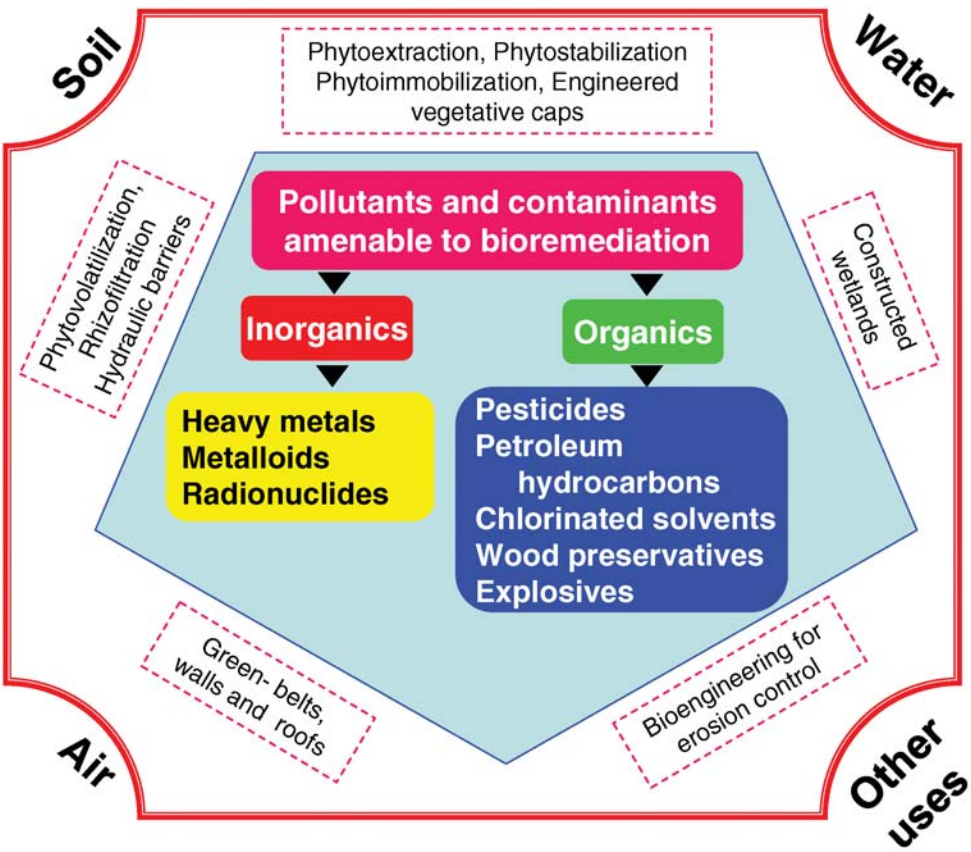


Figure 8: Multiple mechanisms that are involved in bioremediation of soil, water, air and other miscellaneous uses

9

Figure 9: Green roofs

10 a

10 b

Figure 10a and b: Green walls for cleanup of particulate and gaseous contaminants from air and also to enhance aesthetics

2.1. Phytosequestration

The three mechanisms of phytosequestration that reduce the mobility of the contaminant and prevent migration to soil, water and air are as follows:

* Phytochemical complexation in the root zone: Phytochemicals can be exuded into the rhizosphere, leading to the precipitation or immobilization of target contaminants in the root zone. This mechanism of phytosequestration may reduce the fraction of the contaminant that is bioavailable.

* Transport protein inhibition on the root membrane: Transport proteins associated with the exterior root membrane can irreversibly bind and stabilize contaminants on the root surfaces, preventing contaminants from entering the plant.

* Vacuolar storage in the root cells: Transport proteins are also present that facilitate transfer of contaminants between cells. However, plant cells contain a compartment (the "vacuole") that acts, in part, as a storage and waste receptacle for the plant. Contaminants can be sequestered into the vacuoles of root cells, preventing further translocation to the xylem.

2.2. Phytodegradation

Specifically, phytodegradation, also called "phyto-transformation," 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.

Plants catalyze several internal reactions by producing enzymes with various activities and functions (Box 1). Specifically, oxygenases have been identified in plants that are able to address hydrocarbons such as aliphatic and aromatic compounds. Similarly, nitroreductases are produced in some plants that can reduce and breakdown energetic compounds such as the explosives trinitrotoluene (TNT), 1, 3, 5-trinitroperhydro -1, 3, 5- triazine (RDX) and 1,3,5,7-tetranitro -1,3,5,7-tetrazocine (HMX High melting explosive). (Anonymous 2009).

Box 1: Some important enzymes associated with bioremediation (for details, see Husain *et al* 2009)

Enzyme	Target pollutant
Aromatic dehalogenase	Chlorinated aromatics (DDT, PCBs etc.)
Carboxyl esterases	Xenobiotics
Cytochrome P450	Xenobiotics (PCBs)
Dehalogenase	Chlorinated solvents and Ethylene
Glutathione s-transferase	Xenobiotics
Peroxygenases	Xenobiotics
Peroxidases	Xenobiotics
Laccase	Oxidative step in degradation of explosives
N-glucosyl transferases	Xenobiotics
Nitrilase	Herbicides
Nitroreductase	Explosives (RDX and TNT)
N-malonyl transferases	Xenobiotics
O-demethylase	Alachlor, metalachor
O-glucosyl transferases	Xenobiotics
O-malonyl transferases	Xenobiotics
Peroxdase	Phenols
Phosphatase	Organophosphates

Many of the plant enzymes may even be able to metabolize or mineralize several chemicals completely to carbon dioxide and water (McCutcheon and Schnoor 2003). In addition, research has shown that the endophytic symbiotic bacteria *Methylbacterium populum* that lives within poplar can mineralize RDX and HMX (Van Aken 2009). Further, the oxidation and reduction cycle operating during photosynthesis offers additional contaminant breakdown potential. Stronger oxidants and reductants are produced in the plant system (from + 1.1 V to –1.3 V) than are commonly available in biodegradation processes (from + 0.5 V to –0.3 V). Specifically, the redox potential for aerobic reactions with dissolved oxygen as the electron acceptor range +0.25 V and higher, possibly up to + 0.5 V, while other electron acceptors (nitrate, iron-III, Mn, sulfate) range from + 0.25 V down to –0.2 V. Below this redox potential, perhaps to – 0.3 V, methanogenesis may occur. Therefore, organic chemicals (electron donors) in the transpiration stream reaching the photosynthetic centers of a plant are potentially subject to these strong redox conditions as well. This effect has been observed for RDX (Van Aken 2009).

2.3. 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 (Figure 11). 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.

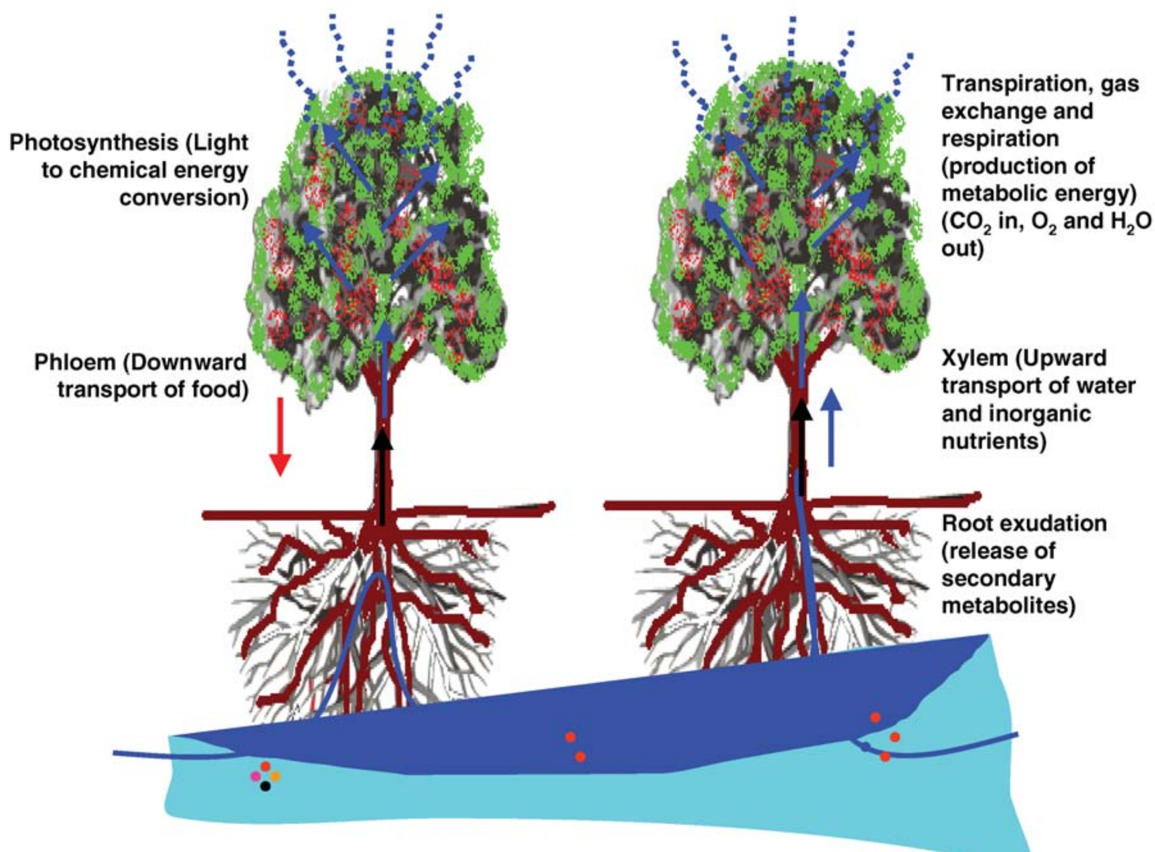


Figure 11: Phytovolatilization mechanism

Phytovolatilization occurs as growing trees and other plants take up water and the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Mercury has been shown to move through a plant and into the air in a plant that was genetically altered to allow it to do so. The thought behind this media switching is that elemental Hg in the air poses less risk than other Hg forms in the soil. This method is a specialized form of phytoextraction, that can be used only for those contaminants that are highly volatile. Mercury or selenium, once taken up by the plant roots, can be converted into non-toxic forms and volatilized into the atmosphere from the roots, shoots, or leaves. For example, Se can be taken up by *Brassica* and other wetland plants, and converted (for example, by methylation to the volatile dimethyl selenium) into non-toxic forms which are volatilized by the plants. Field testing has shown this to be a potentially effective method. A similar mechanism can be exploited for Hg, although there are no naturally occurring plants that can accomplish this. The goal here is to engineer bacterial genes for mercury reduction into plants, and here too laboratory experiments are highly encouraging as mercury breathing out plants are developed *in vitro*. (Heaton *et al* 1998)

2.4. Phytostabilization

Phytostabilization refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils (Figure 12). 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.

a) Soil/Sediment Stabilization: Soil and sediment can mobilize (vertically and laterally) when exposed to uncontrolled water flows. Soil can also mobilize by blowing wind. Both of these modes of soil/sediment migration are known as “erosion” or “leaching.” If the soil or sediment is impacted, the migration of the contaminants through these modes is generally considered non-point source (NPS) pollution. Phytostabilization covers provide a natural barrier and resistance to erosion and leaching and can be further used to minimize NPS pollution if the soil or sediment is impacted.

The main mechanism contributing to stabilizing erosion is the infusion of plant roots into the soil or sediment. Typically, plants with fibrous root systems are used, such as many grasses, herbaceous species, and wetland species. Typical rooting depths for these species are about 30-60 cms for upland species and < 30 cm for wetland species (Anonymous 2009). Therefore, phytostabilization covers are simply soil or sediment that are planted with vegetation selected specifically to control bulk soil migration and/or prevent contaminant migration through phytosequestration.

In addition to phytosequestering contaminants in the rhizosphere, other plants, such as halophytes and hyperaccumulators, can be selected based on their ability to phytoextract and accumulate contaminants into the aboveground tissues. Obviously, additional risks are involved with moving contaminants into the plant; however, this aspect of a phytostabilization cover application for soil/sediments may still be acceptable, depending on the overall human health and ecological risks associated with the site. This is a decision factor to consider when selecting this phytotechnology application as the site remedy . If a harvesting and removal plan is implemented for the application to mitigate the additional risks, then the application is classified as a phytoremediation groundcover.

b) Infiltration Control: Another method to stabilize contaminants in the subsurface is to prevent water from interacting with the waste, possibly leading to its migration. This is a common approach for landfill covers but can also be applied to minimize surface water recharge of groundwater plumes.

Phytostabilization covers for infiltration control, also known as evapotranspiration, waterbalance, or vegetative covers, use the ability of plants to intercept rain to prevent infiltration and take up and remove significant volumes of water after it has entered the subsurface to minimize the percolation into the contained waste (Anonymous 2009). The main phytotechnology mechanism for these applications is phytohydraulics. Phytostabilization covers for infiltration control are composed of soil and plants that maximize evaporation from the soil and plant evapotranspiration processes from the system. To allow these time-dependent (and climate-dependent) processes to occur and successfully remove water from the system, the soil component of the cover is specifically designed and installed such that the available water storage capacity in the soil is maximized.

The vegetation component of the cover usually entails specially formulated seed mixes or mixed communities of plants/trees that can access the stored water as well as create the intercepting canopy. Furthermore, the entire cover is often contoured to promote runoff as another significant loss mechanism for the overall water balance. Different water balance models are available with additional information in ITRC 2009 report (Anonymous 2009).

When minimizing infiltration, one of the potential outcomes is to create an anaerobic zone underneath the phytostabilization cover. In some cases, the subsurface conditions will be driven into methanogenic (methane-producing) conditions. These covers may not be appropriate for sites that can lead to the production of chronic, large, or uncontrolled amounts of this landfill gas. While the methane itself may or may not be toxic to the plants, the presence of the gas in the vadose zone may restrict the oxygen transport needed for cell respiration in the root system. Furthermore, these covers have not been shown to be able to prevent the diffusion of landfill gases to the surface. Therefore, these gases must be controlled through other means.

Phytoremediation groundcovers: In addition to the ability of cover systems to stabilize soil/sediment and control hydraulics, densely rooted groundcover plants and grasses can also be used in bioremediation. Phytoremediation ground covers are one of the most widely used applications and have been applied at various bench-to-full-scale remediation projects. It is the "classic" application often referred to as "phytoremediation" (distinguishing it from the nonremediation aspects of phytotechnologies such as phytostabilization covers and hydraulic tree stands). Furthermore, in the context of this document, phytoremediation groundcovers are vegetated systems typically applied to surface soils as opposed to phytoremediation tree stands , which refers to phytoremediation systems for deep soils and/or groundwater. The typical range of effectiveness for phytoremediation groundcovers is 30-60 cms below ground surface; however, depths down to 1.5 meters have been reported as within the range of influence under some situations.

Phytoremediation groundcovers have been widely applied to soils impacted with recalcitrant compounds such as PAHs, PCBs, and other persistent organic pollutants that are typically less mobile, soluble, biodegradable, and bioavailable. Furthermore, these groundcover systems can also be used as certain types of landfill covers that also promote the degradation of the underlying waste .These have been referred to as bioreactor landfills. Finally, phytoremediation groundcovers have been used to extract specific inorganic contaminants such as metals, salts, and radionuclides in concentrations higher than what existed in the soil.

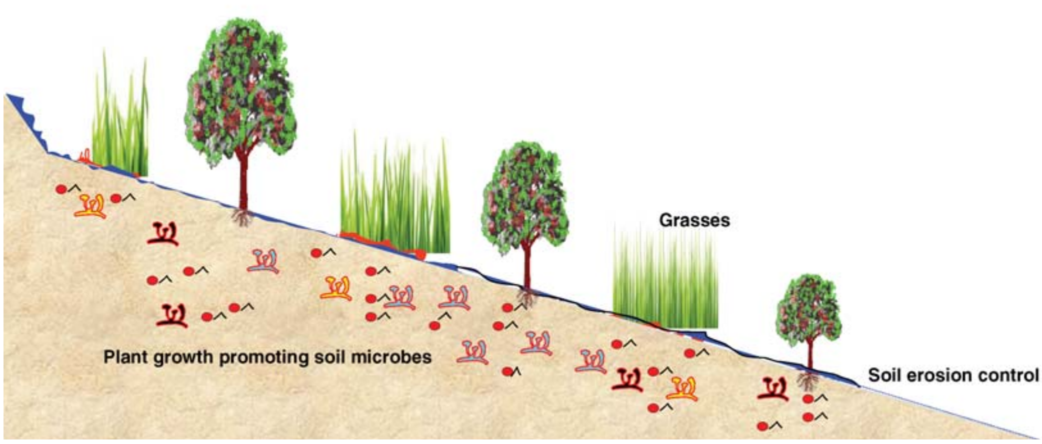


Figure 12. Grasses acclerate the phytostabilization of soils contaminated with trace metals. Their unique adventitious root system coupled with plant growth microbes are implicated for this process. (Schematic presentation).

2.5. 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. 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 (Figure 13).

Once a chemical is taken up, the plant may store the chemical and/or its by-products in the plant biomass via lignification (covalent bonding of the chemical or its by-products into the lignin of the plant), sequester it into the cell vacuoles of aboveground tissues (as opposed to in root cells) as part of phytosequestration. Alternatively, the contaminant may be metabolized through phytodegradation mechanisms (Figure 14) and/or phytovolatilized in the transpiration stream existing in the plant. For organic chemicals, factors that affect the potential uptake into plants through the transpiration stream include hydro-phobicity, polarity, sorption properties and solubility. One characteristic that has been shown to correlate to uptake into a plant is the octanol-water partition coefficient, log Kow. Specifically, organic chemicals having log Kow values between 1 and 3.5 have been shown to enter into plants. The plant root is an organic membrane consisting of a lipid bilayer. The organic characteristics of the lipids make the root partially hydrophobic while the bilayering aspects make it also nonpolar. Therefore, hydrophobic chemicals (log Kow >3.5) are generally not sufficiently soluble in the transpiration stream or are bound so strongly to the surface of the roots that they cannot be easily translocated into the plant xylem. On the other hand, chemicals that are highly polar and water soluble (log Kow <1.0) are not sufficiently sorbed by the roots, nor are they actively transported through plant membranes due to their high polarity. Mostly benzene, toluene, ethylbenzene, and xylene (BTEX); chlorinated solvents; and short-chain aliphatic chemicals fall within the log Kow range that allow them to be susceptible to phytoextraction.

The vapor uptake pathway into plants was specifically identified for chlorinated solvents such as perchloroethene (PCE, also known as "tetrachloroethene"), where partitioning coefficients between plant tissue and air and between plant tissue and water were measured to be 0.0081 L/g and 0.049 L/g, respectively (Struckhoff *et al* 2005). Volatile hydrocarbons such as BTEX constituents are often rhizodegraded to an extent that limits measurable phytoextraction (Fiorenza *et al*. 2005).

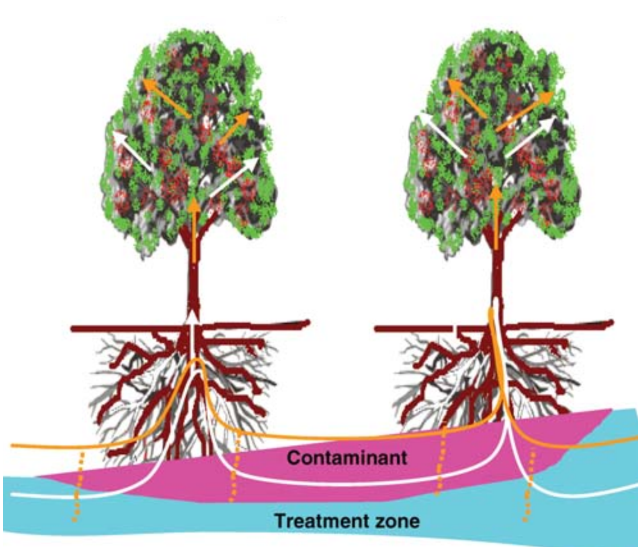


Figure 13. Phytoextraction mechanisms

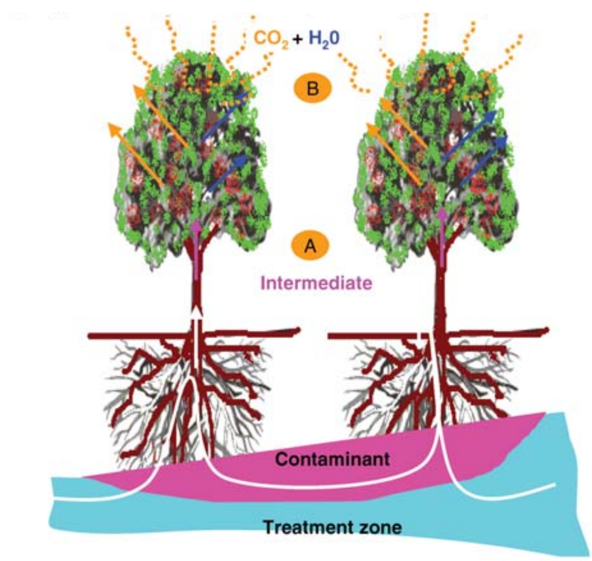


Figure 14. Phytodegradation mechanisms A: plant enzymatic activity, B: photosynthetic oxidation

The relative ability of a plant to take up a chemical from the soil or groundwater into its roots is described by the root concentration factor (RCF), measured as the ratio of the concentration in the root (mg/kg) to the concentration in the external solution (mg/L). Furthermore, translocating the chemical to its shoots is described by the transpiration stream concentration factor (TSCF), measured as the ratio of the concentration in the xylem sap (mg/L) to the concentration in external solution (mg/L). The field values will typically depend on soil properties, chemical partitioning, and the plant species. Higher RCF and TSCF values are an indication of enhanced contaminant uptake by plants and vary directly with the log Kow of the chemical. Contaminants in solution with the highest TSCF contained a log Kow in the range of 1–3.5

For inorganic constituents such as salts, metals, and radionuclides, the uptake into plants and translocation into the aboveground tissues depends on the redox state, chemical speciation in the soil, sediment or groundwater, and the plant species. As a general rule, readily bioavailable inorganics for plant uptake include As, Cd, Cu, Ni, Se, and Zn. Moderately bioavailable metals are Co, Fe, and Mn, whereas Cr, Pb, and U are not easily bioavailable. Several of these constituents, often considered as environmental contaminants in sufficient concentration, are also essential plant nutrients.

Certain plants called "hyperaccumulators" (McIntyre 2001) absorb unusually large amounts of metals in comparison to other plants and the ambient metal concentration. For a plant to be classified as a hyperaccumulator, it must be able to accumulate at least 1,000 mg/kg (dry weight) of a specific metal or metalloid (for some metals or metalloids, the concentration must be 10,000 mg/kg) (Baker 1981). Similarly, "halophytes" are plants that can tolerate and, in many cases, accumulate large quantities of salt (typically, NaCl but also Ca and Mg chlorides). Hyperaccumulators and halophytes are often discovered as being selected to grow at a site based on the metals or salts naturally present, forming their own niche through evolution.

The remediation aspects for these contaminants/pollutants occur when the aboveground portions of the plant where the inorganic contaminant accumulates are harvested with conventional agricultural methods and removed from the site. To enhance the phytoextraction capabilities, several strategies have been attempted. Lead can be made much more bioavailable with the addition of chelating agents such as ethylene diamine tetra-acetic acid (EDTA) to soils. Similarly, a considerable body of information exists on the uptake of radionuclides into plants, including laboratory and field studies where radionuclides from nuclear weapons complexes or test sites have been transferred into plants.

Specifically, the availability of uranium and ^{137}Cs has been enhanced using citric acid and ammonium nitrate, respectively. However, adding these enhancing agents also increases the inherent risks associated with the application since they can also mobilize target contaminants and other constituents deeper into the soil or into groundwater. This is a decision factor to consider when selecting this phytotechnology application as the site remedy. Furthermore, the timing of the application should be thoroughly designed, planned, and managed during implementation .

Some halophytes in tropical and near tropical environments such as salt cedars take up saline water and exude the excess salt through the stomata back onto the ground as a means to create the niche. Furthermore, some plants may produce and exude specific phytochemicals directly into the soil environment that alter the chemistry and speciation of constituents to promote the mobilization and uptake into the plant, particularly for enhancing the uptake of essential nutrients through the release of acidic phytochemicals. In this process of planting a crop of a species known to accumulate metals, metalloids or, radionuclides in plants, and then harvesting the crop the contaminant is recovered.

2.6. 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 (Figure 15). Hydroponically cultivated plants rapidly remove heavy metals from water and concentrate them in the roots and shoots. 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. Shallow lagoons have been engineered as wetlands and maintained as facultative microbial systems with low dissolved oxygen in the sediment. Groundwater or wastewater is pumped through the system for the removal of contaminants by rhizofiltration. Wetlands have been used with great success in treating metals for many years. Long-term utilization of wetland plants and sulfate-reducing conditions result in an increase in pH and a decrease in toxic metals concentrations for treatment of acid mine drainage. Root systems and sediments in wetlands are facultative (aerobic and anaerobic zones) which facilitates sorption and precipitation of toxic metals.

Harvested plants containing heavy metals can be disposed of or treated to recycle the metal. Today scientists have identified plants demonstrating high biomass production and metal removal capacity for a wide variety of metals. Rhizofiltration has many of the benefits of other phytoextraction techniques, including low cost and minimal environmental disruption. A continuous flow system circulates the contaminated water through specially designed plant containment units. Periodically, older plants are harvested and replaced.

Experimental evidence showing nonlinear kinetics of disappearance of metals from solution suggests that several different mechanisms, of differing speeds, operate simultaneously. Surface absorption by the roots, the fastest and often the most prevalent mechanism, most likely depends on physicochemical processes (e.g., ion exchange, chelation) and can even take place on dead roots (Anonymous 2009). In its reliance on surface absorption as the primary mechanism for removing metals from waste streams, rhizofiltration is related to the process known as biosorption, in which microbial, fungal or other biomass, living or dead, is used to absorb large quantities of materials such as heavy metals. In addition to surface absorption, other, slower mechanisms underlying rhizofiltration may also occur: these might include biological processes (intracellular uptake, deposition in vacuoles, and translocation to the shoot), or precipitation of the metal from solution by plant exudates (the slowest mechanism of the three). Thus, negatively affecting economics and efficiency. Rhizofiltration is believed to be effective (and perhaps most economically attractive) for dilute concentrations of contaminants in large volumes of water, and this feature may make it especially attractive for radionuclide decontamination.

2.7. Rhizoremediation

Well established rhizoremediation processes are: a) Sequestration or immobilization or retention of toxicants within a confined area i.e. the soil at the site of their release or in contaminated soil placed in a landfill; b) Removal of contaminants from the soil/waste water, and c) Destruction/degradation of organic pollutants by plant-microbial association. These three strategies either individually or in combination with each other have been routinely

Figure 15. Rhizofiltration of contaminants of concern

implemented to successfully treat contaminated soil. Partial immobilization of water soluble contaminants is brought about by plant transpiration (soil water taken up, transported, and evaporated from leaf surfaces) since the process removes soil water that would otherwise cause contaminant leaching and movement. Removal of toxic metals from contaminated soil occurs when inorganic ions are taken up by plant roots and translocated through the stem to aboveground plant parts. Soil microflora of plant roots (rhizosphere zone) is involved in xenobiotic metabolism. The catabolic activity within the rhizosphere has been attributed to both bacteria and fungi whose presence and enzymatic expression are believed to be modulated by organic chemicals released from both living and dead roots.. Both the direct and indirect degradation of soil contaminants by plant root physiology and biosynthetic pathways can potentially occur at the lowest depth of root penetration, a special feature of plant remediation.

Rhizodeposition and Root exudates: The roots of plants deposit high amounts of photosynthetically derived hydrocarbons into the surrounding soil as well. Annually, plants transfer 40–90 % of the net fixed carbon (as primary and secondary metabolites) to roots. Organic compounds that are released as rhizodeposits can be categorized as exudates, secretions, plant mucilages, mucigel, and root lysates. These organic substances (e.g. organic acids of low and high molecular weight, sugars, and amino acids) play an important role in interactions of plants with their environment and consequently in the stimulation of microbial degradation of soil contaminants by plants. They stimulate the growth of microorganisms in the root zone of plants leading to an enhanced abundance of bacteria and fungi. This so-called rhizosphere effect is supported by physical impacts of the plant roots on the soil (i.e. gas exchange, soil moisture).

No single plant or microbe excels in a) immobilization, b) removal, and c) destruction properties, nor does any single species show maximum uptake of all toxic metals or faster degradation of all organic contaminants. Therefore, successful treatment of soils with mixed waste requires a combination of plant species with appropriate remediation properties, and also the inclusion of plant species hosting rhizosphere communities (bacteria and fungi) active against specific contaminants that are present. Rhizosphere microorganisms, which are closely associated with roots, have been termed Plant Growth Promoting Rhizobacteria (PGPR). Further, rhizosphere microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests (Rajkumar 2009, 2010, Mackova *et al* 2006) On the other hand, the plant root exudates provide nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn, stimulate plant growth and reduce the metal toxicity in plants. Among the rhizosphere microorganisms involved in plant interactions with the soil milieu, the Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) have gained prominence all over the world to treat soil (Figure 16). (Ma *et al* 2011)

The presence of a contaminant in a soil tends to naturally select organisms such as bacteria, yeast, and fungi that prefer that chemical as a source of food and energy. Microbial populations of specific organisms selected by using the contaminant as a primary food source can be several orders of magnitude higher than other organisms that do not metabolize the contaminant. The rate of degradation, metabolization, or mineralization of the contaminant in the soil depends on the bioactivity in the soil that is derived primarily from the proteins and enzymes from the soil organisms. However, contaminant breakdown is often limited by the availability of electron acceptors or donors, cometabolites, inorganic nutrients, plant vitamins and hormones, pH, and/or water.

In general, a symbiotic relationship evolves between plants and soil microbes in the rhizosphere. Plants provide nutrients necessary for the microbes to thrive, while the microbes provide a healthier soil environment where plant roots can grow. Specifically, plants loosen soil and transport oxygen and water into the rhizosphere. Furthermore, plants exude specific phytochemicals (sugars, alcohols, carbohydrates, etc.) that are primary sources of food (carbon) for the specific soil organisms that aid in providing the healthier soil environment. Alternatively, the exuded phytochemical may be an allelopathic agent meant to suppress other plants from growing in the same soil. In return for exporting these phytochemicals, plants are protected from competition, soil pathogens, toxins, and other chemicals that are naturally present or would otherwise be growing in the soil environment.

Microbial populations can be several orders of magnitude higher in a vegetated soil compared to an unvegetated soil. Rhizodegradation, sometimes called phytostimulation, rhizosphere biodegradation, or plant assisted bioremediation/degradation, is the enhanced breakdown of a contaminant by increasing the bioactivity using the plant rhizosphere environment to stimulate the microbial populations. This enhanced bioactivity represents the primary means through which organic contaminants can be remediated, including into harmless products that can be converted into a source of food and energy for the plants or soil organisms. The specific proteins and enzymes, or analogs to those produced by the soil organism needed to break down the contaminant, may be produced and exuded by the plant itself.

2.8. Phytohydraulics

Plants significantly affect local hydrology. Phytohydraulics is the ability of vegetation to evapotranspire sources of surface water and groundwater. The vertical migration of water from the surface downward can be limited by the water interception capacity of the aboveground canopy and subsequent evapotranspiration through the root system. If water infiltrating from the surface is able to percolate below the root zone, it can recharge groundwater. However, the rate of recharge depends not only on the rooting depth of the species, but on the soil characteristics as well. The horizontal migration of groundwater can be contained or controlled using deep-rooted species such as prairie plants and trees to intercept, take up, and transpire the water. One class of trees that has been widely studied in phytotechnologies is phreatophytes, which are deep-rooted, high-transpiring, water-loving trees that send their roots into regions of high moisture and that can survive in conditions of temporary saturation (Anonymous 2009). Salicaceae comprises typical phreatophytes e.g. poplars and willows. Trees such as *Prosopis* and *Eucalyptus* are typical phreatophytes useful in bioremedidation (Figure 17 a and b).

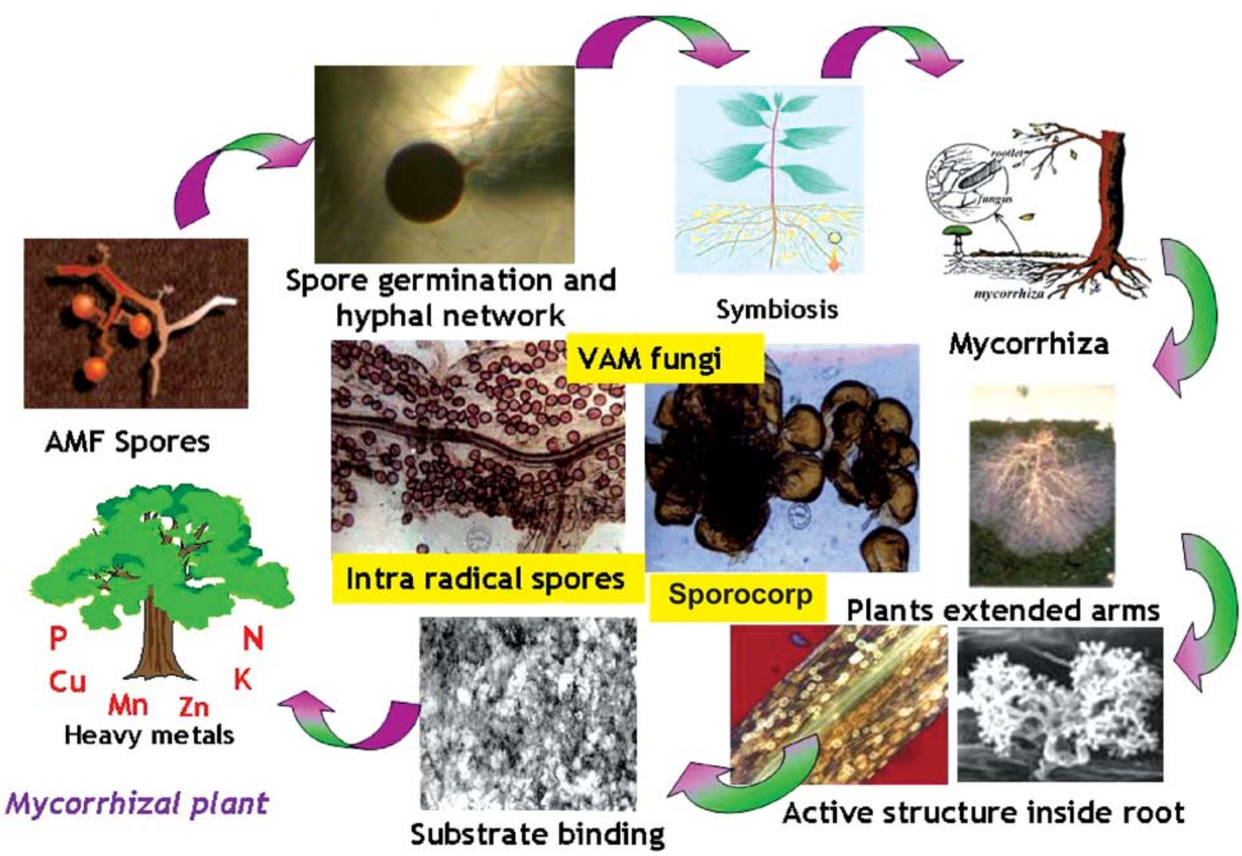


Figure 16. Mycorrhizal fungal networks connect the roots of the same or different plant species, provide pathway for nutrient transfer. Associated plant growth promoting rhizobacteria foster rhizoremediation of inorganic and organic pollutants.

a b

Figure 17. a and b *Prosopis sp.* (phreatophyte)

2.9. Tree Hydraulic Barriers

Groundwater naturally migrates from higher to lower elevations in the subsurface, typically along the path of least resistance (i.e., higher permeable zones or aquifers). Contaminants present in the groundwater can likewise migrate in the subsurface, potentially impacting downgradient receptors. However, many contaminants can interact with the subsurface environment through adsorption and electrostatic forces to retard the contaminant plume compared to the bulk groundwater. To contain the hydraulic flow, groundwater extraction can be used to further limit the migration of groundwater plumes. When groundwater is extracted downgradient of the plume, the hydraulic gradient is reduced in a cone (or zone) of depression creating a capture zone. When groundwater is extracted upgradient of the plume, the hydraulic gradient within the plume is reduced, causing slower plume migration. Most tree hydraulic barrier applications concentrate the plantings above and at the downgradient edge of the plume. All applications use the phytohydraulic mechanisms. In general, the deep-rooted, high-transpiring trees must be actively tapping into the Groundwater to create the barrier. Furthermore, a relatively large number of trees (and associated area) are generally required to extract the volumes necessary to achieve containment. Certain trees may have high transpiration rates at various ages. Although this type of phytotechnology application has generally focused on the use of trees, other species such as prairie grasses have root systems that can reach 3 to 4.5 meters below ground given optimal soil and moisture conditions (Figure 18). The transpiration rate may depend upon many other factors, including the depth of groundwater, soil conditions, and climate in the region where the site is located. These factors must be considered when selecting and designing bioremediation.

Phytoremediation tree stands: In addition to the ability of deeper rooted plants and trees to take up and transpire groundwater, they can also be used to phytoremediate deeper soils and contaminated plumes that are located near the top of the water table. While phytohydraulics can be used to bring the contaminants into the root zone (Figure 19), rhizodegradation, phytodegradation, and/or phytovolatilization mechanisms can reduce contaminant concentrations at depth. Furthermore, phytoremediation also includes phytoextraction as long as harvesting and contaminant removal is included in the application. These mechanisms further reduce the migration of contaminated groundwater plumes through destruction. Phytoremediation tree stands have been widely applied to soluble contaminants that commonly impact groundwater such as petroleum products BTEX (benzene, toluene, ethylbenzene, and (o-, m-, p-) xylenes), MTBE (methyl-tert-butyl ether,) aliphatics, gasoline-range organics (GRO), diesel-range organics (DRO), TPH (total petroleum hydrocarbon) and chlorinated hydrocarbons, PCE (perchloroethene), TCE (trichloroethylene), DCE (dichloroethene), VC (vinyl chloride), PAH (polycyclic aromatic hydrocarbons); PCB (polychlorinated biphenyl); TNT (2,4,6-trinitrotoluene) etc. The lighter fractions of these constituents are generally mobile, soluble, and bioavailable with log Kow values in the range where uptake into plants is expected.

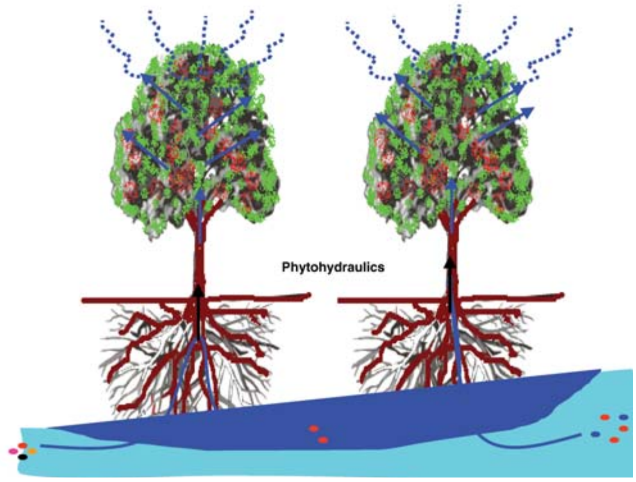


Figure 18. Depth of plant root penetration is crucial for successful bioremediation

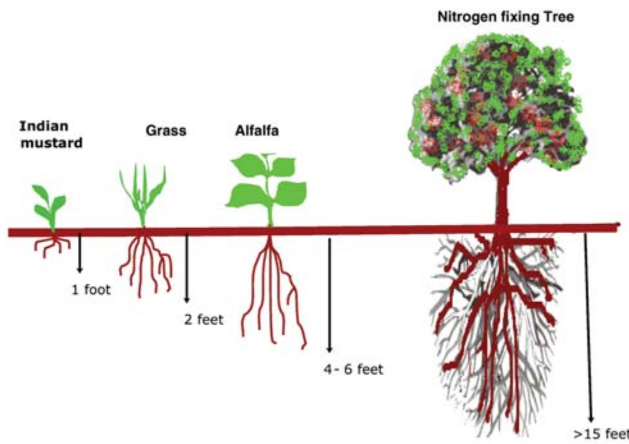


Figure 19. Phytohydraulics and groundwater hydraulic depression bioremediation

2. 10. Riparian Buffers

Riparian buffers are vegetated areas that protect adjacent water resources from Non-Point Source (NPS) pollution. In addition, these buffers provide bank stabilization and habitat for aquatic and other wildlife. Similar situations that threaten surface water bodies are groundwater seeps that contain environmental pollutants. Typically, where these seeps daylight is just upgradient of a surface water body (i.e., a gaining water body) that then flows directly into the receptor. In some cases, including seasonal variations, the groundwater may not always daylight and may simply feed the surface water body through a subsurface hydrologic connection. Placement of a riparian buffer would be along and upgradient of the groundwater-surface water interface.

It has long been recognized that riparian buffers (also known as riparian corridors/zones) are vital to controlling the hydrology and cleansing the runoff and near-surface groundwater. Specifically, the surface runoff or seep requires that the flow of water be sufficiently slow or contained to allow sediments and other particulate matter to settle out. The rate of flow is often measured as the hydraulic retention time (HRT) and can be designed using the width, grade (vertical drop over horizontal distance), and contouring of the system as well as soil characteristics. Furthermore, runoff water must flow evenly across the buffer to be effective. If channels develop due to erosion, the effectiveness of the buffer is greatly reduced due to the water "short-circuiting" the system and reducing the HRT. For the contaminants in the runoff to be adequately remediated, the HRT must be sufficient to match the rates of attenuation from various mechanisms.. The hydrology is also affected by the vegetation in the riparian buffer with the same mechanisms driving phytohydraulics while their root systems promote phytosequestration, rhizodegradation, phytoextraction, phytodegradation, and/or phytovolatilization. Bioremediation is profitable for countries like India with vast biodiversity. Its advantages and limitations are mentioned in Box 2.

Box 2 : Advantages and limitations of bioremediation	
Advantages	**Limitations**
In situ	Limited to shallow soils, streams, and groundwater
Passive	High concentrations of hazardous materials can be toxic to plants
Solar driven	Mass transfer limitations associated with other biotreatments
Costs 10% to 20% of mechanical treatments	Slower than mechanical treatments
Transfer is faster than natural attenuation	Only effective for moderately hydrophobic contaminants
High public acceptance	Toxicity and bioavailability of degradation products is not known
Fewer air and water emissions	Contaminants may be mobilized into the groundwater
Generate less secondary wastes	Potential for contaminants to enter food chain through animal consumption
Soils remain in place and are usable following treatment	Unfamiliar to many regulators
Phytovolatilized contaminants could be transformed to less toxic forms (e.g. elemental mercury and dimethyl selenite gas) ii) phytovolatilization acclerates degradation processes	The contaminant or a hazardous metabolite might accumulate in vegetation and be passed on in later products such as fruit or lumber. Low levels of metabolites have been found in plant tissue.
Phytostabilization: i) circumvents the removal of soil, ii) It has a lower cost and is less disruptive than other more-vigorous soil remedial technologies, iii) Revegetation enhances ecosystem restoration.	i) The contaminants remain in place. ii) The vegetation and soil may require long-term maintenance to prevent re-release of the contaminants and future leaching. iii) Require extensive fertilization or application of soil amendments. iv) Plant uptake of metals and translocation to the aboveground portion must be avoided. v) The root zone must be monitored to prevent metal leaching.
In phytoextraction , the plant biomass containing the extracted contaminant can be a resource (phytoextraction). For example, biomass that contains selenium (Se), an essential nutrient, has been transported to areas that are deficient in Se and used for animal feed. In green house experiments, gold was harvested from plants.	i) Metal hyper accumulators are generally slow-growing with a small biomass and shallow root systems. ii) Plants harvested must be properly disposed. iii) Phytoextraction studies conducted using hydroponically grown plants, with the contaminant added in solution, may not reflect actual conditions and results occurring in soil. v) Phytoextraction coefficients measured under field conditions are likely to be less than those determined in the laboratory.
Rhizofiltration using terrestrial plants removes contaminants more efficiently than aquatic plants. ii) This system can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system). iii) An *ex situ* system can be placed anywhere because the treatment does not have to be at the original location of contamination.	i) The pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake. ii) The chemical speciation and interaction of all species in the influent have to be understood and accounted for. iii) A well-engineered system is required to control influent concentration and flow rate. iv) The plants (especially terrestrial plants) may have to be grown in a greenhouse or nursery and then placed in the rhizofiltration system. v) Periodic harvesting and plant disposal are required. vi) Metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field.

* This chapter is based on the information of: ITRC (Interstate Technology & Regulatory Council) (2009). Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised.PHYTO-3.Washington, D.C.

Chapter 3
Inorganics

3.1 Heavy metals

Inorganic pollutants which contaminate land and water bodies include heavy metals, metalloids fluoride and cyanide etc. Heavy metals can occur in different valence states, so that one element may be more or less toxic in different states. One example is Cr(III) and the more toxic Cr(VI). Normally heavy metals in the environment are in low concentrations but may be elevated because of human activities, fossil fuel combustion, mining, smelting, sludge amendment to soil, fertilizer application, and agrochemical application (Figure 20). At low concentrations some trace elements e.g. 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 e.g. As, Cd, Hg, and Pb etc. are extremely toxic to biota even at very low concentrations.

3.1.1 Arsenic

Arsenic (As) is one of the most toxic elements present in soils and water. Over the years, arsenic has been widely used in agriculture and industrial practices such as pesticides, fertilizers, wood preservatives, smelter wastes and coal combustion ash, which are of great environmental concern, apart from the natural sources. Arsenic contamination affects biological activities as a teratogen, carcinogen and mutagen as well as having detrimental effects on the immune system. The most common manifestations are skin melanosis and keratosis (Figure 21).

a) West Bengal: As-enriched groundwater from the vast alluvial aquifer in the Bengal Delta Plain is a subject of global concern. In West Bengal: Malda 7 districts viz., Murshidabad, Burdwan, Nadia, Hoogly, 24 Parganas (North)and 24 Parganas (South) are the

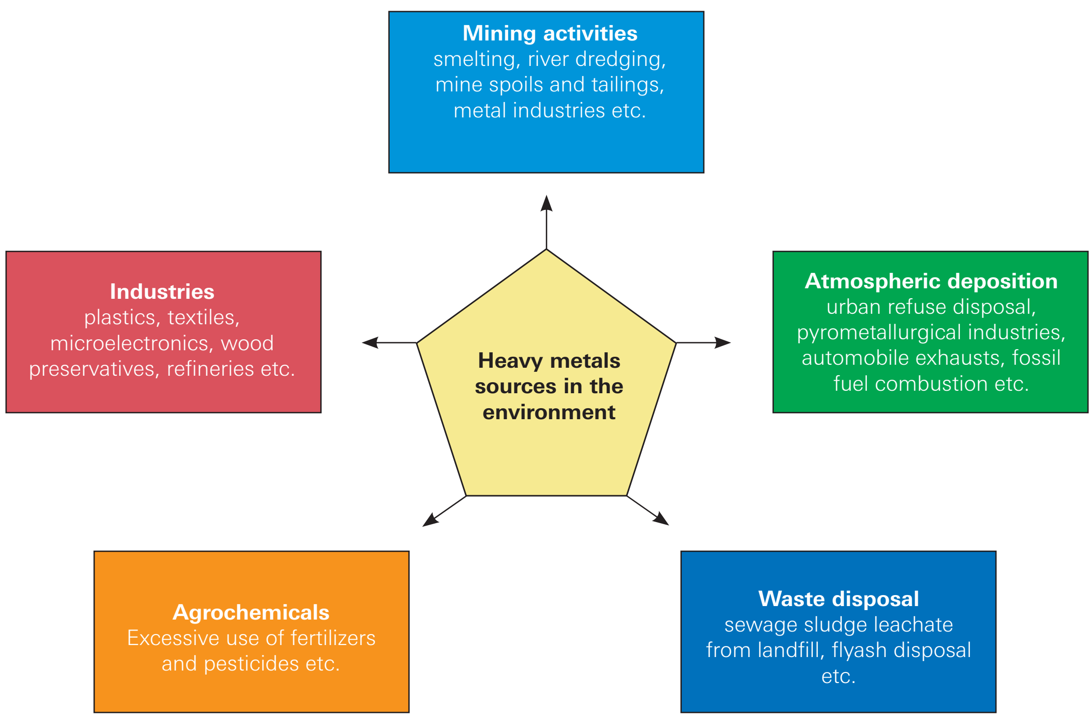


Figure 20. Sources of heavy metals in the environment

Figure 21. Arsenic toxicity symptoms

most severely affected. About 38.4% of the area of West Bengal and about 44.4% of the total population appear to be affected by As poisoning. The As content in groundwater in the Bengal Basin varies from 0.05 to 3.7 mg/l, with an average of 0.2 mg/l, which is much higher than the limit of 0.010 mg/l recommended by WHO in drinking water. The occurrence of elevated level of arsenic in groundwater of West Bengal is due to geochemical reactions such as oxidation of arsenic rich pyrites during the water extraction process or reductive dissolution of arsenic-rich iron hydroxides.

b) Chhattisgarh: Parts of Chhattisgarh state have been identified as arsenic contaminated. The major locations are in Rajnandgaon district, Chhattisgarh. The area between Dongargaon to Mohla (about 500 sq. km.) is affected by the presence of arsenic in varying concentrations.

c) Patancheru: Industrial area in Hyderabad and around Khazipally are polluted. There are more than 40 pesticide and agrochemical producing industries of various capacities. Almost all industrial effluents from these industries ultimately join the Khazipally lake.

Strategies for arsenic phytoremediation: Plants exhibit four different strategies when exposed to elevated concentration of metals. Hyperaccumulators: Plants in which metal concentration is upto 1% in dry matter (metal specific and variable). Accumulators: Plants in which uptake and translocation reflect metal concentration in interstitial water without showing toxic symptoms. Indicators: Plants in which uptake and translocation reflect metal concentration in interstitial water and show toxic symptoms. Excluders: Plants restrict the uptake of toxic metals into shoot over a wide range of background concentrations (Baker 1981) (Figure 22).

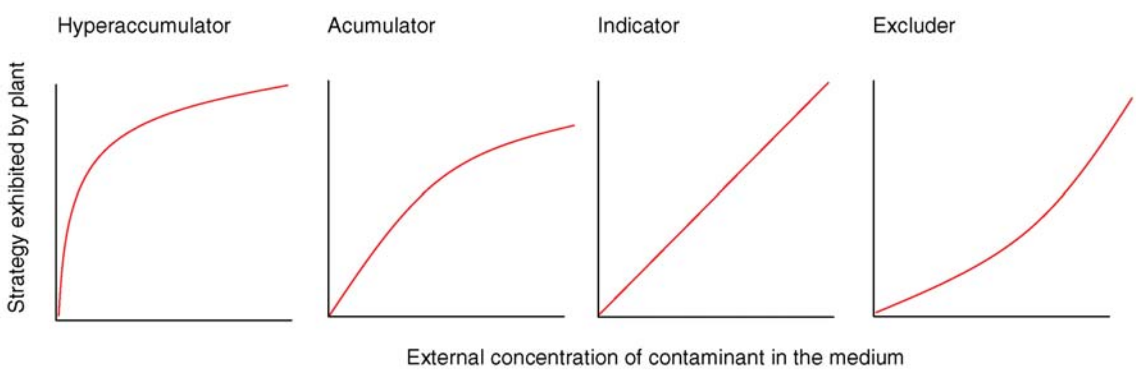


Figure 22. Plants exhibit four strategies when exposed to elevated concentration of metals

a) The first known arsenic hyperaccumulating plant is *Pteris vittata*. Also known as Chinese brake fern, it was discovered from an arsenic-contaminated site that was contaminated from pressure-treating lumber using chromated-copper-arsenate (CCA). *P. vittata* is reported to accumulate 23 g kg-1 of arsenic in its fronds (Figures 23 and 24). In addition to *P. vittata* and *P. cretica*, several other arsenic hyperaccumulating plants have been reported recently including *Pityrogramma calomelanos* and *Pteris longifolia* and *Pteris umbrosa*.

b) Phosphate fertilization: Phosphate fertilization of arsenic contaminated soils seems to be one of the feasible strategies for successful phytoremediation using As hyperaccumulating fern viz. *Pteris vittata.* Arsenic is toxic whereas phosphorus is essential for plants. They are both have similar electron configurations and chemical properties. Therefore, arsenate and phosphate will compete with each other for soil sorption sites, resulting in a reduction in their sorption by soil and an increase in solution concentrations. Phosphate significantly suppressed the sorption of arsenate. Arsenate may replace phosphate in ATP synthesis, and/or in various phosphorolysis reactions, thus interfering with phosphate metabolisms and causing toxicity to a plant. In contrast, phosphate may be able to alleviate arsenate toxicity by improving phosphate nutrition. The effects of arsenate and phosphate interactions on fern biomass production and uptake of arsenate and phosphate, and optimal molar ratios of phosphate to arsenate in both soil and better fern growth were

Figure 23. Pilot scale setup using *Pteris vittata* for removal of As from contaminated water. Courtesy Prof L.Q. Ma. Univ. Florida, USA

Figure 24. Scheme for phytofiltration of As contaminated water using *Pteris vittata* (a) *Pteris vittata* (fern) in pot, (b) Plant suspension tray, (c) Entire wrapped fern plant, (d) tray of eight fern plants, (e) Consortium of ferns in phytofiltration of As contaminated water. Source: Elless MP, Poynton CY,. Willms CA, Doyle MP, Lopez AC, Sokkary DA, FergusonBW and Blaylock MJ (2005) Pilot-scale demonstration of phytofiltration for treatment of arsenic in New Mexico drinking water. Water Research, 39: 3863-3872

studied. These results provided critical information for better understanding of arsenate hyperaccumulation by Chinese brake and optimizing soil conditions for arsenate phytoextraction.

c) Rhizospheric processes: Root exudates (metabolites that are released to the root surface) are generally classified into two types, High Molecular Weight (HMW = polysaccharides and polyuronic acid) and Low Molecular Weight (LMW = organic acids, sugars, phenols and various amino acids, including non-protein amino acids such as phytosiderophores). The composition and quantity of root exudates vary from plant to plant based on i) plant biology, such as plant species, growth and developmental period and nutrient status and ii) the soil and its elemental content. Characterizations of root exudates, which include the movement of root exudates in the photosphere mobilization of soil nutrients, have yielded significant results (Figure 25).

d) Prospects of arbuscular mycorrhizal fungi: It has been observed that the Arbuscular mycorrhizal fungus Glomus mosseae formed a stable association with Chinese brake fern (*P. vittata* L.) and possessed substantial resistance to arsenic toxicity. Mycorrhizal colonization increased plant biomass and consequently increased the quantity of arsenic removed from the soil by the hyperaccumulator.

e) Grasses as ideal plants for remediation of arsenic contaminated in soil: Poaceae members (grasses) such as *Agrostis castellana, A. delicatula,* and *Holcus lanatus* have played a significant role in revegetation of the arsenic contaminated soil in SW Europe. Arsenic accumulation was found in all parts of the grasses with different levels of concentration. Vetiver is a perennial grass with strong ecological adaptability, large biomass and is easy to manage and grow in different soil conditions. It has great potential for various applications including hillside soil and water conservation, sustainable agriculture, fixing sandy riverbank and pollution control.

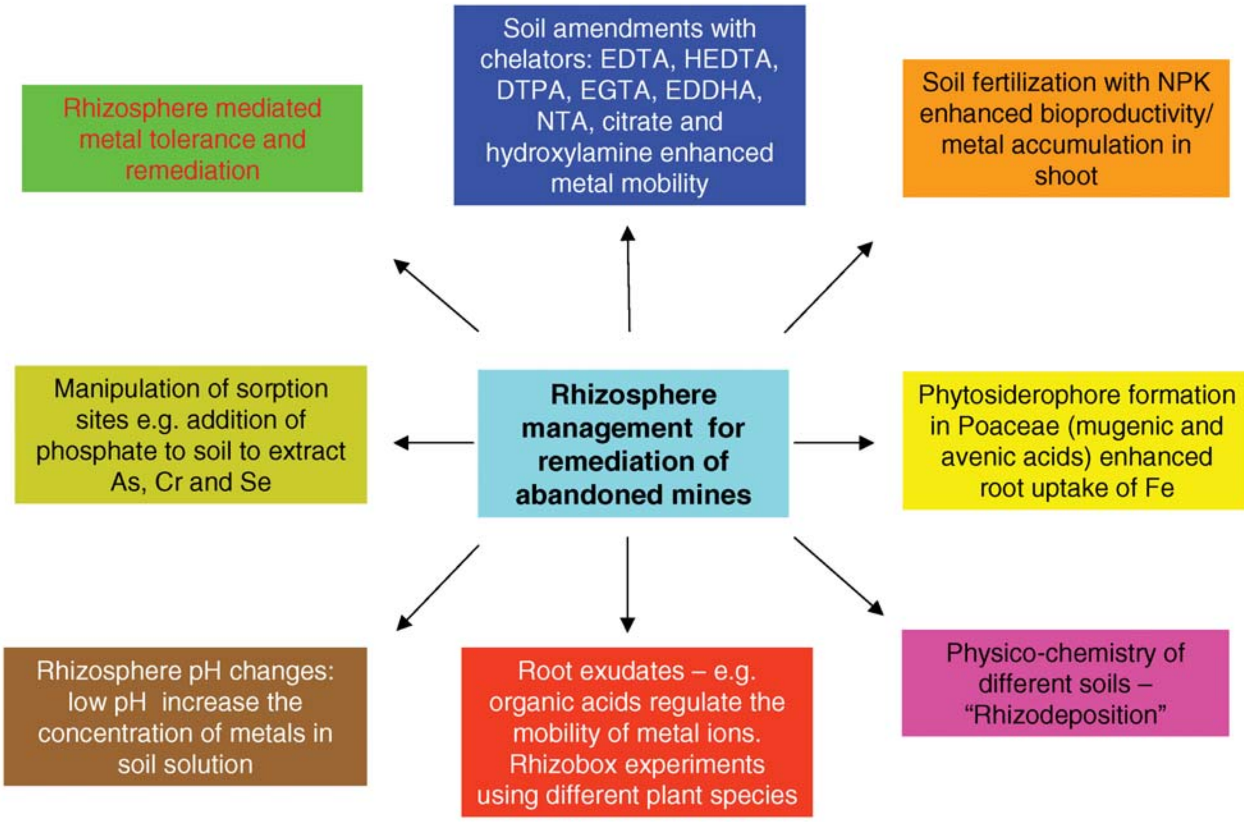


Figure 25. Rhizospheric processes influencing bioremediation changes

3.1.2 Mercury

Contamination of mercury is reported to be widespread in India (Box 3). In a recent study, Mukherjee *et al*., 2008 reported the industrial emissions of mercury from coal combustion, iron and steel industry, non-ferrous metallurgical plants, chloralkali plants, cement industry, waste disposal and other minor sources (i.e. brick manufacturing, instruments, clinical thermometers). No information was found in the literature for the pulp and paper industry or for the oil and petrochemical industry in India as well as natural sources. It should be stated that the lack of true emission data make it very uncertain to estimate anthropogenic emissions of mercury for India (Figures 26-27).

Box 3: Mercury emission from different source categories in India (Metric tons/year (Mukherjee *et al*., 2008)			
Source	**Mercury emission:**	**2000**	**2004**
Coal fired power plants		100.44	120.85
Residential & Commercial boiler		3.65	3.70
Pig iron & steel production		3.84	4.56
E-waste		NA	0.82
Biomass burning			
Forest		7.74	7.74
Crop		4.76	4.76
Cheor-alkali plants		132	6.2
Brick Manufacturing		7.49	
Residual fuel oil consumption		0.52	0.47
Cement Production		4.2	4.66
Municipal solid waste		50	70.00
Medical waste		6.6	6.60
Pb-production		2.49	1.83
Zn-production		1.41	1.90
Cu-production		3.84	11.78
Total		**321.49**	**253.36**

Figure 26. Electronic waste containing mercury, lead and cadmium etc.

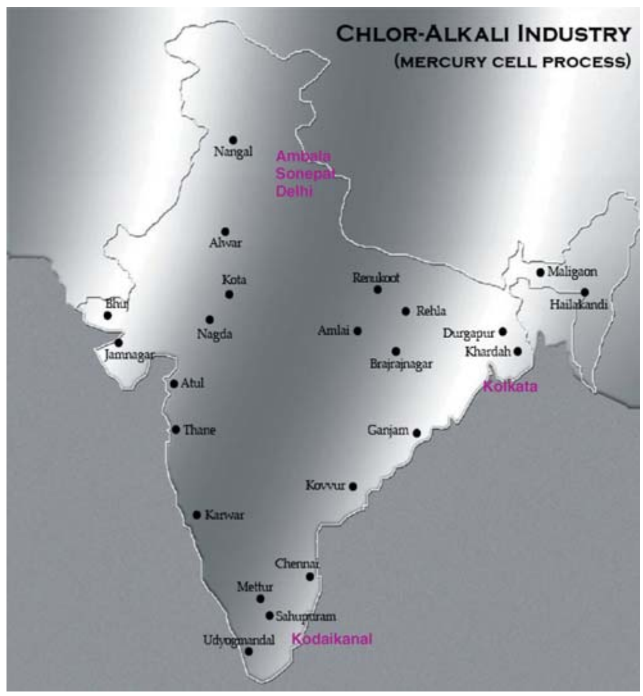


Figure 27. Chlor-alkali industry sites in India

Source: Toxic links, Mukherjee *et al* 2008

UNEP-Global Partnership for Mercury Transport and Fate Research (UNEP-MFTP) initiative prepared global mercury emission budget (Box 4)

a) Mercury contamination at thermometer factory in Kodaikanal:

The site is approximately 85,000 m^2 and is located in a notified industrial area, on top of a cliff at an elevation of approximately 2,180 m above sea level. A former mercury thermometer factory is located on this site which ceased manufacturing operations in March 2001. The site comprises of residential and recreational areas. The site slopes steeply into the Pambar Shola forest (a protected nature sanctuary in the state of Tamil Nadu). This site is predominant with overgrown grass and dense vegetation. Sectional view of the site is shown in Figures 28 and 29. Most of the mercury contaminated equipment and soil has been removed from the site.

Box 4: Mercury – a global scenario (Source UNEP-MFTP report 2008)			
Source Natural	**Hg emission in atmosphere (Mg y^{-1})**	**Source Anthropogenic**	**Hg emission in atmosphere (Mg y^{-1})**
Oceans	2682	Coal combustion, oil combustion	1422
Biomass burning	675	Artisanal Gold Mining Production	400
Desert/Metalliferrous/ Non-vegetated Zones	546	Waste disposal	166
Tundra/Grassland/Savannah/Prairie/ Chaparral	448	Non-ferrous metal production	156
Forest	342	Cement production	140
Evasion after mercury depletion events	200	Caustic soda production	65
Agricultural areas	128	Other	65
Lakes	96	Mercury production	50
Volcanoes and geothermal areas	90	Pig iron and steel production	31
		Coal bed fires	6
A. Sub-total (Natural)	**5207**	**B. Sub-total (Anthropogenic)**	**2501**
Total A+B		**7708**	

b) Bioremediation and enhanced phytovolatilization via Molecular genetic and transgenic strategies.

Mercury cleanup via phytovolatilization has been established (Figures 30 a-c). Genetic strategies, transgenic approaches including the use of microbes will fetch phytoremediation lab and field applications. Mercury is a worldwide problem as a result of its many diverse uses in industry (chlorine production, paper, textiles, lamps, fungicide and antibacterial agent etc.)

Elemental mercury, Hg (0), can be a problem because it is oxidized to Hg^{2+} by biological systems and subsequently is leached into wetlands, waterways, and estuaries. Additionally, mercury can accumulate in animals as methyl mercury ($CH_{3-}Hg^{+}$), dimethylmercury (CH3)2-Hg) or other organomercury salts. Organic mercury, produced by some anaerobic bacteria, is 1-2 orders of magnitude more toxic in some eukaryotes, is more likely to biomagnify than ionic mercury, and efficiently permeates biological membranes. Monomethyl-Hg is responsible for severe neurological degeneration in birds, cats, and humans.

Certain bacteria are capable of pumping metals out of their cell, and/or oxidizing, reducing, or modifying the metal ions to less toxic species. One example is the mer operon. The mer operon contains genes that sense mercury (merB), transport mercury (merT), sequester mercury to the periplasmic space (merP), and reduce mercury (merA). MerB is a subset of the mer operon and is capable of catalyzing the breakdown of various forms of organic mercury to Hg^{2+}. MerB encodes an enzyme, organomercurial lyase, that catalyses the protonolysis of the carbon-mercury bond. One of the products of this reaction is ionic mercury (Heaton *et al* 1998; Pilon-Smits 2000; Rugh *et al* 1996):

Hg^{2+}. $R\text{-}CH_{2-}Hg^{+}$ → merB →$R\text{-}CH_3$ + Hg(II)
Hg(II) + NADPH → merA→Hg(0) + $NADP^{+}$ + H^{+}
Hg (0) (elemental mercury) can be volatilized by the cell.

Figure 28. Mercury contaminated site (former thermometer manufacturing site) at Kodaikanal. The site comprises of residential and recreational areas on the steep slopes of the Pambar Shola Forest, a protected nature sanctuary in the state of Tamil Nadu.

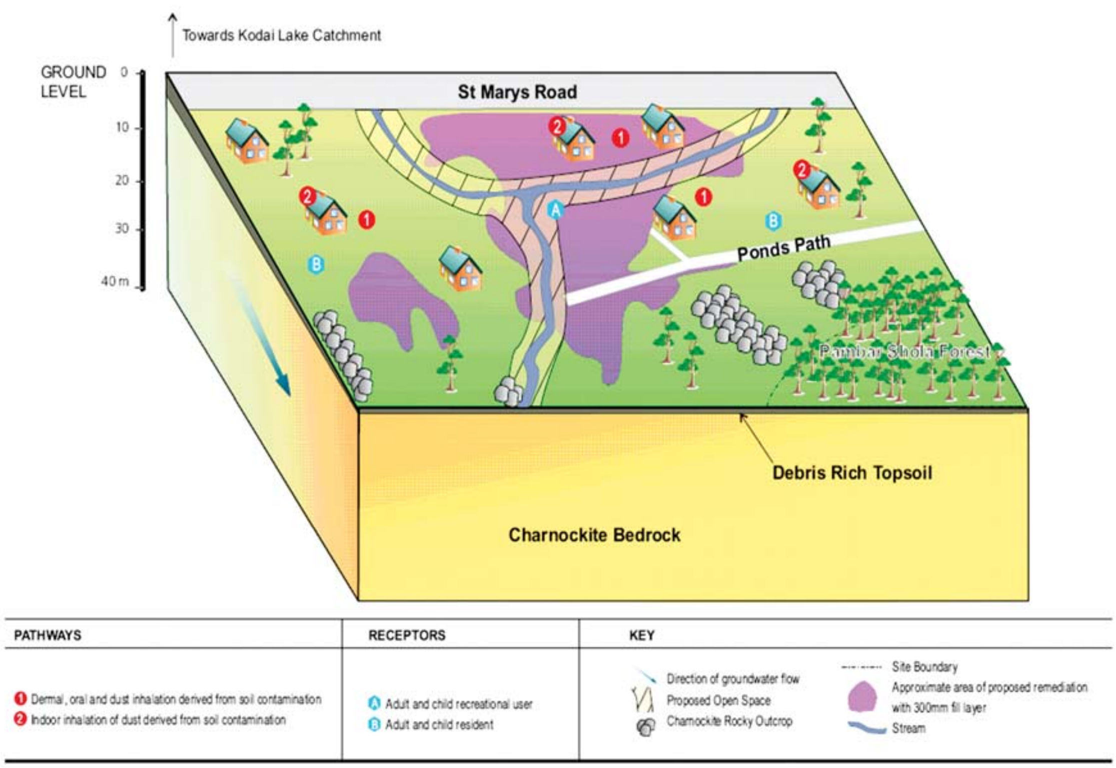
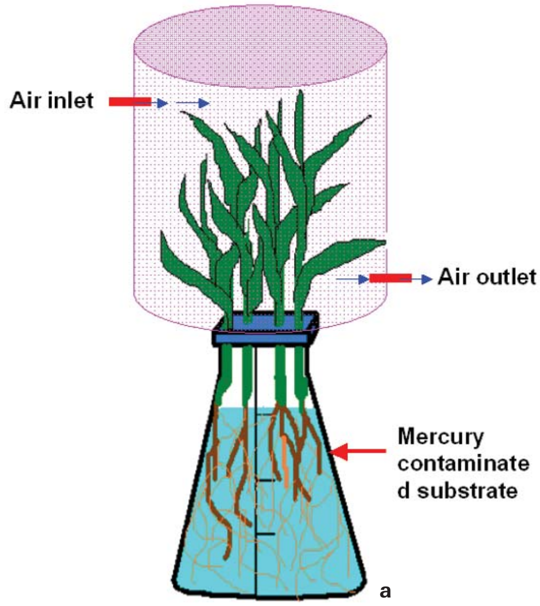


Figure 29. Sectional view of the mercury contaminated site at Kodaikanal. Source: Belinda Thompson, Environmental Resources Management, Australia

b

c



a

Figure 30. a) Mercury phytovolatilization experimental setup using *Salix* stem cuttings
b) *Salix* sp. (fast growing and high biomass producing tree species) is suitable for mercury phytovolatilization
c) Schematic view of mercury phytovolatilization-experimental setup

3.1.3 Chromium

Chromium (Cr) is the chief heavy metal contaminant found in the tannery effluent. Cr used by the leather industry to tan hides is not taken up completely by leather and relatively large amounts escapes into the effluent. Due to chrome leather tanning processes, large quantities of Cr compounds are discharged through liquid, solid, and gaseous wastes into the environment and can have significant adverse biological and ecological effects. Several reports have shown that the values for Cr in tannery effluent are considerably higher than the safe limits prescribed by National and International standards. Cr is a toxic element to higher vascular plants and is detrimental to its growth, development and reproduction. The physiological impact of Cr contamination in soil and water is dependant on the speciation. These factors are responsible for the mobilisation of the metal, subsequent uptake and resultant toxicity in the plant system. The action of Cr is seen at the whole plant level as reduced growth, and at the organ level through leaf symptoms. On a smaller scale, the effects of Cr can be seen as cellular symptoms. Symptoms, both macro cellular and growth effects are side effects of the direct mode of action. The two common oxidation states of Cr present in the environment, viz., Cr(III) and Cr(VI), are drastically different in charge, physicochemical properties as well as chemical and biochemical reactivity. The toxicological impact of Cr(VI) originates from the action of this form itself as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr(VI) to Cr(III) occurring inside the cell. Cr(III) on the other hand if present in significant concentration can cause further adverse effects because of its high capability to coordinate various organic compounds resulting in inhibition of some metallo-enzyme systems.

Mycorrhizal fungi have greatest impact on elements with narrow diffusion zones around plant roots, including heavy metals and phosphorus. An important arbuscular mycorrhizal genus is Glomus, which colonize a variety of host species, including crops and tree species. Mycorrhizal fungi are a direct link between soil and roots, and consequently of great importance in phytoremediation. Little is known of the ability of mycorrhizal fungi to enhance plant tolerance or phytoaccumulation of Cr. Furthermore, information is lacking on the influence of mycorrhizae on the uptake of other essential plant macro- and micronutrients in soils contaminated with Cr. Since ascorbate–glutathione pathway plays a major role in the antioxidant response of plants under abiotic environmental stress and that increased sulphur enhances the components of the pathway, it is possible that supplemented sulphur could enhance the antioxidant response of stressed plants. Chromium is a known competitive inhibitor of iron uptake in plants due to its similar atomic configuration, hence it is possible that supplementation of iron can suppress Cr induced toxicity.

Cleaning up of the Cr contaminated sites is a challenging task. Phytoremediation is an emerging technology that can be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable; at sites with low concentrations of contaminants, where treatment is required over long periods of time. Phytoextraction refers to the use of metal accumulating plants that translocate and concentrate metals from the soil in roots and above ground shoots or leaves. Tree species in association with mycorrhizae have shown promising prospects for phytoremediation of Cr contaminated lands in and around tannery industrial areas. Organic acids have been used to enhance extraction of immobile metals from soils due its ability to complex with metals and increase its availability. Chromiteore processing industries release Cr. Sewage treatment plants from industrial and residential sources discharge substantial amounts of Cr. The leather industry is the major reason for the environmental influx of chromium. Tamil Nadu is a leading finished leather producer in India. Over 250 tanneries had been functioning in the past decade and were actively involved in chrome tanning processes. Presently there are 6000 tanneries out of which a sizable percentage is actively involved in the chrome tanning process.

3.2 Fluoride

Excess fluoride in drinking water causes harmful effects such as dental fluorosis and skeletal fluorosis. The high fluoride levels in drinking water and its impact on human health in many parts of India have increased the importance of defluoridation studies. The fluoride -bearing minerals or fluoride-rich minerals in the rocks and soils are the cause of high fluoride content in the groundwater, which is the main source of drinking water in India.

Adsorption technique using naturally available adsorbents, especially clays which contain oxides of iron, aluminium and silicon are appropriate for removal of fluoride. Nayagarh district of Orissa, Nalgonda and Mahaboobnagar districts of Andhra Pradesh are some of the the hot spots of fluoride contamination in ground water in India. Effects of prolonged use of fluoride contaminated drinking water on human health are shown in Box 5 ; Figure 31).

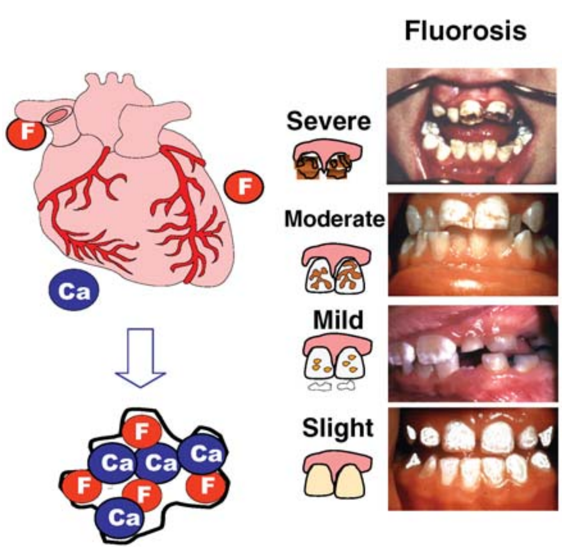


Figure 31. Binding of F to Ca is a serious health concern. Heart, teeth and bones are vulnerable

Box 5 : Fluoride concentration
mg/L Health outcome
<0.5 Dental caries
0.5–1.5 Optimum dental health
1.5–4.0 Dental fluorosis
4.0–10 Dental /skeletal fluorosis
>10 Crippling fluorosis

3.3 Cyanide

The Hutti Gold Mines Company Limited, in Karnataka produces about three tonnes of the yellow metal. The Hutti gold deposits are located in the Hutti-Muski Precambrian greenstone belt and both free milling ores as well as gold-bearing sulphides are present. Cyanide leaching practice has been followed in the Hutti and Chitradurga plants of the Hutti Gold Mines

Highly concentrated cyanide solution is used to extract gold from fine-grounded ore [Cyanide Leaching Gold Recovery (CLGR) process]. The chemistry of the cyanide leaching is as follows:

$2Au_{(s)} + 4\ NaCN_{(aq)} + H_2O + ½\ O_{2(aq)} \rightarrow 2Na[Au(CN)_2]$ $(aq) + 2NaOH_{(aq)}$

Hundreds of tons of CN is consumed in gold mining annually. Water hyacinth has vast potential, however, it is yet to be exploited in our situation (Ebel *et al* 2007). Cyanide forms very stable complexes with gold. CLGR makes it economically worthwhile to extract gold from a very low-grade ore, i.e., containing 0.5–13.7 g gold per 1000 kg rock (Korte *et al*., 2000). Water hyacinth (*Eichhornia crassipes*) is an obnoxious and invasive weed and is omnipresent in wetlands of India. It is useful in treating cyanide containing water (Malik 2007) (Figure 32).

The detoxifying enzyme is ß - cyanoalanine synthase (CAS) and it catalyzes the conversion of free cyanide and cysteine to ß cyanoalanine. The final metabolite is asparagine. The detoxifying enzyme b-cyanoalanine synthase converts cyanide and cysteine to cyanoalanine.

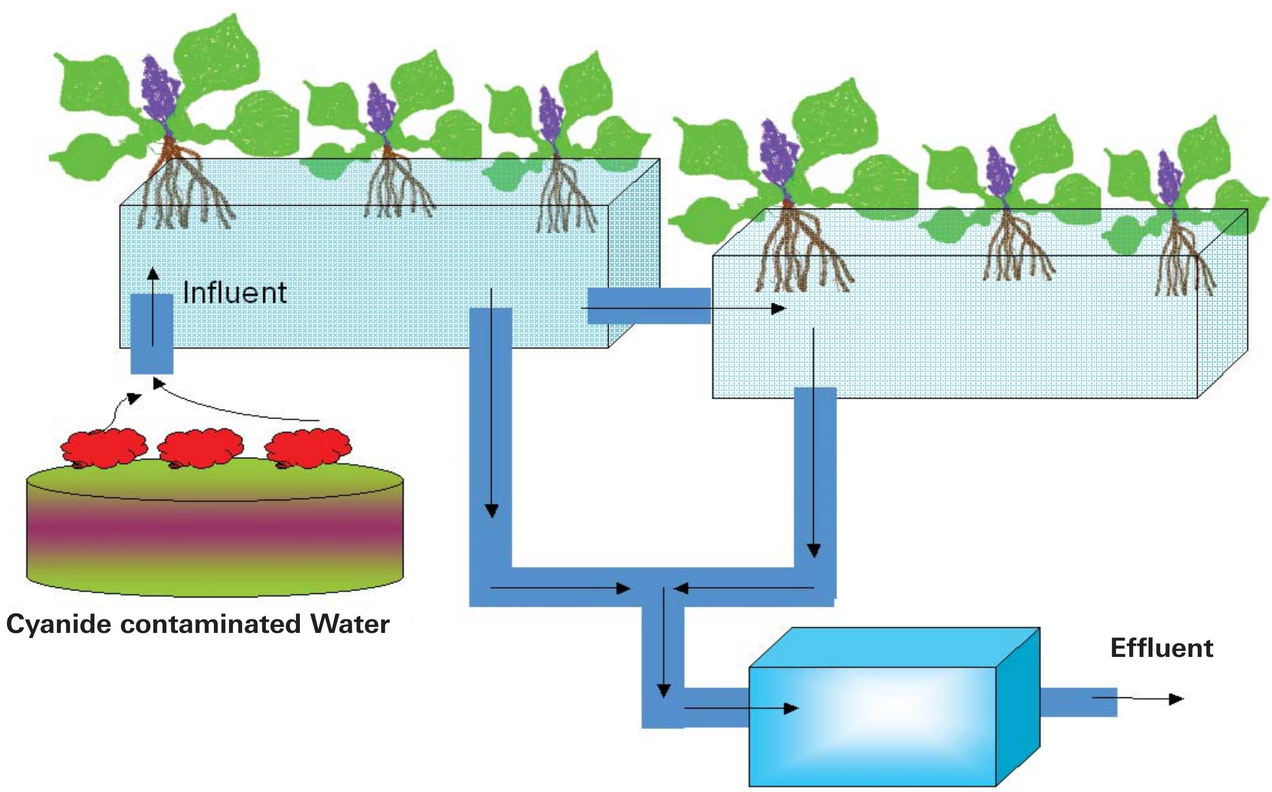


Figure 32. Cyanide cleanup using water hyacinth *(Eichhornia crassipes)*

3.4. Reclamation of abandoned mine sites

India is rich in a wide variety of mineral sources (Figure 33). The environmental implications of abandoned mines are shown in Figure 34 a,b.

The key processes involved in reclamation of abandoned mine sites are i) metal uptake, transport, accumulation and ii) phytostabilization. Other related applications are: erosion control of mine tailings and metals. Reclamation of abandoned mine sites would depend on successful immobilisation of metalliferous substrates.

The restoration of a dense vegetation cover is the most useful to physically stabilise the mine wastes and to reduce metal pollution effects. Different plant species that are well adapted to the local conditions, capable of excluding and accumulating heavy metals without showing toxic symptoms are the ideal species that should be considered for early stages of revegetation

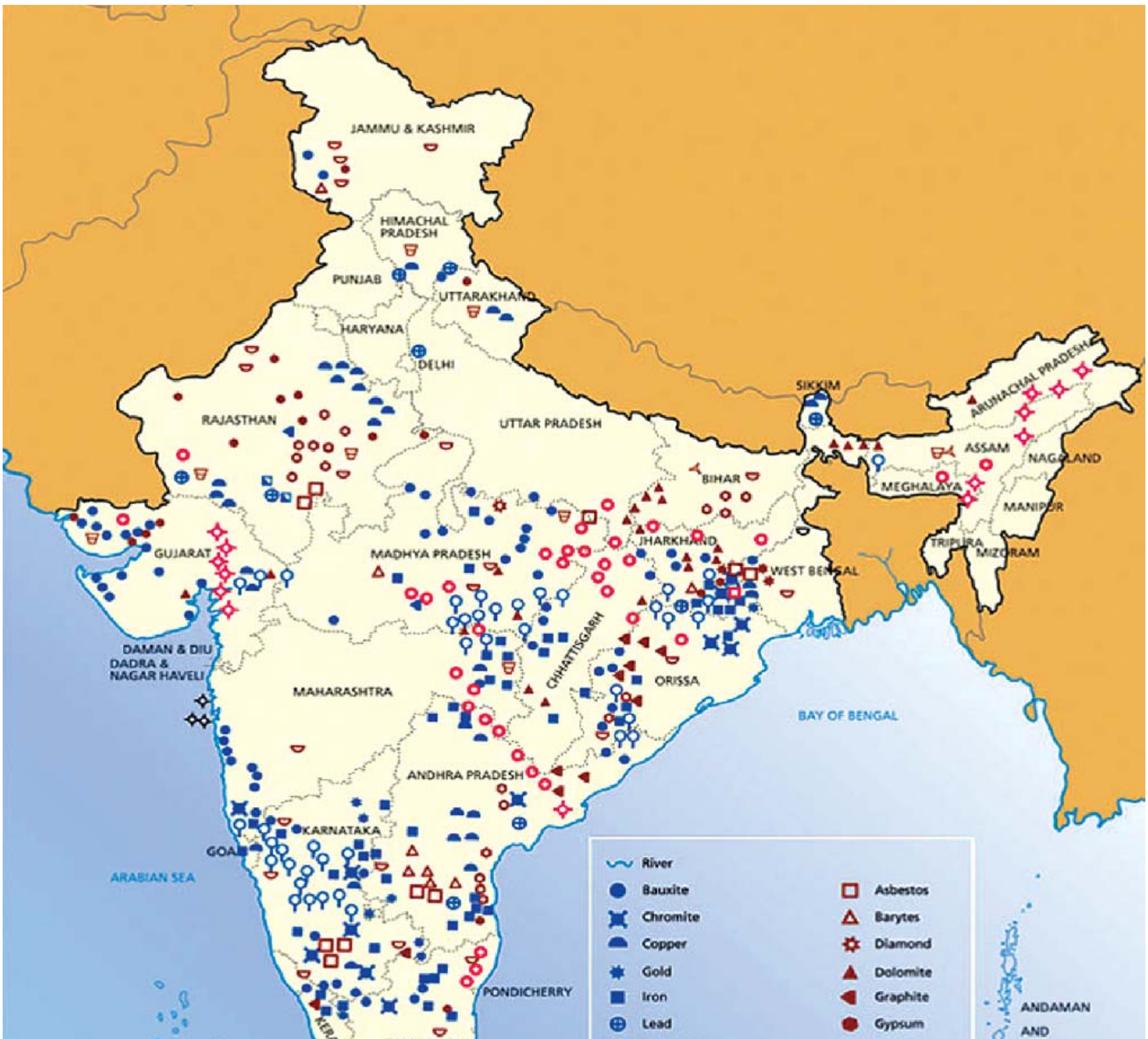


Figure 33. Mineral resources of India.

of the 'green corridor' or establishment of 'green belt'. Several of the grasses, legumes and trees can be a suitable material for this purpose. Bermuda grass (*Cynodon dactylon*), has been suggested for stabilising metalliferous soils.

Restoration of a vegetation cover can fulfil the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings. The constraints related to plant establishment, and amendment of the physical and chemical properties of the toxic metal-mined soils, and the choice of appropriate plant species will depend upon several aspects. Traditional remedies of soil amelioration are very costly and can only affect the upper layers of the soil. Populations of a variety of higher plant species are known to colonise degraded mine soils in which other cultivated plants cannot survive. Thus the plant community tolerant to heavy metals play a major role in remediation of degraded mine soils. Plants tolerant to toxic levels of heavy metals respond by exclusion, indication or accumulation of metals. A number of plant species endemic to metalliferous soils have been found to accumulate metals at extraordinarily high levels (1%) in contrast to normal concentrations in plants. So far, approximately 400 metal hyper-accumulators have been identified. It was not until the early 1980s that it was realised that hyperaccumulators might be used to remediate polluted soils by growing these plants and harvesting them to remove the pollutants. The success of any phytoremediation technique depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amount of biomass using established crop production and management. Tree-grass-legume association was found to be the best combination for restoration of mica, copper, tungsten, marble, dolomite, limestone, and mine spoils of Rajasthan state and elsewhere in India.

According to the UN habitat best practices data base for improving the living environment, NEERI reported Ecological Restoration of Degraded Lands through biotechnological approaches at various mining public industries located in India to improve the environmental and socio-economic status of local population. Bioremediation of mine spoil dumps is being conducted since 1989, which enabled restoration of soil productivity over 247 hectares of mine soil dumps/ (including coal mines) and restoration of silted soil in 2004 at different locations in India. It is a biocompatible technology which involves isolation and inoculation of site-specific specialized nitrogen fixing strains of *Bradyrhizobium* and *Azotobacter* species and nutrient mobilizing vesicular arbuscular mycorrhizal spores of *Glomus* and *Gigaspora* species in combination with industrial waste material available near the vicinity of mine site used as organic amendments to ameliorate the mine spoil and encourage revegetation. Plant species of ecological and economical importance were planted on mine spoil dumps using appropriate blends of organic waste along with site-specific biofertilizers. Plant species suitable for revegetation of mine spoils and commercialization of integrated biotechnological approaches for reclamation of abandoned mines are shown in Box 6 and 7.

Figure 34. Environmental implications of abandoned mines are a) acid mine drainage formation b) red mud and air pollution due to aerosols which poses risk to national heritage. The detrimental effects would be loss of cultivated land, forest or grazing land and the overall loss of natural wealth

Box 6: Plant species suitable for revegetation of mine spoils (Prasad 2007)	
Mine spoil category	**Suitable plant species**
Bauxite mined area of Madhya Pradesh	*Grevillea pteridifolia. Eucalyptus camaldlllenis, Shorea robusta*
Coal mine spoils of Madhya Pradesh	*Eucalyptus hybrid, Eucalyptus camaldulensis, Acacia aurifuliformis, Acacia nilotica, Dalbergia sissoo, Pongamia pinnata*
Lime stone mine spoils of outer Himalayas	*Salix tetrasperma, Leucaena lellcocephala, Bauhinia retusa, Acacia catechu, Ipomea cornea, Eulaliopsis binata, Chrysopogon fulvus, Arllndo donax, Agave americana, Pennisetum purpureum, Erythrina subersosa*
Rock-phosphate mine spoils of Musoorie	*Pennisetum purpureum, Saccharum spontaneum, Vitex negundo, Rumes hastatus. Mimosa himalayana, Buddlea asiatica, Dalbergia sissoo, Acacia catechu, Leucaena leucocephela* and *Salix letrasperma,* etc.
Lignite mine spoils of Tamil Nadu	*Eucalyptus species, Leucaena leucocephala, Acacia* and *Agave*
Mica, copper, tungiston, marble, dool, mite, limestone, and mine spoils of Rajasthan	*Acacia tortilis. Prosopis juliflora, Acacia Senegal, Salvadora oleodes, Tamarix articulata. Zizyphus nummularia, Grewia tenax, Cenchrus setigerus. Cymbopogon, Cynodon dactylon. Sporobollis marginatus D. annlllalum*
Iron ore wastes of Orissa	*Leucaena leucocephala*
Haematite, magnetite, manganese spoil from Karnataka	*Albizia lebeck*

Box 7: Commercialization of Integrated Biotechnological Approach to different Industrial Sectors				
Name of Site	**Year of commercialization**	**Area in Hectares**	**No. of plant species**	**Total no. of plants planted**
Manganese Mines				
Gumgaon, Chikla and Dongribuzurg	1993-95	62	12	1,60,000
Tirodi	1997	10	16	16,000
Iron Mines Kudremukh Iron Ore Company Limited, Mangalore				
Kudremukh	1996	4	10	10,000
Kodli Mines, Goa	1999 - 2003	5	28	8,000
Copper Mine HCL – Hindustan Copper Limited, Malanjkhand				
Malanjkhand	1999 - 2003	5	28	10,000
Zinc Mine HZL – Hindustan Zinc Limited, Udaipur				
Udaipur, HZL	1999 - 2003	5	30	8,000
Fly Ash Dumps Maharashtra State Electricity Board, Khaperkheda				
Khaperkheda	1997	10	16	16,000
Source:NEERI, in UN habitat best practices database for improving the environment, 2006				

The rat-hole mining of coal and its leachate poses a threat to the survival of *Nepenthes khasiana* an endemic to Meghalaya (Figures 35-37). Mine leachates into shallow aquifers, runoff to surface water is of human health concern. Therefore, remediation of these sites is essential for meeting the environmental regulations and also fro the human health point of view.

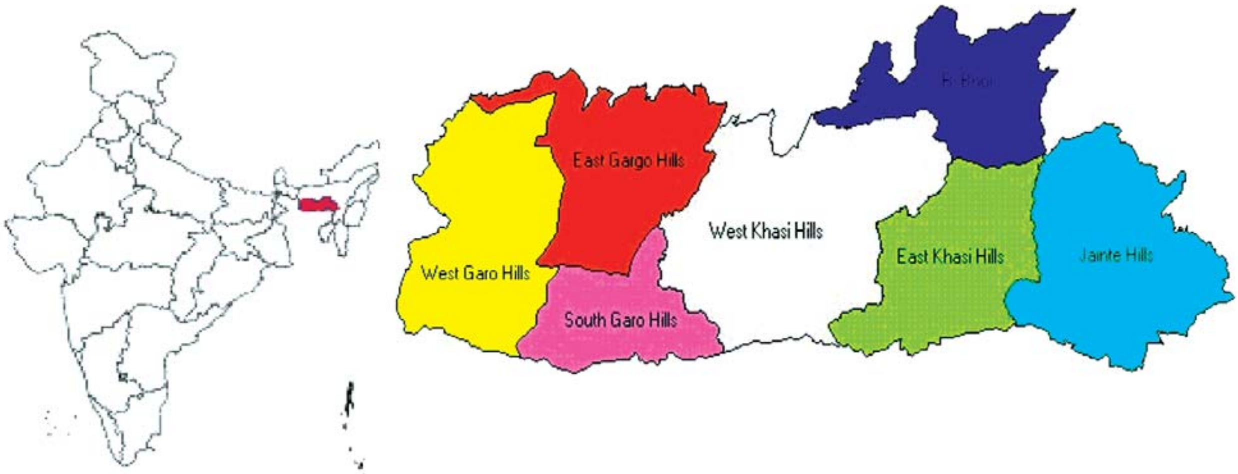


Figure 35. The Khasi hills and Jaintia hills, of the State of Meghalaya, the habitat of *Nepenthes khasiana* (pitcher plant , see inset in bottom figure)

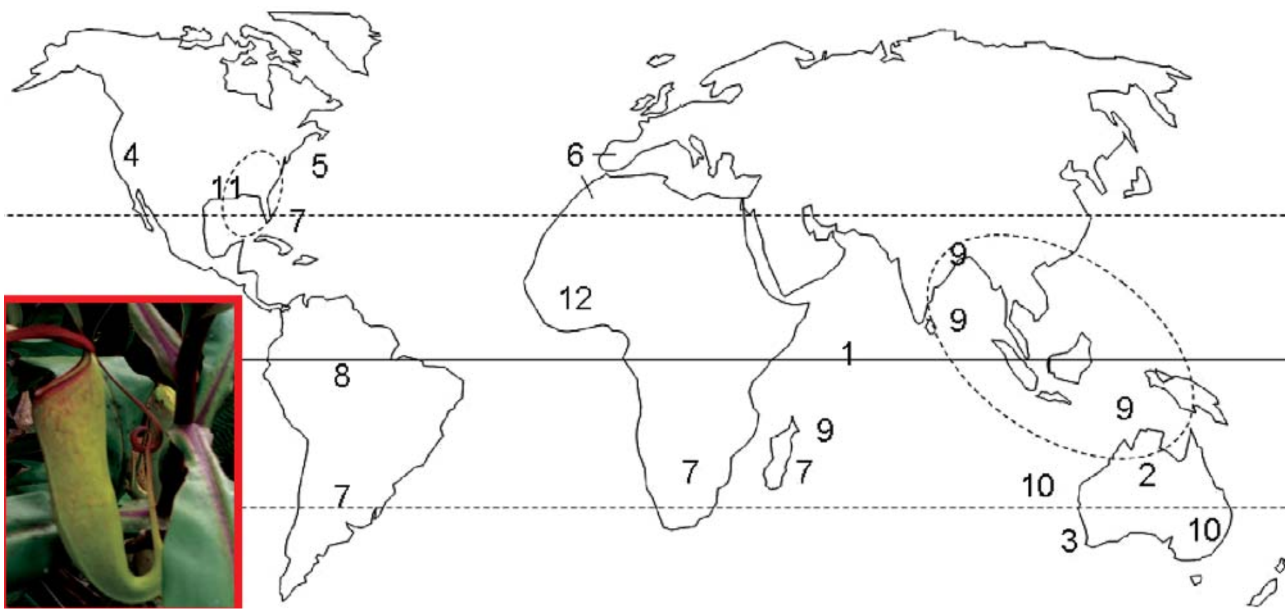


Figure 36. Endemic plants that prey on insects

1 *Anurosperma* – Seychelles, 2 *Byblis* – N & W Australia, 3 *Cephalotus* – SW coast of Australia, 4 *Darlibgtonia* – California & Oregon, USA, 5 *Dionea* – N carolina USA, 6 *Drosophyllum* – Spain, Portugal & Morocco, 7 *Genlisea* Tropical waters of Madagascar, Africa, W Indies and S America, 8 *Heliamphora* – Venezula, 9 *Nepenthes* – NE India, Madagascar, Sri Lanka, N Australia, SE Asia, 10 *Polypompholyx* – West and South Australia, 11 *Sarracenia* SE USA, 12 *Triphyophyllum* – Sierra Leone in Africa

Drosera, Pinguicula and *Utricularia* are wide spread in tropical and temperate countries.
Aldrovanda is widespread in South Africa, SE Asia and Australia. It is rare in Europe.

Figure 37. a. Habitat of *Nepenthes khasiana* (endemic to Meghalaya), b) and c) Rat-hole mining of coal and leaching of acidic water posing threat to its survival. d –f) *N. khasiana* with necrotic spots on leaf and pitcher (toxic symptoms)

3.5 Engineered phyto-covers for hazardous landfills

Mining activities generate a large amount of waste rocks and tailings which are deposited at the surface. The land surface is damaged and the waste rocks and tailings are often very unstable and become sources of pollution. The direct effects are loss of cultivated land, forest or grazing land, and the overall loss of production. The indirect effects include air and water pollution and siltation of rivers. These eventually lead to the loss of biodiversity, amenity and economic wealth. National Aluminium Company Ltd. (NALCO) exploits large deposits of bauxite discovered in the east coast of India. NALCO's Captive Power Plant (CPP) & Smelter Plant are situated near Angul. Captive Power Plant of 720 MW capacity, comprising 6 x 120 MW clusters, has been established to supply power to the Smelter. The vegetative caps (phyto-cover technology) help in containment of hazardous wastes because of the expense and risk associated with treating or removing large volumes of hazardous wastes. Both regulators and the public usually accept phyto-covers as part of remediation. Therefore, phyto-covers enhance *in situ* remediation. Phyto-covers protect the public health and the environment. In this case, the hazardous waste is isolated from receptors and contained in the landfill with the help of phyto-cover. Prior to the establishment of vegetative covers, hazardous wastes were stored in warehouses which has its own disadvantages (Figure 38 a-d).

There are fundamental scientific and technical reasons for placing vegetative covers on hazardous waste landfill sites. The three primary requirements for landfill phyto-covers are to:

a) Minimize infiltration: Water that percolates through the waste may dissolve contaminants and form leachate, which can pollute both soil and groundwater as it is washed away from the site.

Figure 38. a) and b) Hazardous mine waste storehouse at NALCO Angul site. c and d) Established phytocover system

b) Isolate wastes: A phyto-cover over the hazardous wastes prevents direct contact with potential receptors at the surface and prevents movement by wind or water.

c) Control landfill gas: Landfills may produce explosive or toxic gases, which, if allowed to accumulate or to escape without control, can be hazardous. Landfills have been covered by barriers for years, usually built with little regard for the monetary and environmental costs associated with constructing and maintaining them. A typical landfill cover design consists of a sequence of layered materials to control landfill gas infiltration and promote internal lateral drainage. The uppermost layer of a landfill cover consists of a vegetative soil layer to prevent erosion, promote runoff, and insulate deeper layers from temperature changes. The landfill cover is not a single element but a series of components functioning together.

Numerousl and fill investigation studies have suggested that the stabilization of waste proceeds in sequential and distinct phases. The rate and characteristics of leachate produced and biogas generated from a landfill vary from one phase to another and reflect the processes taking place inside the landfill. Typical single barrier phyto-cover system is depicted in Figure 39 consisting of vegetation, topsoil, common borrow material, geocomposite (geotextile) and low density polyethylene barrier. The typical components of an engineered phyto-cover system consist of vegetative cover soils (existing and supplementary), soil amendments, non-soil amendments, understory grasses and plants and trees. An irrigation system is an optional component to ensure sufficient water for tree growth in case of drought.

The existing cover soil at many sites is sufficient to support an adequate root system for healthy tree growth. This is evidenced by the vigorous growth of trees often seen at abandoned landfills. Typically, natural stands of vegetation are not effective at controlling percolation. Therefore, sufficient soil and non-soil amendments (viz., compost, chipped wood, digested sewage biosolids, lime-stabilized sludge, manure, and other organic biomass) may need to be added to meet the requirements for tree growth, and to achieve minimum land surface slopes to promote surface drainage and to provide sufficient soil water holding capacity for storage to function as an adequate "sponge." The amount of soil and nonsoil amendments would depend upon site-specific information.

The trees normally selected for construction of a phyto-cover are *Dalbergia sisso, Eucalyptus* sp., *Cassia siamea, Acacia auriculiformis, Leucaena leucocephala,* and *Tectona grandis*. In addition to trees, grasses such as *Vetiveria zizanioides* and industrial crops like *Jatropha curcas*, *Ricinus Phyto*-cover systems have been designed to minimize percolation to the waste by incorporating a landfill soil cover with sufficient evapotranspirative and water holding capacity to store precipitation temporarily in the nongrowing season for subsequent evapotranspiration by vegetation in the growing season. The two key design elements in engineering a phyto-cover system are. 1) determining the thickness and material composition of the soil cover system required to provide sufficient water storage capacity; and 2) incorporating a supportive phyto-cover system to access water stored in the soil cover system for evapotranspiration to the atmosphere. Moisture flow and moisture content in a landfill are extremely important to the dynamic processes of decomposition and potential leachate generation.

The primary elements of a water mass balance include precipitation, surface runoff (R/O), potential evapotranspiration (PET), infiltration (I), soil moisture storage (ST), actual evapotranspiration (AET), and flux (or percolation) of water through the system. The water shedding efficiency of a cap is then derived by calculating the percentage of flux relative to total precipitation. The phyto-cover system design concept involves maximizing efficiency by optimizing ET and runoff (Figure 40). The engineered phyto-cover functions as a sponge and pump system, with the root zone acting as the sponge, and trees acting as the solar-driven pumps. In contrast to restrictive permeability barrier design, the engineered phyto-cover design involves the storage of free water in soil pores and the extraction of stored water by the tree roots. Surface flow constructed wetlands are being designed for the treatment of municipal waste waters in developed nations. Use of constructed wetlands is spreading rapidly in developed nations. However, in tropical nations due to water scarcity and high surface evapotranspitration, the constructed wetlands for treatment of waste waters is not gaining significance.

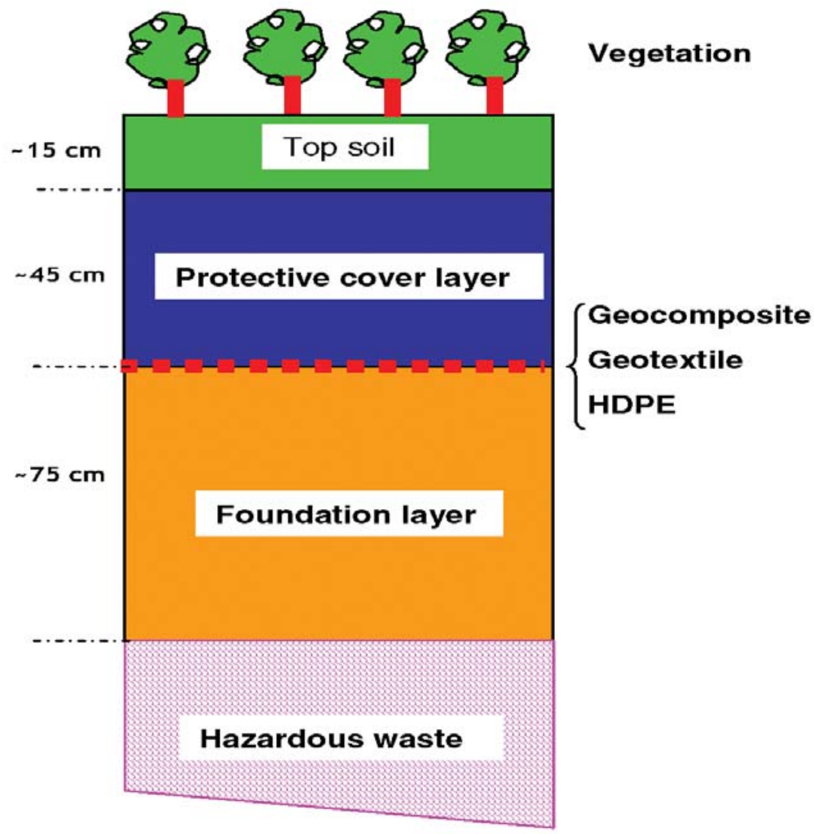


Figure 39. Sectional view of single barrier phyto-cover systems

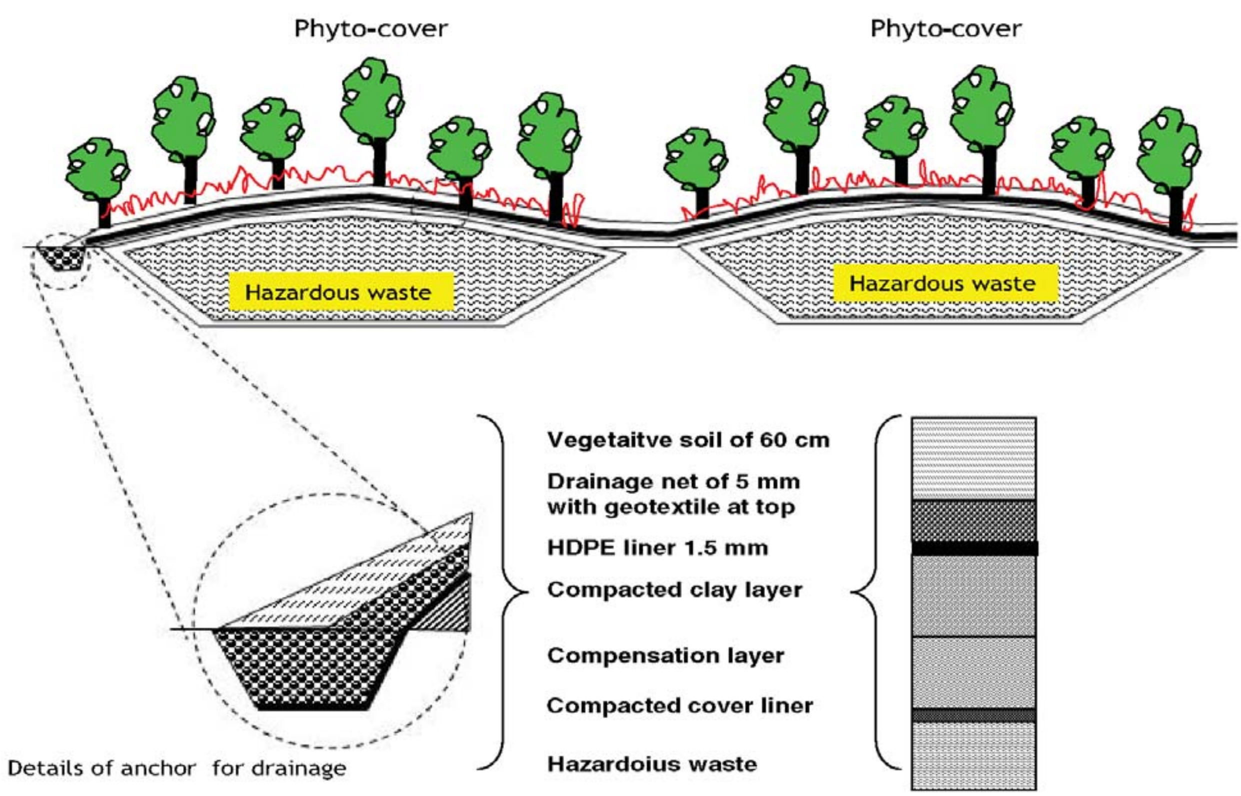


Figure 40. Sectional views of phytocover and anchoring drainage established at NALCO, Angul to contain the hazardous waste (contains fluoride cyanide) from spent spot line of aluminium smelter

3.6. Fly ash disposal sites

Nearly 75% of India's power generation capacity is thermal, of which coal based generation is ~ 90% Most of the thermal power plants use bituminous or sub-bituminous coal and produce large volumes of fly ash. The high ash content (30-50%) of Indian coals contributes to these large volumes of fly ash (Figure 41-43). The 'Mission Mode' experiment of fly ash management has brought into focus that fly ash is an important resource. At present, nearly 90 million tonnes of fly ash is being generated annually in India and nearly 65,000 acres of land is presently occupied by ash ponds. It is a siliceous or aluminous material with pozzolanic properties. It is refractory and alkaline in nature, having fineness in the range of 3000-6000 sq.cm/gm. It is desirable to revegetate these sites for aesthetic purposes, to stabilize the surface ash against wind and water erosion and to reduce the quantity of water leaching through the deposit.

Limitations to plant establishment and growth in fly ash can include a high pH (and consequent deficiencies of all essential elements), high soluble salts, toxic levels of elements such as boron (B)., pozzalanic properties of ash resulting in cemented/ compacted layers and lack of microbial activity. An integrated organic/ biotechnological approach to revegetation seems appropriate and should be investigated further. This would include incorporation of organic matter into the surface layer of ash, mycorrhizal inoculation of establishing vegetation and use of inoculated legumes to add Nitrogen. Leaching losses from ash disposal sites are likely to be site-specific but a sparse number of studies have revealed enriched concentrations of toxic heavy metals in surrounding groundwater. This aspect deserves further study particularly in the longer-term. In addition, during weathering of the ash and deposition of organic matter during plant growth, a soil will form with properties vastly different to that of the parent ash. In turn, this will influence the effect that the disposal site has on the surrounding environment.

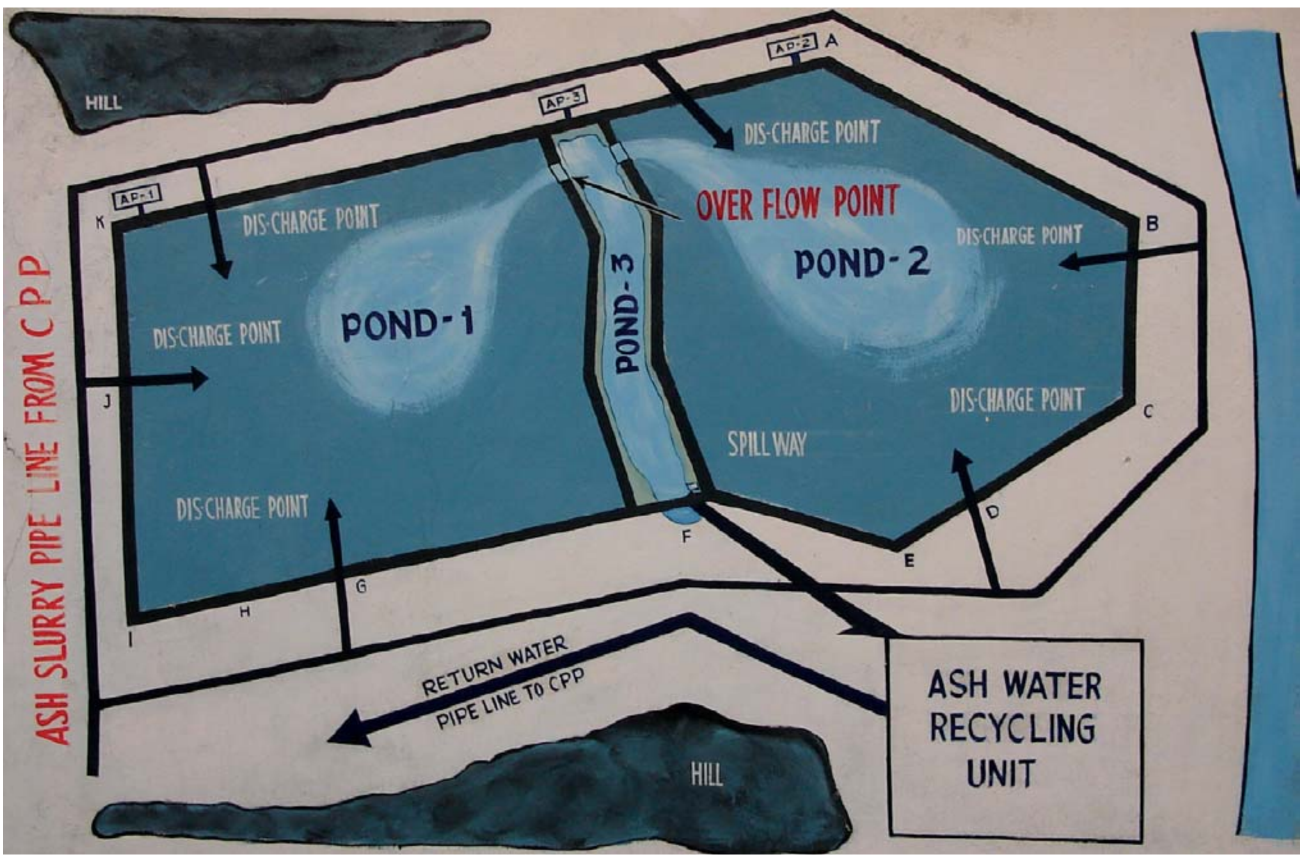


Figure 41. Schematic view of planned constructed wetland to treat ash slurry from NALCO's Angul 720 MW captive power plant

Figure 42. Granite lined canal is discharging ash slurry to wetland

Figure 43. Constructed wetland for treatment of fly ash slurry

3.7. Biological permeable barriers

Biosorption process removes heavy metals which can be quite toxic even at low concentrations. Biosorption is particularly suited as a polishing step whereby wastewater with a low to medium initial metal concentration from a few to about 100 ppm can be deicontaminated. It offers high effluent quality and avoids the generation of toxic sludge. Figure 44 show the cross section, 3-dimensional views of utilizing treatment walls and permeable reactive barriers for treatment of contaminated ground water. The biosorption process can be applied *in situ* without the expense of pumping out the contaminated groundwater or excavating the soil. This technique provides low-cost, easy operating, and safe treatment of contaminants in groundwater. It is particularly useful for treatment of high volume low concentrations of waste water. The immobilized microbial stratum may be placed in an engineered trench across the flow path of a contaminated plume to create a Biological Permeable Barrier (BPB) (Figure 45). Contaminated groundwater enters the BPB to which electron donor and nutrients may be supplied through the groundwater gradient, while the remediated groundwater exits the BPB. Biobarriers serve as an alternative technology for controlling the migration of contaminants from hazardous waste sites. The biobarrier can be applied in the field by injecting starved bacteria and then nutrients into a series of injection wells. The pore space is sealed by bacterial growth and external polysaccharides production and then biobarrier is formed in soil. Biobarrier has the applicability as an alternative liner material in landfill. It is able to immobilize heavy metals *in situ* protecting the environment from the hazardous leachate (Prasad et al 2006).

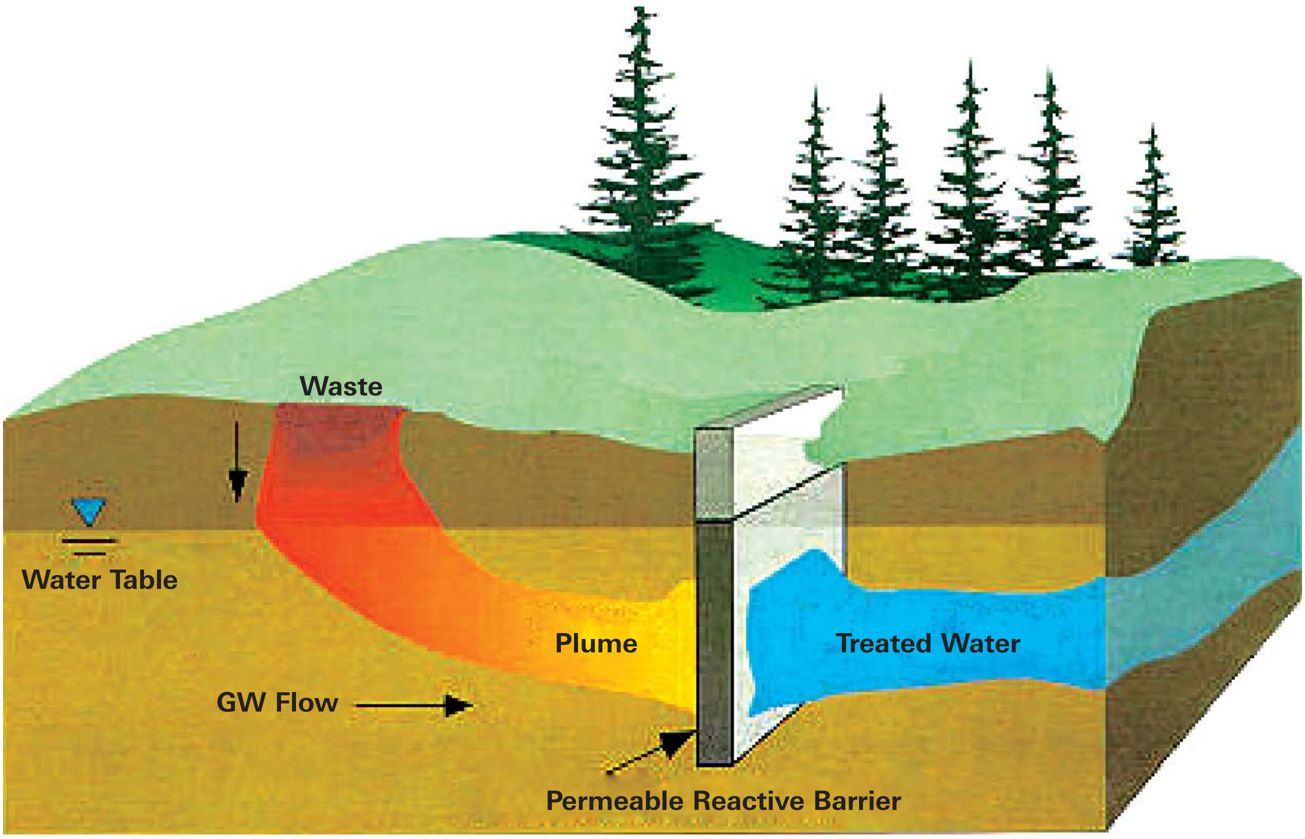


Figure 44. Bioremediation in contaminated groundwater using treatment walls (cross section) (Source: US-EPA)

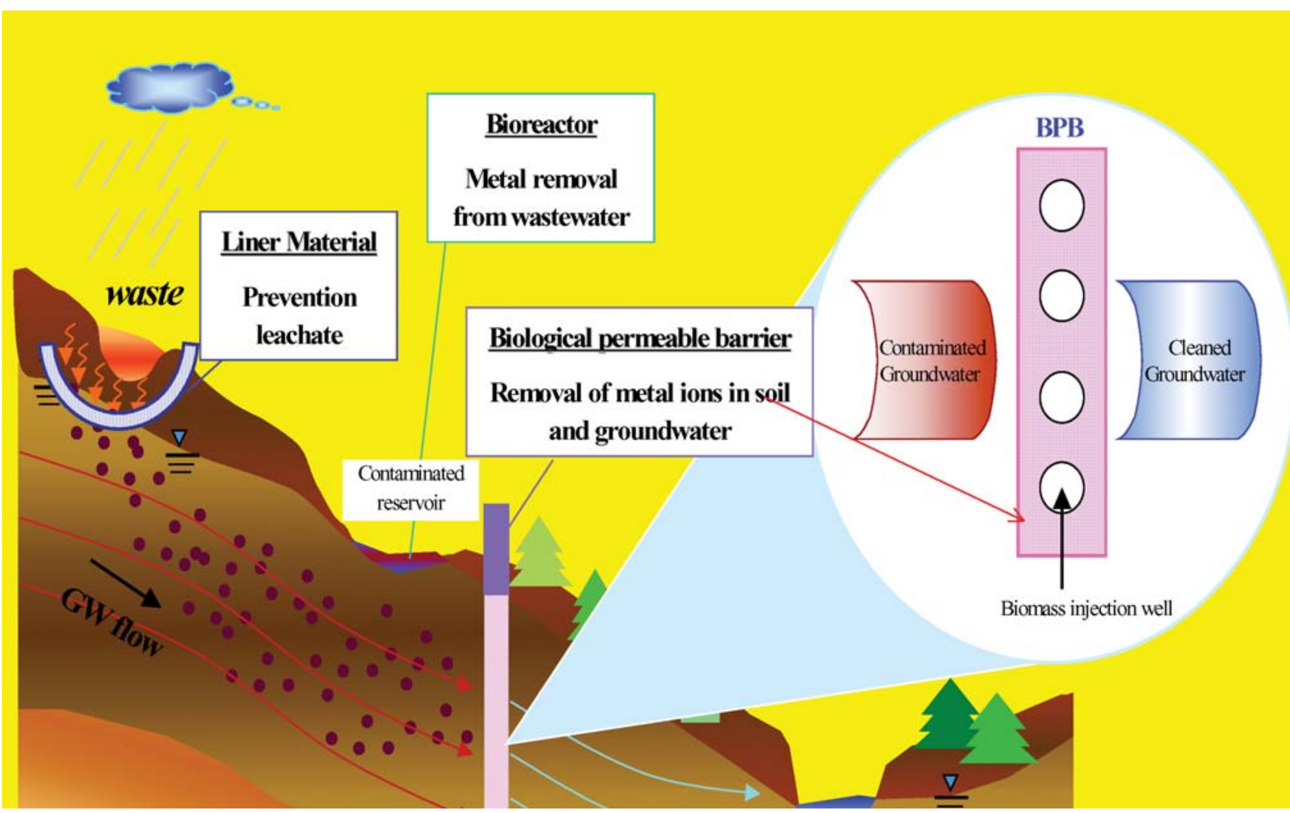


Figure 45. Bioremediation of groundwater using bioreactor approach (see Prasad *et al* 2006)

Chapter 4
Organics

Uptake of organic pollutants by plants through microbes is the first crucial step in biodegradation of organics. In case of constant plant and environmental features, the lipophilicity of the organic compound is for root entry and translocation. Organic contaminants with a log Kow < 1 are considered to be very water-soluble, and plant roots do not generally accumulate them at a rate surpassing passive influx into the transpiration stream. Contaminants with a log Kow > 3.5 show high sorption to the roots but slow or no translocation to the stems and leaves. However, plants readily take up organic contaminants with a log Kow between 0.5 and 3.5, as well as weak electrolytes (weak acids and bases or amphoteres as herbicides) (Box 8). Plant endophytes relationships and the microbial communities play a key role in degrading the hazardous contaminants in rhizosphere to varying extents (Figures 46).

Box 8: log Kow (octanol–water partition coefficient) values of some frequently found organic contaminants.

2-Butanone	0.3	3-Chlorobenzoic acid	2.7
4-Acetylpyridine	0.5	Toluene	2.7
Aniline	0.9	1-Naphthol	2.7
Acetanilide	1.0	2,3-Dichloro aniline	2.8
Benzyl alcohol	1.1	Chlorobenzene	2.8
4-Methoxyphenol	1.3	Allyl phenyl ether	2.9
Phenoxyacetic acid	1.4	Bromobenzene	3.0
Phenol	1.5	Ethyl benzene	3.2
2,4-Dinitrophenol	1.5	Benzophenone	3.2
Benzonitrile	1.6	4-Phenyl phenol	3.2
Phenylacetonitrile	1.6	Thymol	3.3
4-Methylbenzyl alcohol	1.6	1,4-Dichlorobenzene	3.4
Acetophenone	1.7	Diphenylamine	3.4
2-Nitrophenol	1.8	Naphthalene	3.6
3-Nitrobenzoic acid	1.8	Phenyl benzoate	3.6
4-Chloraniline	1.8	Isopropylbenzene	3.7
Nitrobenzene	1.9	2,4,6-Trichlorophenol	3.7
Cinnamic alcohol	1.9	Biphenyl	4.0
Benzoic acid	1.9	Benzyl benzoate	4.0
p-Cresol	1.9	2,4-Dinitro-6-sec-butylphenol	4.1
cis-Cinnamic acid	2.1	1,2, 4-Trichlorobenzene	4.2
trans-Cinnamic acid	2.1	Dodecanoic acid	4.2
Anisole	2.1	Diphenyl ether	4.2
Methyl benzoate	2.1	Phenanthrene	4.5
Benzene	2.1	n-Butylbenzene	4.6
3-Methylbenzoic acid	2.4	Fluoranthene	4.7
4-Chlorophenol	2.4	Dibenzyl	4.8
Trichloroethene	2.4	2,6-Diphenylpyridine	4.9
Atrazine	2.6	Triphenylamine	5.7
Ethyl benzoate	2.6	DDT	6.2
2, 6-Dichlorobenzonitrile	2.6		

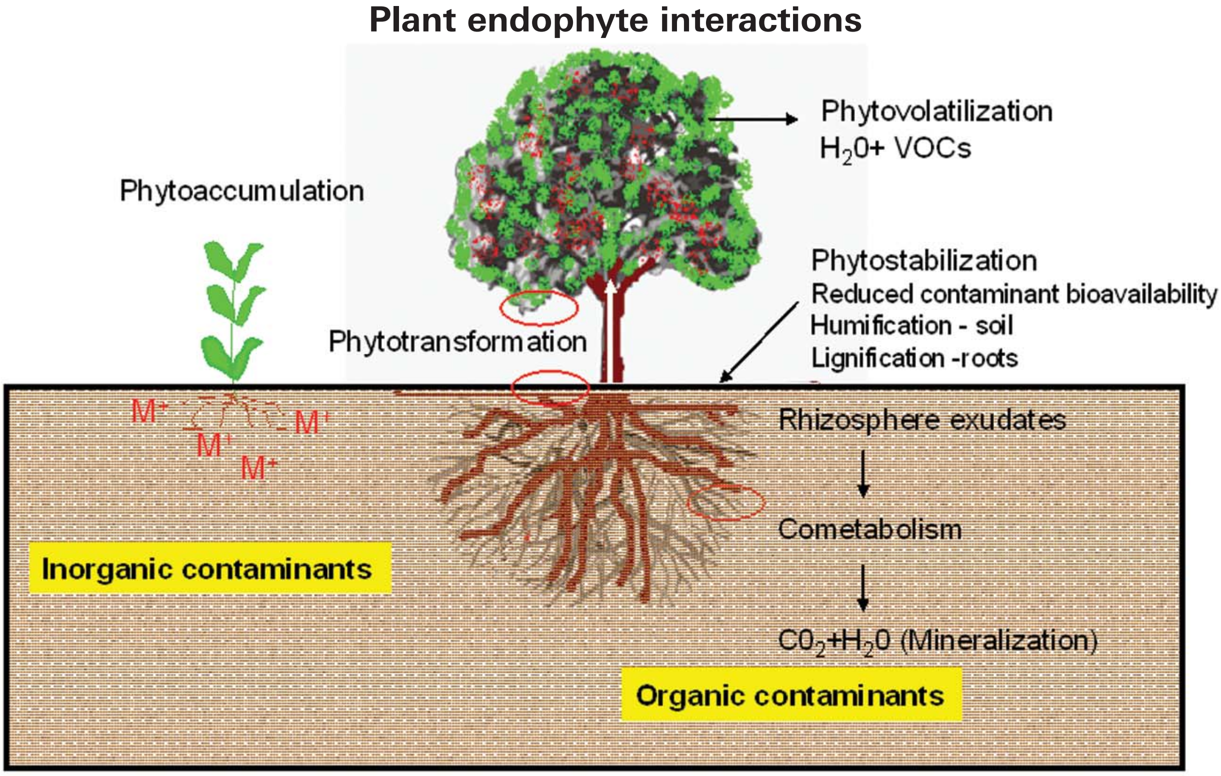


Figure 46. Plant-rhizosphere interactions including plant-endophytes relationships in environmental decontamination

Phenols, anilines and polyaromatic hydrocarbons (PAHs) have common patterns of distribution and are toxic to human health. (Figures 47 and 48). Mycorrhizosphere-bacteria and fungi may play a crucial role in establishing plants in degraded ecosystems. Within the rhizosphere, microbial degradative activities prevail in order to extract energy and carbon skeletons from the pollutants for microbial cell growth. There has been little systematic analysis of the changing dynamics of pollutant degradation within the rhizosphere; however, the importance of plants in supplying oxygen and nutrients to the rhizosphere via fine roots, and of the beneficial effect of micro-organisms on plant root growth are crucial.

In those situations where uptake of contaminant does occur (i.e. only limited microbial activity in the rhizosphere) there is good evidence that the pollutant may be metabolised. However, plant uptake is frequently associated with the inhibition of plant growth and an increasing tendency to oxidant stress. Pollutant tolerance seems to correlate with the ability to deposit large quantities of pollutant metabolites in the 'bound' residue fraction of plant cell walls compared to the vacuole. In this regard, particular attention is paid to the activities of peroxidases, laccases, cytochromes P450, glucosyltransferases and ATP-binding casette (ABC) transporters. However, despite the seemingly large diversity of these proteins, direct proof of their participation in the metabolism of industrial aromatic pollutants is surprisingly scarce and little is known about their control in the overall metabolic scheme. Little is known about the bioavailability of bound metabolites; however, there may be a need to prevent their movement into food chains and food web. In this regard, composting techniques based on the degradative capacity of white-rot fungi merits considerable attention and investigation.

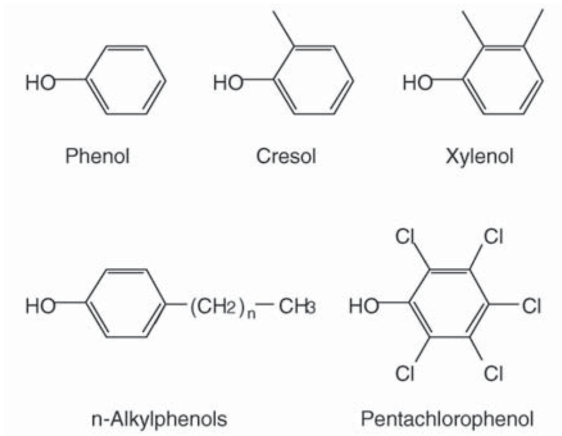


Figure 47. Structure of selected phenols

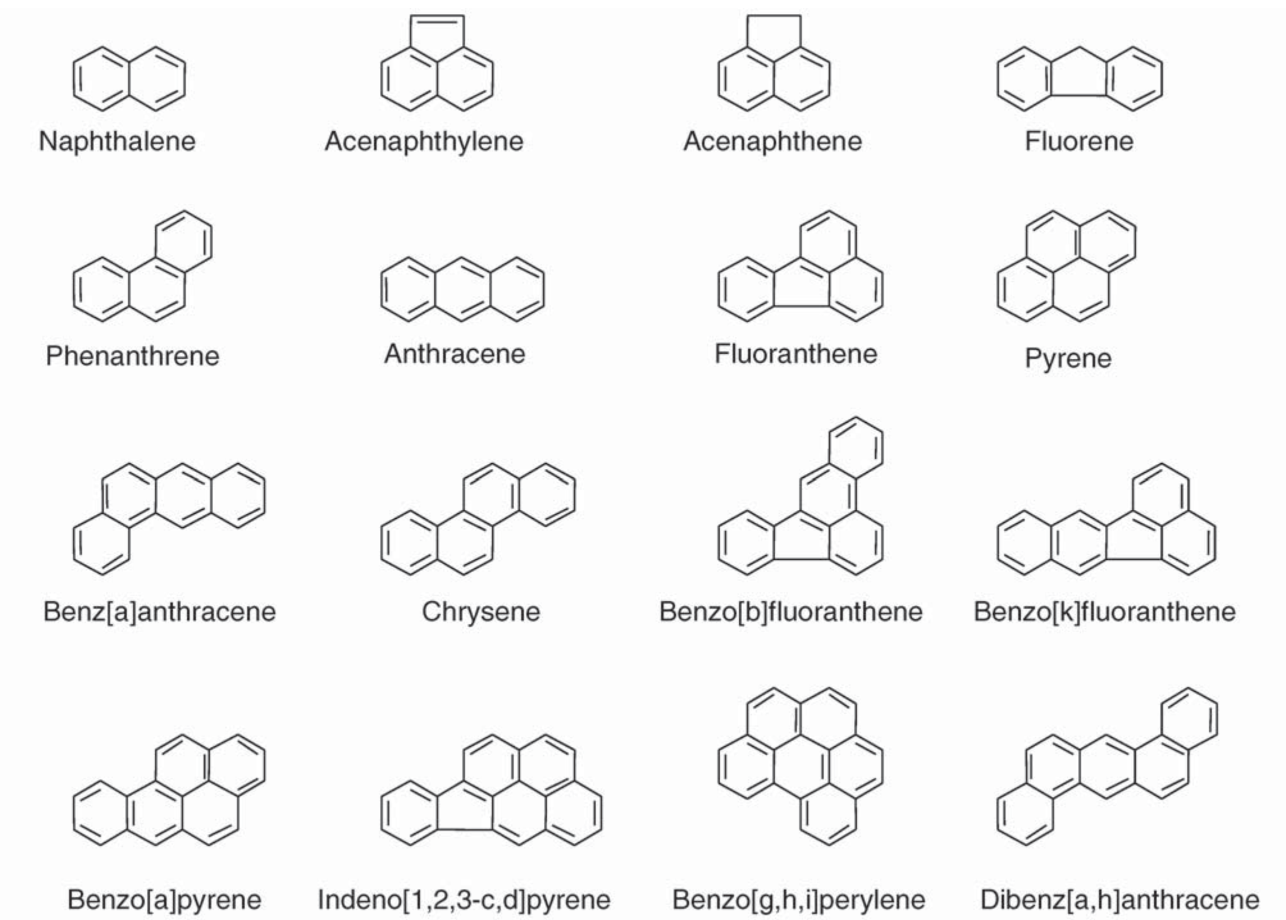


Figure 48. Molecular structure of the 16 PAHs considered as priority pollutants by the American Environmental Protection Agency (EPA).

4.1 Petroleum hydrocarbons

Micro-organisms can adapt and grow at sub-zero temperatures (-20 °F), as well as extreme heat (>200 °F), desert conditions and in water, with excessive oxygen and in anaerobic conditions in the presence of hazardous compounds. Because of the adaptability of microbes, these 'friends of the environment' can be used to degrade petroleum hydrocarbons (Figures 49 and 50)

The Energy Resources Institute (TERI), New Delhi has developed a method of using micro-organisms to clean up oil-contaminated sites. Usually bacteria and fungi, and plant-endophytic associations have been utilized to reduce/eliminate toxic pollutants. These micro-organisms either eat up the contaminants (mostly organic compounds), or assimilate them thus cleaning up the oil contaminated land or waters. TERI prepared consortia of bacteria isolated from nature that eat up the harmful compounds in oil spill sites and oily sludge. TERI named these consortia that it developed for bioremediation of oil-contaminated soils as "Oil zapper". Bioremediation is the most eco-friendly and economically viable amongst all the available methods of sludge management. TERI has reported success of this technology for biodegradation of oil sludge at refineries in India and abroad.

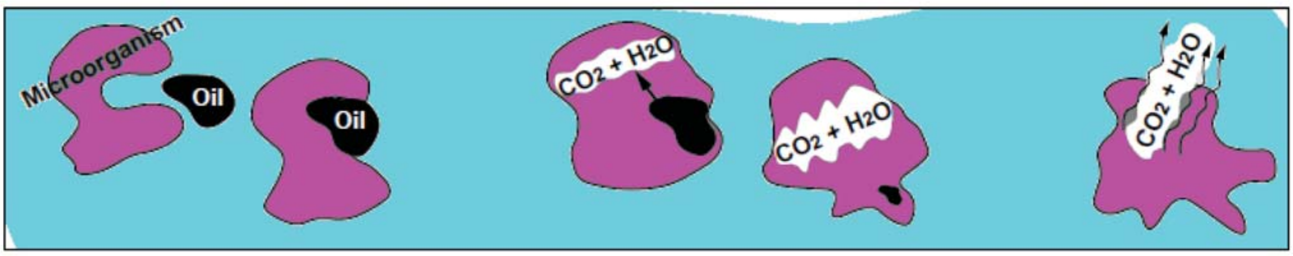


Figure 49. Biodegradation of hydrocarbons. a) Micro organisms eat oil or other organic contaminant, b) Micro organisms digest oil and convert it to carbon dioxide and water c) Micro organisms give off carbon dioxide and water

Figure 50. Different stages of bioremediation of oil sludge. Source: Dr. McIntyre T., Environment Canada

4.2 Drill cuttings and fluids of fossil fuel exploration

Several major oil and gas companies such as Oil and Natural Gas Corporation, Oil India Ltd., Canoro Resource Ltd., Geoenpro Petroleum Ltd., Jubilant Energy, Geopetrol International Inc. and Premier Oil etc. are involved in exploration activities in different parts of India. The Ministry of Environment & Forests, Government of India (MoEF) has accorded environmental clearance (EC) for drilling of over 400 exploratory wells to the ONGC and about 100 exploratory wells to the Oil India Limited in north eastern region of India. Also EC has been accorded to several private companies for carrying out exploratory activities in the north eastern states of Assam, Arunachal Pradesh, Nagaland and Tripura. Since the implementation of Environment Impact Assessment Notification in 2006, handling of drilling cuttings and fluids of fossil fuel exploration has been a subject of environmental concern. The major EC stipulation with regard to management of cuttings and fluids include the use of only Water based mud (WBM). WBM is considered comparatively less hazardous than Oil based mud (OBM) and Synthetic-based mud (SBM). OBM are very effective but highly polluting, and environmental regulations insist on their restricted use in several countries. In order to reduce the mud toxicity, water-based mud systems using biopolymers are being developed. It is generally accepted that the biopolymers exhibit high permeability for complex geometries such as horizontal wells.

Drill cuttings originating from on-shore and separated from WBM should be properly washed and unusable drilling fluids are disposed off in a well designed pit lined with impervious liner located off or on-site. The disposal pit should be provided additionally with leachate collection system. No leachate collection system is provided by the major companies in this region as stipulated. Generally, the exploratory well depth in north eastern India ranges from 2500-3500m. The drill cuttings generated from one exploratory well range from 230-550 m^3. The approximate composition of drilling fluid constituents including the approximate quantities required for drilling an exploratory well are provided in Box 9.

During drilling operations, large amount of drilling mud is lost into the geological formation. In this case, normal mud circulation is no longer possible and the fluid level of the borehole drop drastically creating a dangerous situation. Variety of mixtures are used in different situations, many of the recipes are kept secret by particular companies or individuals. Several chemicals used for exploratory activities are known by trade names only and sometimes the composition of these chemicals is a well guarded secret. Thus,

Box 9: Approximate composition of drill cuttings and fluids

Name of chemical	Purpose	Quantity (Approx.)
Barite	Weighting additive	> 500 tons
Bentonite	Viscosifier	> 25 tons
Caustic soda KOH	pH control	> 8.0 tons
Pot. Sulphate K2SO4	Hole stabilization	> 175.0 tons
Sod bi carbonate	pH control	> 2.0 tons
Calcium carbonate	LCM	> 30.0 tons
Citric acid	pH control	> 1.0 tons
Biocides	Bacterial control	> 300 gallons
Soda ash	Calcium control	> 1.5 tons
Kwikseal	LCM (Lost circulation material)	16.0 tons
Nut plug	LCM	> 7.0 tons
Poly sal/PAC	Filtrate Control	> 40.0 tons
Mica/starch	Filtrate Control	> 4.0 tons
Douvis	Rheology control	> 4.0 tons
EO Lube	Lubricant	>1000.0 gallons
Glycol	Cloud point	> 2700.0 gallons

the major wastes generated during exploratory activities include Drill cuttings and fluids, sludge from wastewater treatment plant and wastewater. Therefore, the WBM from drilling wastes may contain free oil, dissolved aromatic hydrocarbons, heavy metals (chromium, copper, nickel, lead, zinc, barium, mercury, cadmium etc.), radionuclides (minerals such as barite and bentonite and some drilling chemicals may contain minute amount of radium), biocides and other additives(Figure 51 a-d).

Figure 51. a) Drill cutting fluid due to exploration of fossil fuels. b) Stored in valley with polythese sheet lining. c) Leachate through hill crevices d) Concrete leachate collection and treatment system facility at Masimpur, Cachar, Assam. (For details see Prasad and Katiyar, 2010)

Some additives used as defoamers, descalers, thinners, lubricants, stabilizers, surfactants and corrosion inhibitors are reported to have effects on aquatic organisms ranging from minor physiological changes to reduced fertility, lower feeding rates and higher mortality depending on the concentrations.

Drilling for fossil fuel exploration generate a large amount of waste material as stated above which gets eroded and re-transported at the surface. This will cause soil degradation and the waste materials thus generated are often very unstable and become sources of pollution. The direct effects are loss of cultivated land, forest or grazing land, and the overall loss of production (Wong, 2003). The indirect effects will include oil and water pollution and siltation of rivers and ravines. These eventually lead to the loss of biological diversity and economic wealth (Bradshaw, 1993). Bioremediation of such sites can fulfil the objectives of stabilization, pollution control, visual improvement and removal of threat. As of today there is no single management option for drill cuttings. Therefore, integrated remediation technologies are being followed for an effective management of drill cuttings and fluids of fossil fuel exploration (Figure 52). Tropical pasture grass for e.g. *Brachiaria brizantha* is reported to enhance the rhizosphere microflora viz., bacteria, fungi and degraders of alkanes, aromatics, cycloalkanes and crude oil in petroleum hydrocarbon contaminated soil (sludge amended soil). Oil sludge degradation under the influence of *B. brizantha* is due to microbial activity. Other factors like oxygen availability, plant enzymes and synergistic degradation by microbial consortia are also known to play a key role. Fungi play a significant role in degradation of oil sludge, since they tolerate lower pH than bacteria. Native species found in the region are the best candidates to cover a range of physiology and root morphology. Studies on the abandoned disposal sites in N. E. region to assess the actual hazardous potential of these sites are also required to be undertaken as thousands of such abandoned sites exist in this region (Prasad and Katiyar 2010).

4.3 Pesticides

Research in examining the fate and degradation of pesticides in agricultural soils started over 50 years ago. In view of the wide range of catabolic reactions mediated by bacterial enzymes. the capacity of bacteria and fungi to degrade xenobiotics is impressive. (Figures 53 and 54). Options for decontamination of pesticides are: a) Chemical treatment: very expensive b) Incineration: very expensive, c) Landfills: not a permanent solution and d) Bioremediation: is a low cost feasible solution.

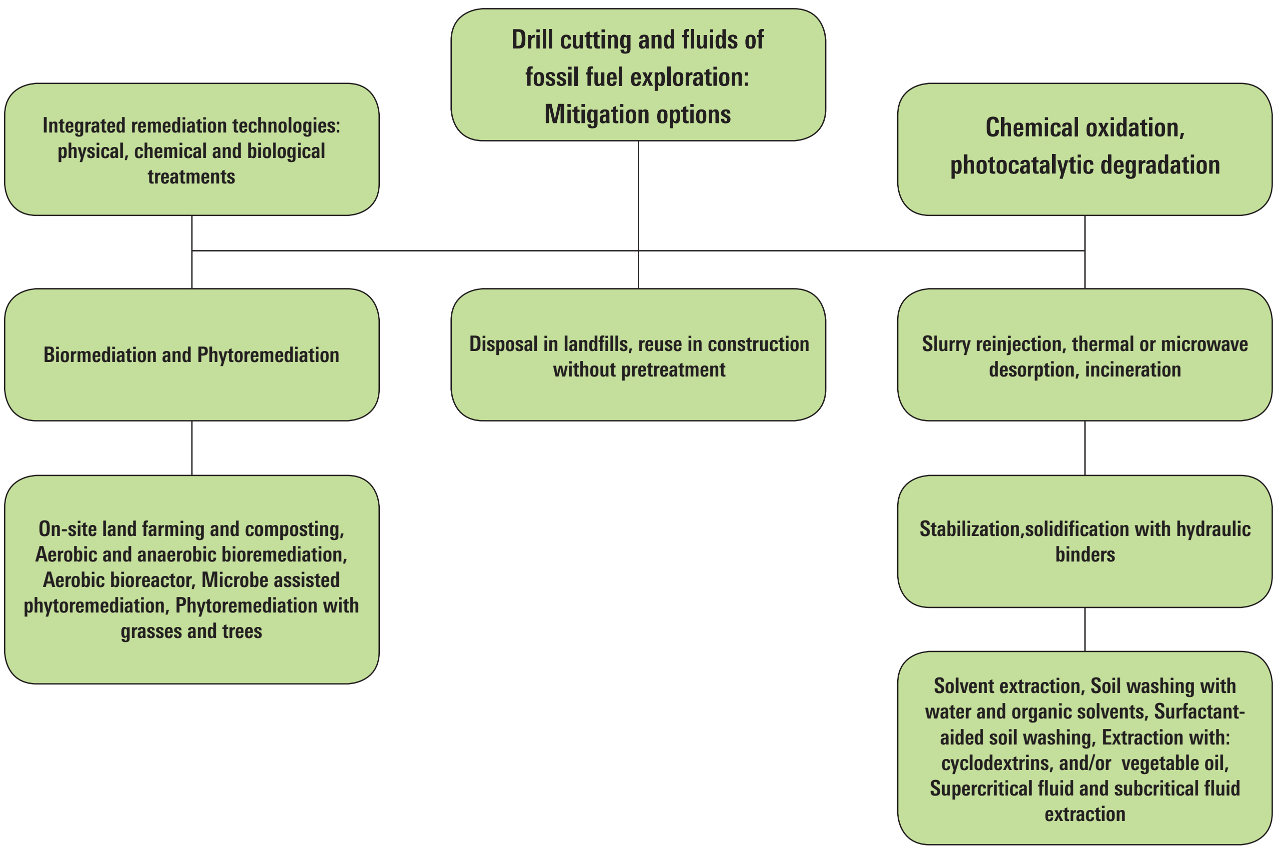


Figure 52. Integrated remediation technologies for drill cutting and fluids of fossil fuel exploration fluids. For details see Prasad and Katiyar (2010)

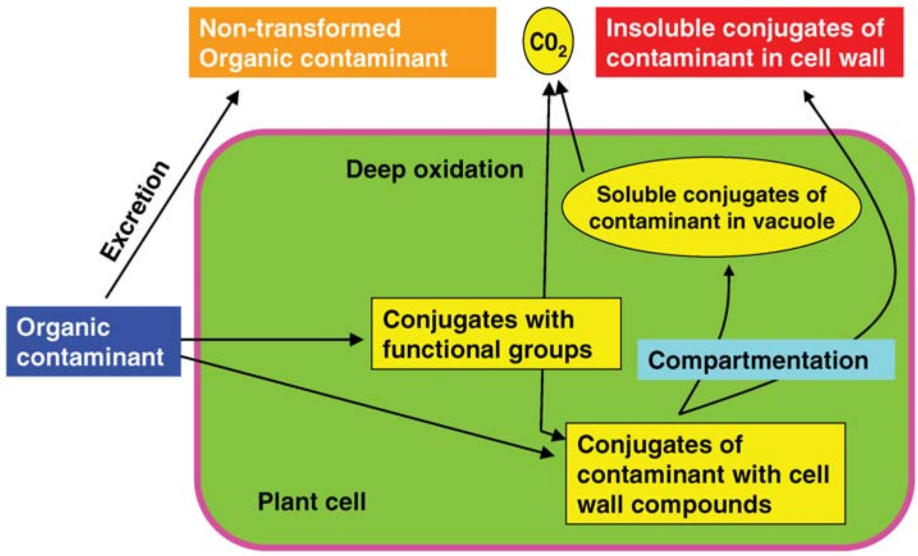


Figure 53. Detoxification of xenobiotics.

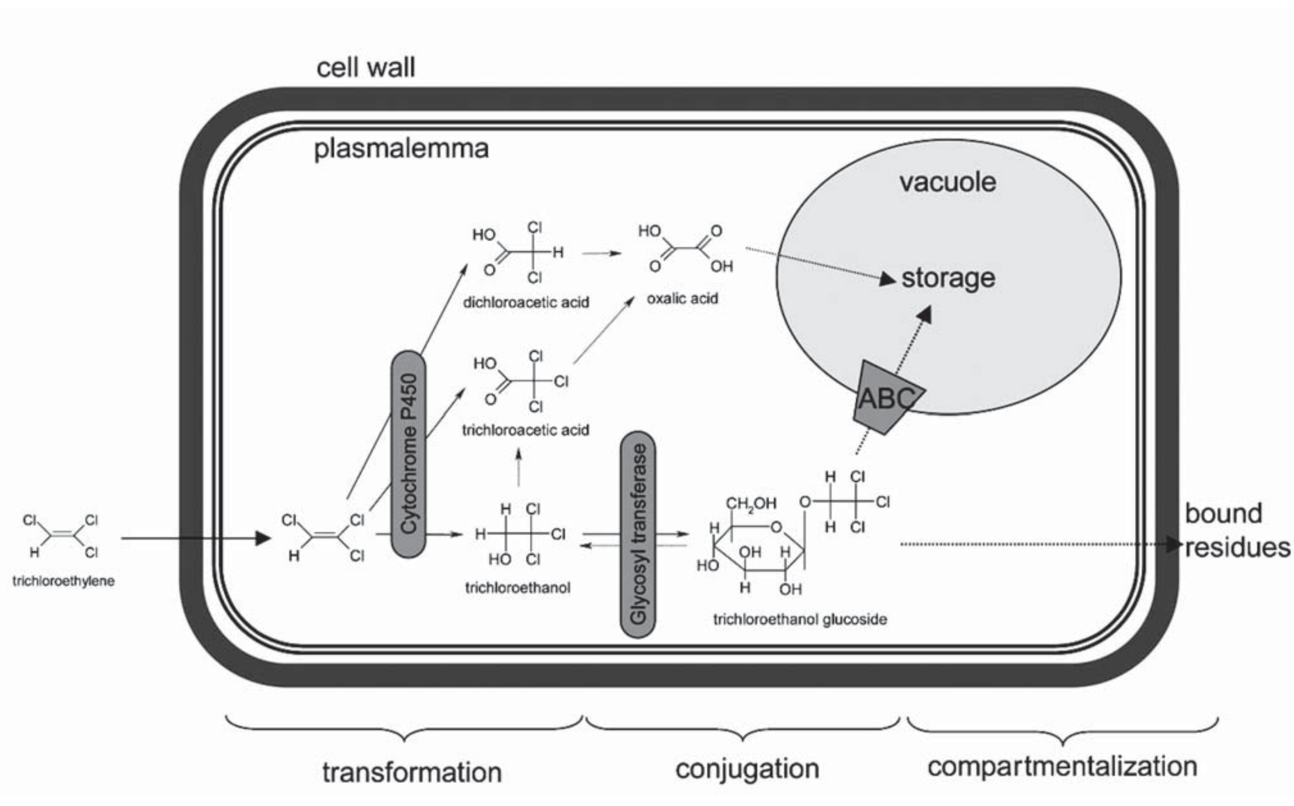
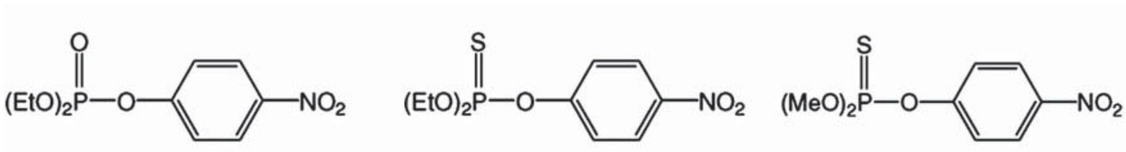


Figure 54. The pathway representing the metabolism of trichloroethylene (TCE) in plant tissues. Phase I, activation / transformation of TCE to trichloroethanol; phase II, conjugation with a plant molecule; phase III, sequestration of the conjugate into the cell wall or within the vacuole (Source: Reichenauer and Germida 2008; Van Aken 2009)

HCH degradation by *Sphingomonas paucimobilis* and endosulfan degrading microbes have been reported (Figure 55 and 56). The widespread use of organophosphates (OPs) in agriculture as pesticides has led to serious environmental pollution by these extremely toxic compounds. OPs are the ester forms of phosphoric acid and most widely used insecticides including paraoxon, parathion, or methyl parathion. Naturally occurring soil bacteria have evolved the ability to degrade OPs with the help of an enzyme called organophosphate hydrolase (OPH or phosphotriesterase). OPH catalyzes the hydrolysis of P-O linkage releasing p-nitrophenol as a leaving group. Since the toxicity of OPs is significantly reduced by hydrolysis of phosphoester bonds, many researchers have focused on the initial hydrolysis by OPH.



Paraoxon **Parathion** **Methyl parathion**

Figure 55. Soil bacteria degrade OPs with the help of an enzyme called organophosphate hydrolase (OPH or phosphotriesterase).

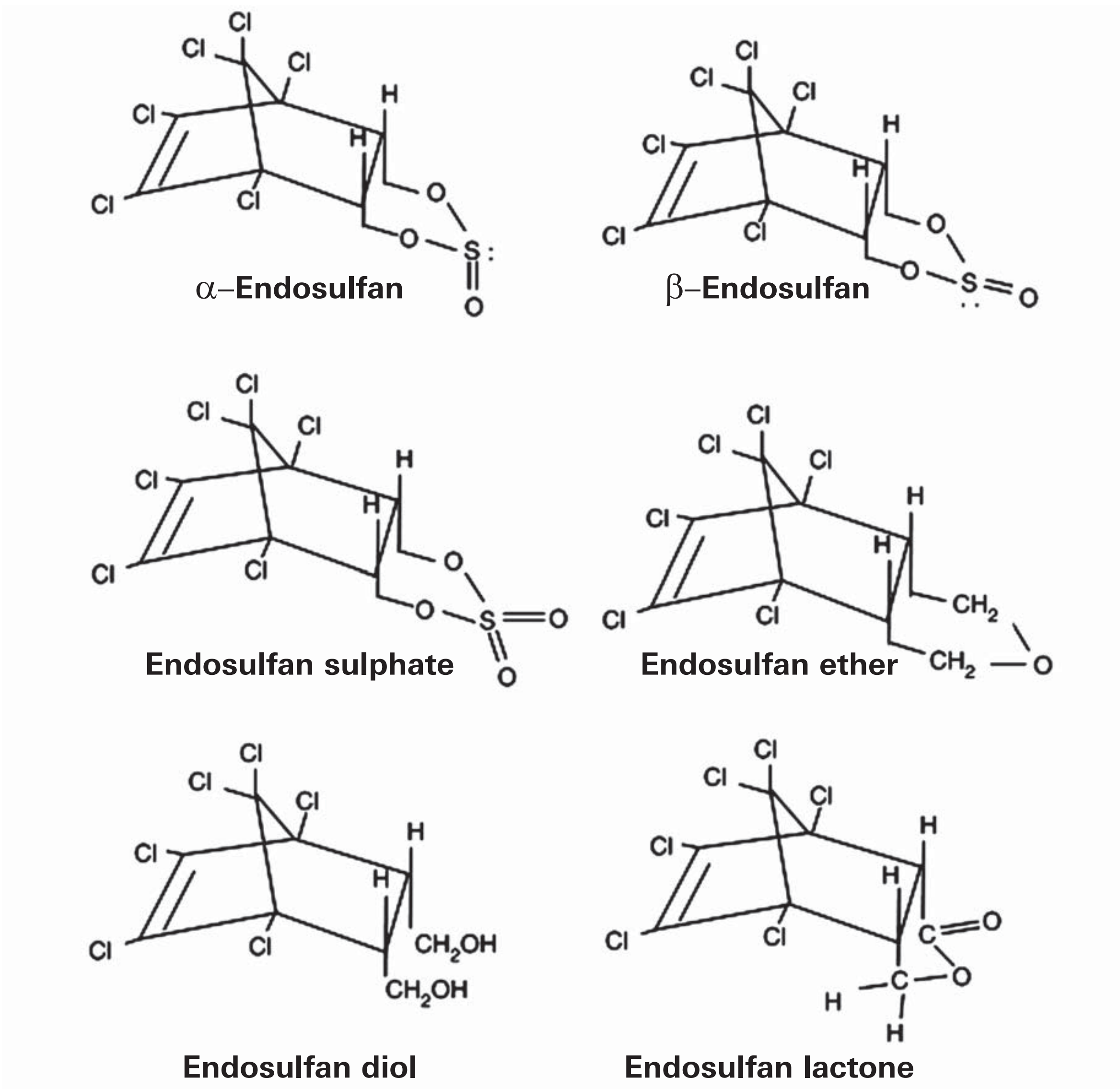


Figure 56. Structures of endosulfan isomers and their metabolites produced during microbial degradation

Both the isomers, α-endosulfan and β-endosulfan, are degraded by attack at the sulphite group via either oxidation to form the toxic metabolite endosulfan sulfate, or by hydrolysis to form the nontoxic metabolite, endosulfan diol. Endosulfan sulfate is produced only through biological transformation, whereas, under alkaline conditions endosulfan is converted to diol. *Klebsiella oxytoca , Bacillus spp. Pandoraea sp. and Micrococcus* sp. are the bacteria reported to degrade endosulfan in solutions and soils. Many fungi viz., *Aspergillus niger, A,. terreus, Cladosporium oxysporum, Mucor thermo-hyalospora, Fusarium ventricosum , Phanerochaete chrysosporium Trichoderma harzianum* and algae such as *Anabaena sp. Chlorococcum sp., and Scenedesmus* sp. are implicated in endosulfan degradation.

4.4 Explosives

Simplified representation of the microbial degradation of the explosives is shown in figure 57.

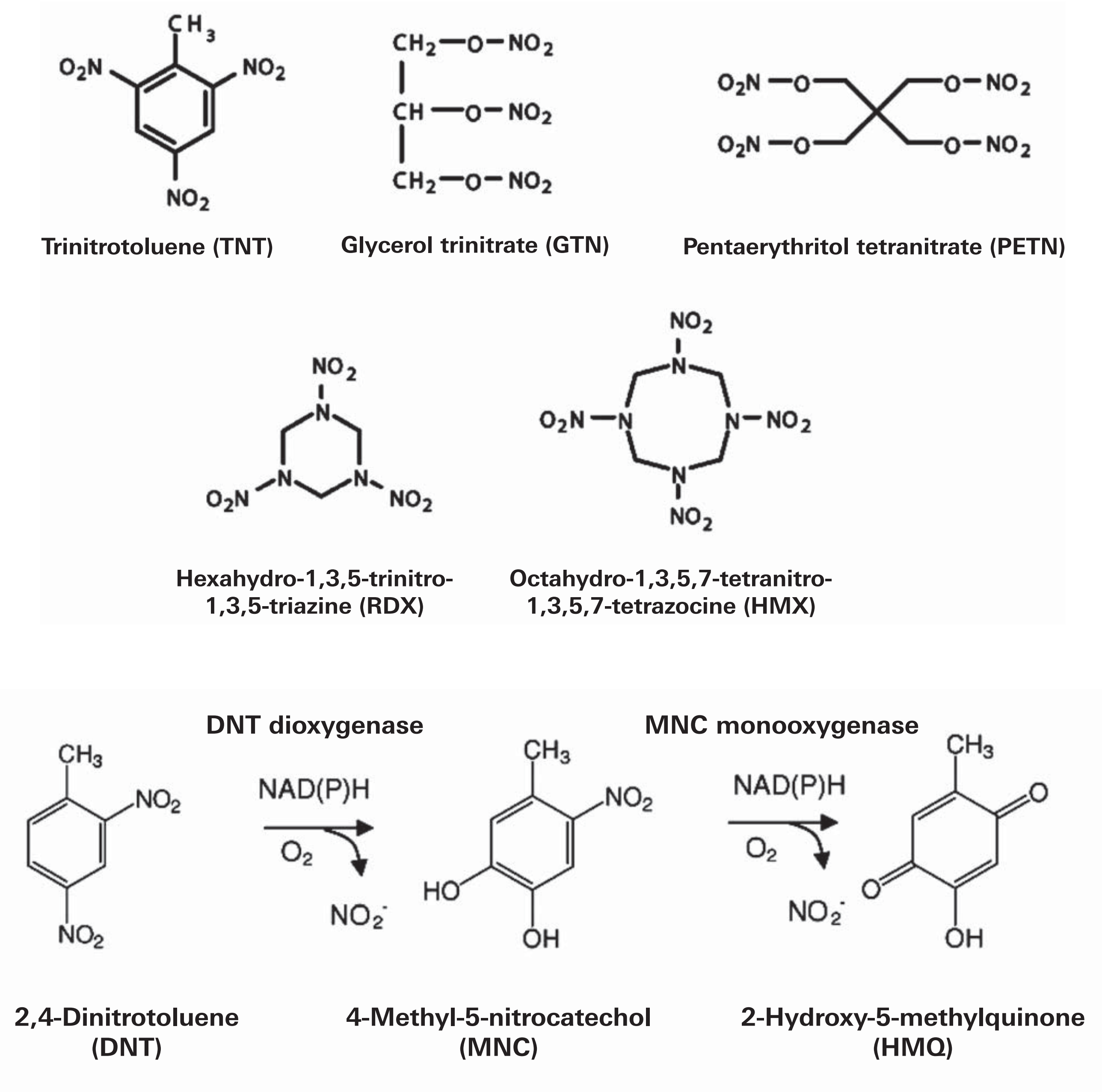


Figure 57. Degradation of explosives. A few common nitro-substituted explosives: 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), glycerol trinitrate (GTN), and pentaerythritol tetranitrate (PETN).

4.5 Endocrine disrupting chemicals

Pharmaceuticals and healthcare products that are refractory in function have been detected in drinking water. These include estrogen and anthropogenic endocrine disrupting chemicals (EDC) (Prasad *et al* 2010). India witnessed upto 95% loss of the vulture population over the past decade. The investigation proves that veterinary diclofenac is the prime cause for the declined vulture populations. The veterinary diclofenac is in heavy use in the livestock sector (buffalos, cattle, sheep, and goat). In the course of treatment, if the cattle dies, and the vultures scavenge the corpses of cattle, It leads to dehydration, visceral gout, and kidney failure in vultures within a few days. There has been increasing concern about the potential adverse effect of endocrine disrupting chemicals (EDC) on environmental health. EDC have existed for a long time in the environment, but not until recently did it catch the attention of scientists. Because the content of EDC is very low and usually accords with current environment standards, it is not easy to recognize its harmful effects on the human endocrine system (Figure 58) . It is believed that EDC might have a serious effect on human health, especially on the generative system. Therefore, it is also called environmental hormone. Biomonitoring and bioremediation of EDC is at the infancy stage in India.

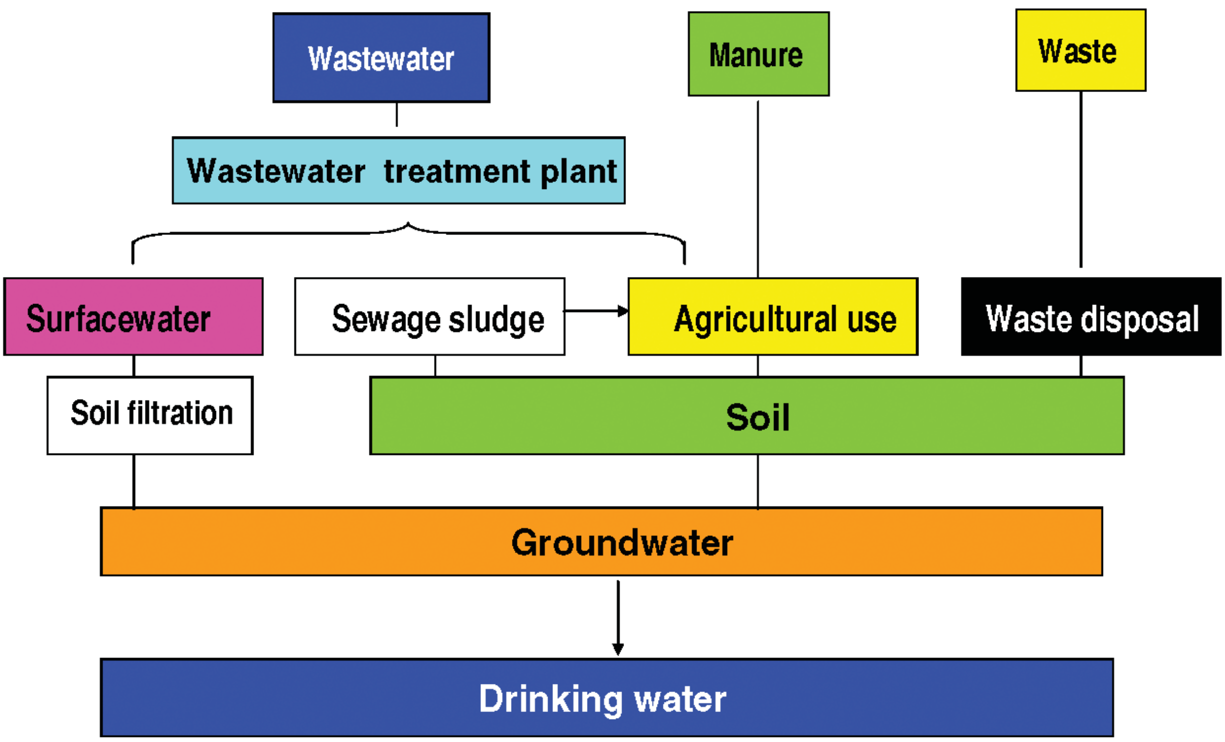


Figure 58. Pharmaceutical residues are common contaminants of groundwater in many cities

Chapter 5
Constructed wetlands for waste treatment

In a climate constrained world, the importance of wetlands is gaining considerably. During the last five years, the publications on various aspects of wetlands have been steeply growing. Data gleaned from www.sciencedirect.com is shown in Figure 59. Aquatic macrophytes e.g. *Typha latifolia, Eichhornia crassipes, Ipomea* sp. *Lemna minor, Polygonum* sp, *Alternanthera philoxeroides, Phragmites* sp..have paramount significance in bioremediation. These are being used in water quality assessment and also as fast track botanical cleanup crews (Prasad 2007, Prasad *et al* 2006b) (Figure 60). They are important in nutrient cycling, controlling water quality, sediment stabilization and provision of habitat for a host of aquatic organisms (Figure 61).

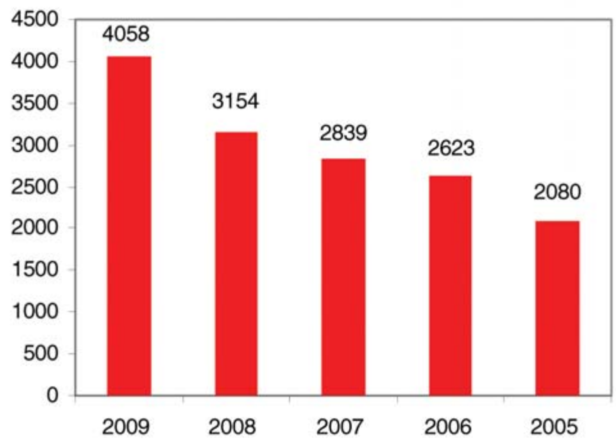


Figure 59. Knowledge explosion in wetland science

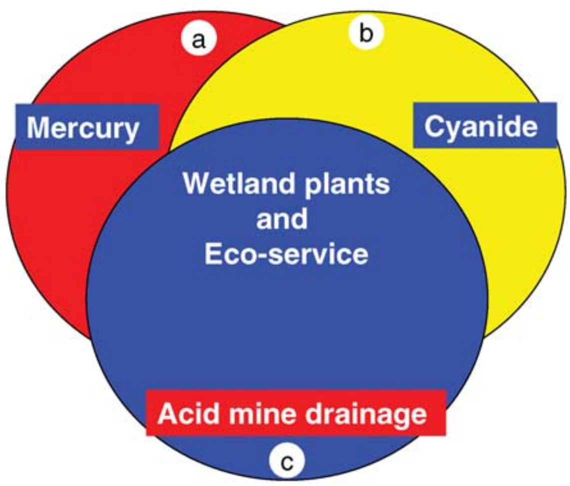


Figure 60. Eco-service of wetland plants for bioremediation of hazardous waste

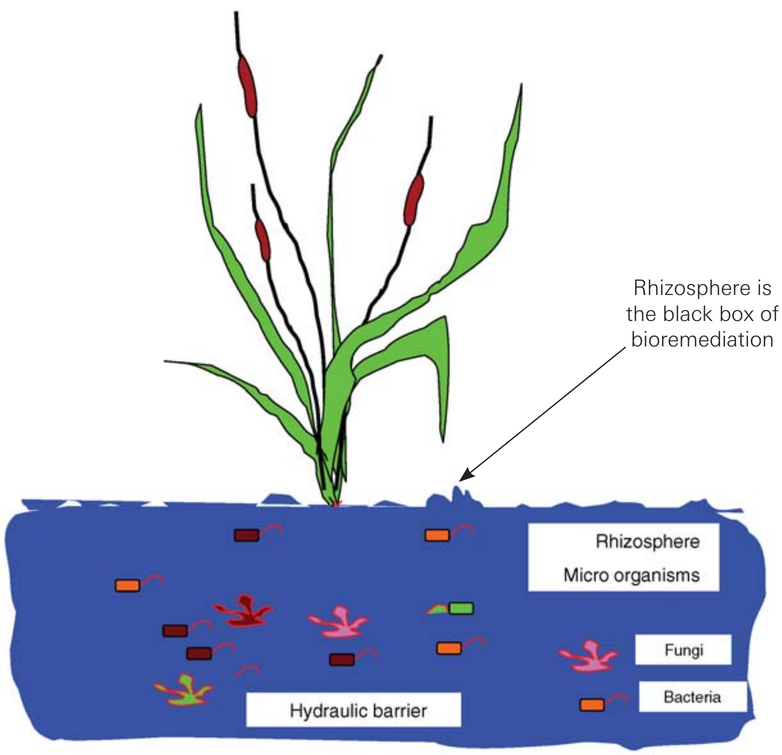


Figure 61. Wetland plant rhizosphere harbours a wide variety of microbes

Two kinds of wetlands are in the service of mankind: a) Natural wetlands - used for wastewater treatment for centuries; b) Constructed wetlands - effective in treating organic matter, nitrogen, phosphorus, decreasing the concentrations of trace metals and organic chemicals (Kadlec and Knight 1996). The submerged aquatic macrophytes have very thin cuticles and therefore, readily take up metals from water through the entire surface. Macrophytes possess extraordinary ability to survive the adverse conditions of pollution and possess high colonization rate that are virtual tools of excellence for phytoremediation. Further they redistribute metals from sediments to water, finally accumulate in the plant tissues, accelerate biogeochemical processes and hence maintain homoeostasis. Both submerged and emergent macrophytes play an important role in metal bioavailability from sediments through rhizosphere exchanges and other carrier chelates. These phenomena facilitate metal uptake by other floating and emergent forms of macrophytes.

A special group of wetland plants reduce element leakage from mine tailings by phytostabilisation. Mine tailings rich in sulphides, e.g. pyrite, can form acid mine drainage (AMD) if it reacts with atmospheric oxygen and water, which may also promote the release of metals and As. To prevent AMD formation, mine tailings rich in sulphides may be saturated with water to reduce the penetration of atmospheric oxygen. An organic layer with plants on top of the mine tailings would consume oxygen, as would plant roots through respiration. Thus, phytostabilisation on water-covered mine tailings may further reduce the oxygen penetration into the mine tailings and prevent the release of elevated levels of elements into the surroundings. Metal tolerance can be evolutionarily developed while some plant species seem to have an inherent tolerance to trace for e.g. *Typha latifolia, Glyceria fluitans* and *Phragmites australis* (Prasad 2001).

Mine tailings weather by penetration of oxygen thus forming free metal ions and sulfuric acid in acidic mine drainage (AMD) water. Wetland plants have the ability to either take up oxygen from the air or use photosynthetic oxygen and translocate the oxygen to the roots and into the rhizosphere (Figure 62). Thereby, they will increase the redox potential and thus also decrease the pH and increase the release of metals. Aquatic plants can tolerate a very low pH, which can be necessary when treating AMD. *Carex rostrata, Phragmites australis, Typha angustifolia, T. latifolia*, have been found growing under field conditions in pH as low as 2-4.4.

Constructed wetlands are being designed for the treatment of municipal waste waters in developed

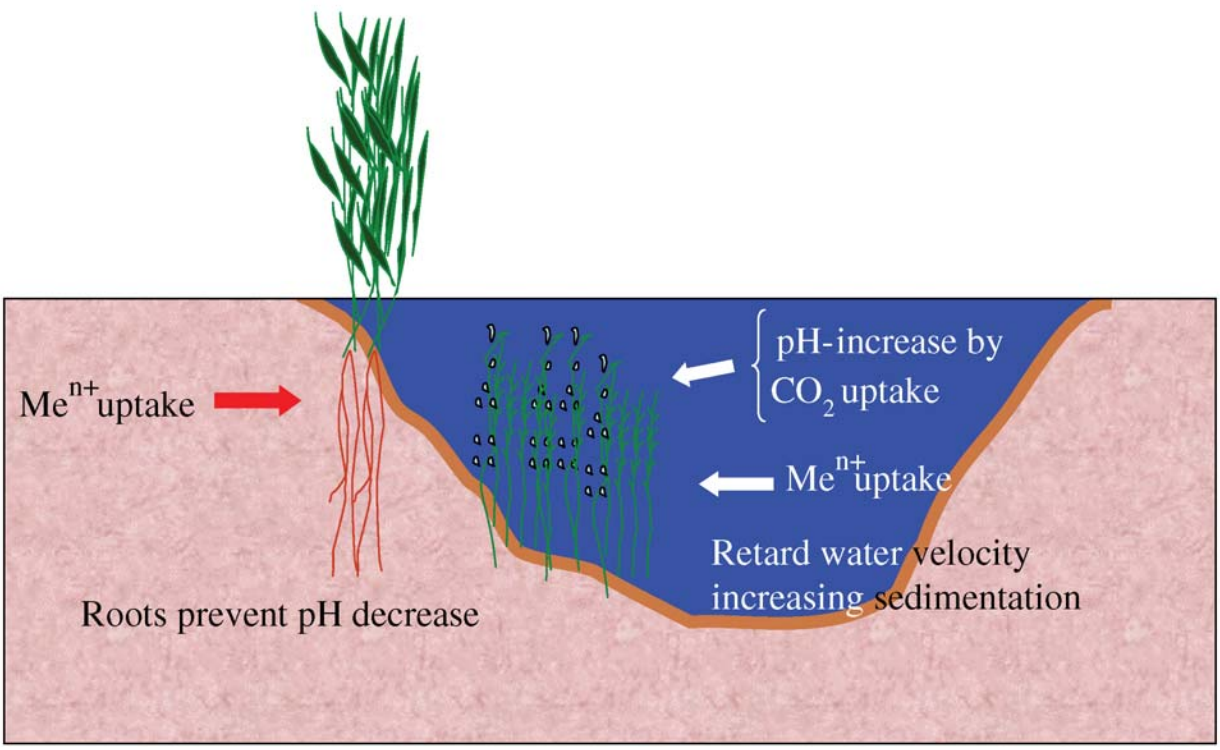


Figure 62. Prevention of formation of acid mine drainage by wetland plants (Prasad *et al* 2006)

nations (Figures 63-65). Due to the cost-effectiveness in the treatment of non-point source pollution, use of constructed wetlands is rapidly spreading in developed nations. However, in tropical nations due to water scarcity and high surface evapo-transpiration, the constructed wetlands for treatment of waste waters is not gaining importance.

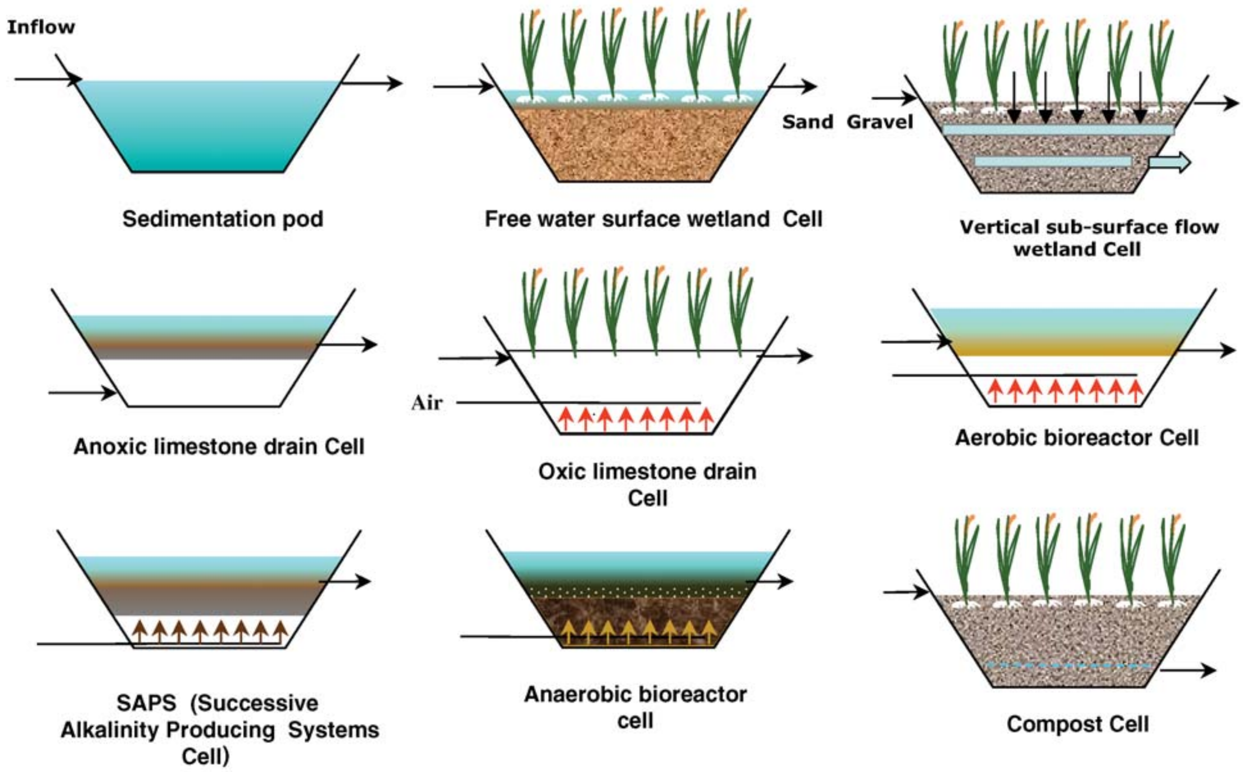


Figure 63. Schematic view of a generalized constructed wetland for waste treatment
Source: Dr. McIntyre T., Environment Canada

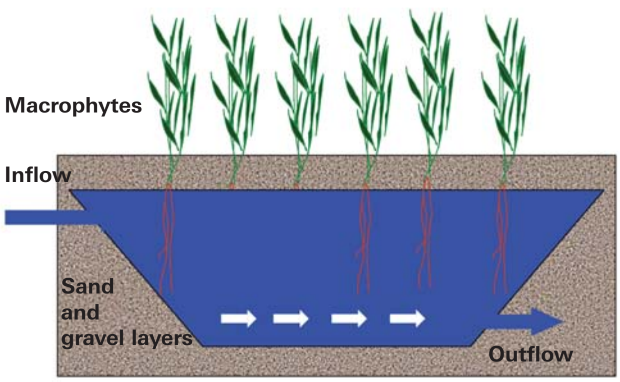


Figure 64. Schematic view of a horizontal flow constructed wetland

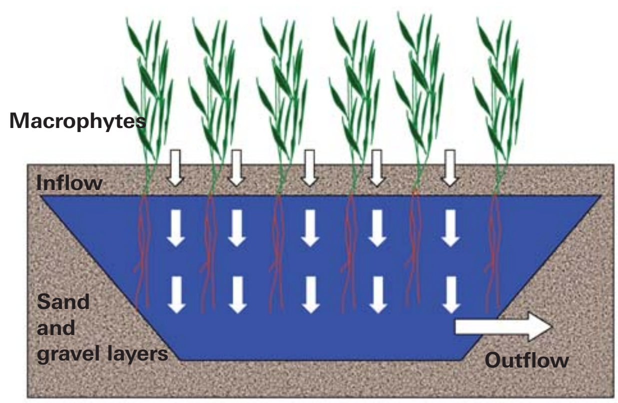


Figure 65. Schematic view of a vertical flow constructed wetland

Chapter 6
Conclusions and Action Plan

As a developing country, India has been paying more attention to economic growth and people's well-being. Due to rapid economic development, in recent times, environmental pollution and resources depletion problems have become major issues affecting the sustainable social and economic development. The balance between the economic growth and environmental/ecological protection has gradually become one of the key issues that should be taken into consideration by government officials, policy makers, company managers, scientists and engineers.

Prevention and control of pollution is one of the most important objectives of the MoEF. These activities are supported by a set of legislative and regulatory and promotional measures such as Policy Statement on Abatement of Pollution, 1992; and the National Environment Policy, 2006. The major actions on Abatement of Pollution and Environmental Cleanup are as follows: Clean Technology (CT), Control of Pollution (CP), Environment Education (EE), Environmental Impact Assessment (IA), Environmental Information (EI), Environmental Information System (ENVIS), Environment Research (RE), Forest Protection (FPR), Hazardous Substances Management (HSM) , Climate Change(CC) , Clean Development Mechanism (CDM), National River Conservation Directorate (NRCD) and Montreal Protocol & Ozone Cell (OC). This report highlights:

1. Bioremediation and its application to contaminated sites in India.
2. Mechanisms responsible for bioremediation have been elucidated for possible implementation on a variety of contaminated and polluted sites in India (only selected).
3. Information related to the experts/institutions that are actively involved in research of the subject area.

Bioremediation is cost effective, solar driven, faster than natural attenuation, high public acceptance including enhancement of aesthetics, and generates less secondary wastes with fewer air and water emissions. Bioremediation has emerged as an integrated 'toolbox' for environmental cleanup and ecosystem service provider. Convincing evidences are forthcoming highlighting its potential for addressing contemporary environmental agenda, with synergies that involve the product development from plants used for bioremediation and integrating them with ecosystem service providers (Dickinson *et al* 2009) (Figure 66).

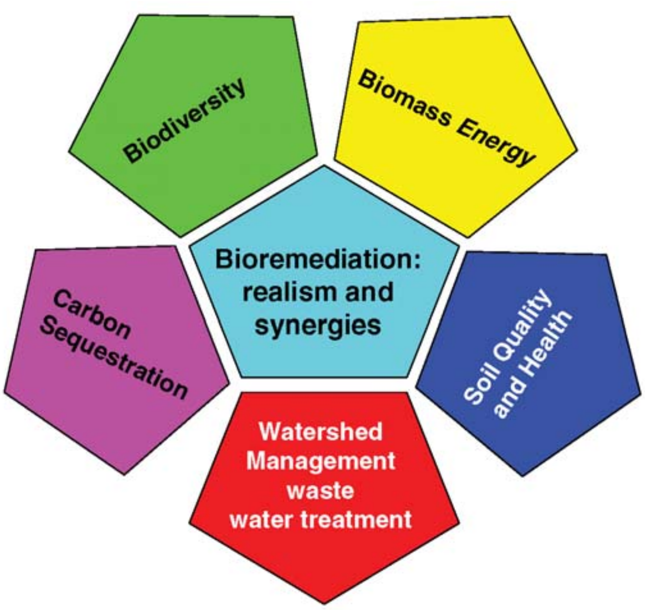


Figure 66. Tangible benefits of bioremediation and synergies with other glocal (global + local) environmental agenda

The Chairman and members of the Advisory Committee for Research Programme on Bioremediation of Contaminated Sites (F.No. 2/29/2008-RE dt 1-10-2008). felt that this report could be utilized to identify specific bioremediation projects and to demonstrate its application on a full scale. Further, the Committee also felt and suggested that techniques given in the report may also be considered for application to contaminated water bodies as well as the industrial effluents. There is an immediate necessity for initiating an "All India Coordinated project on Bioremediation of the Contaminated Sites" involving premier institutions in this field for conducting large scale demonstration projects. Further, various departmental agencies and related ministries in India may plan a joint action plan to launch bioremediation from Lab-Pilot –Field scale projects at specific sites (Figures 67-69).

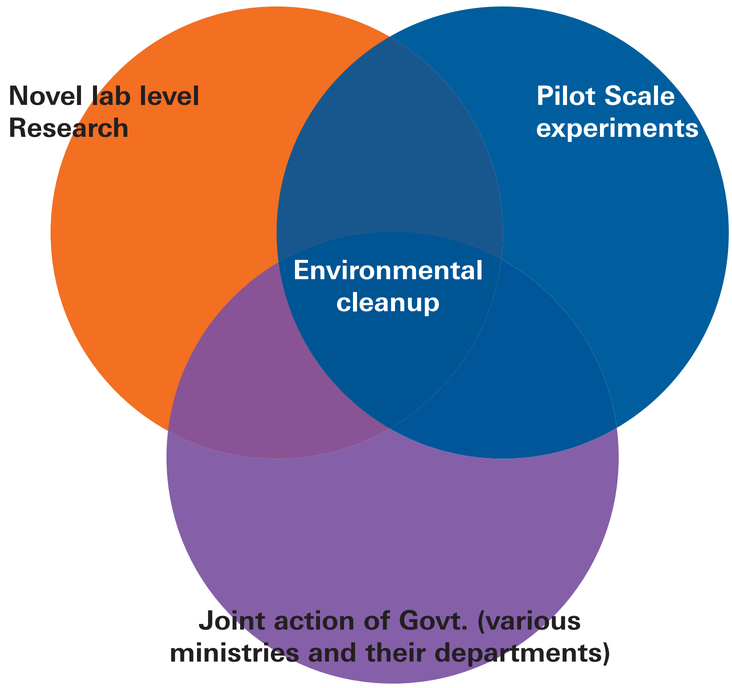


Figure 67. Possible All India coordinated bioremediation demonstration projects at specific sites

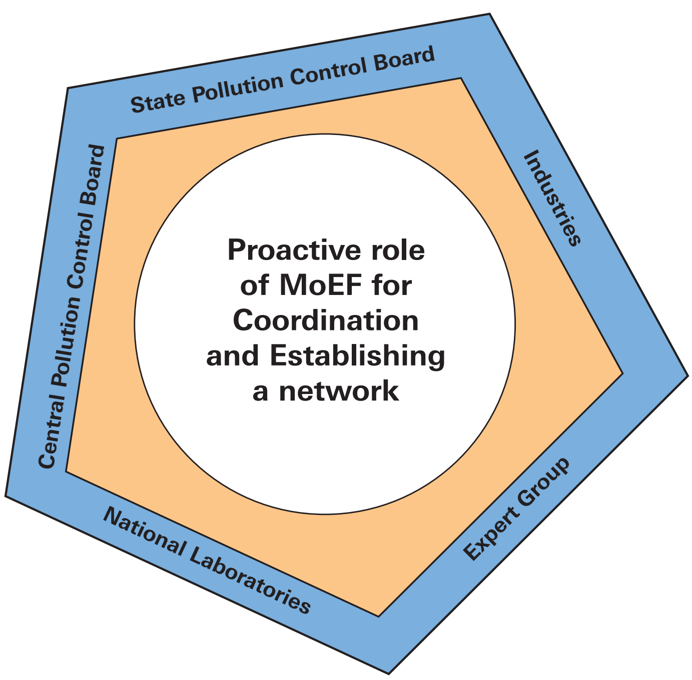


Figure 68. Envisaged action plan for inventorizing and demonstration of large scale full application of bioremediation and replication by respective industries

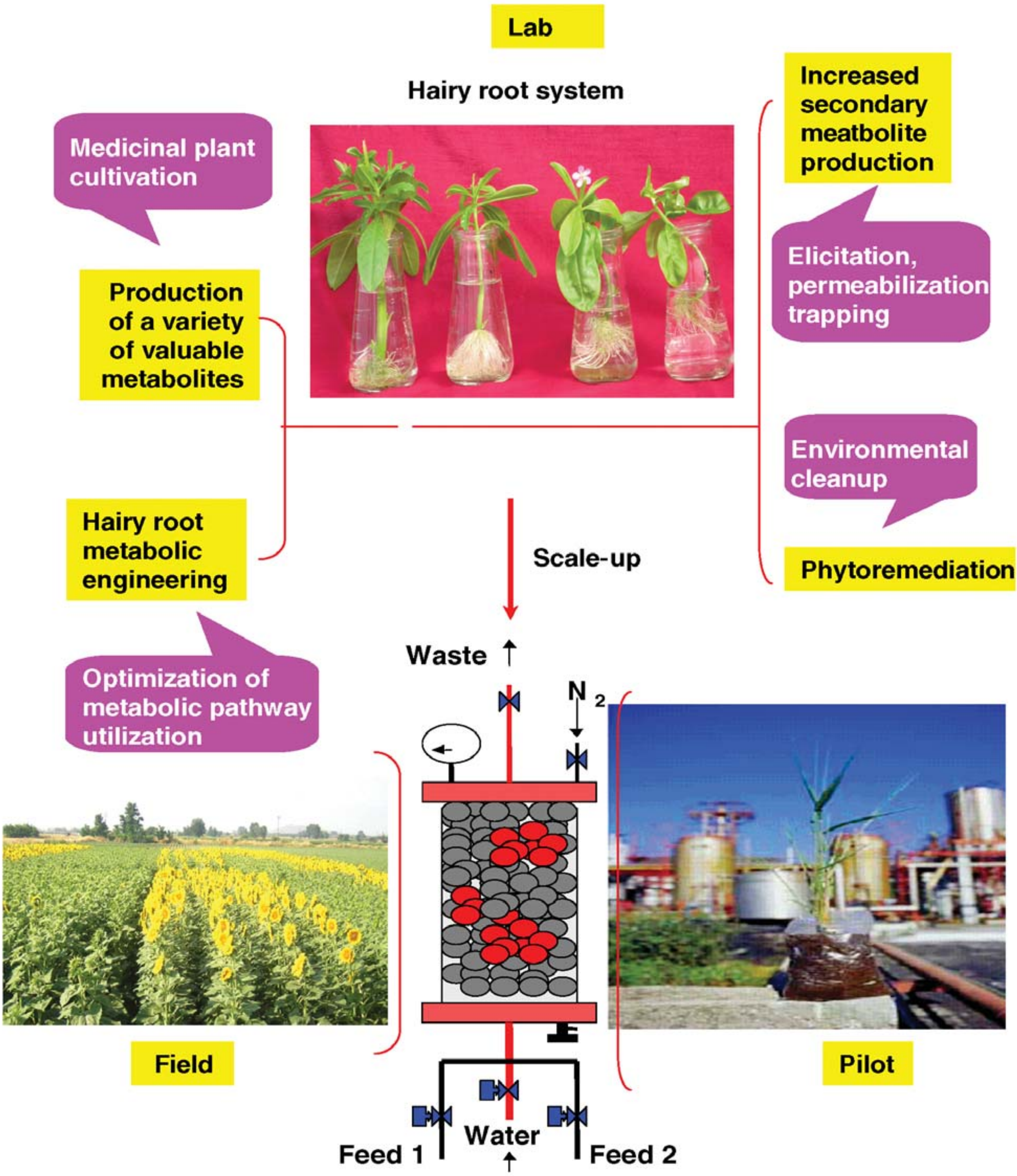


Figure 69. Bioremediation from Lab-Pilot –Field scale

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M.N.V. Prasad
Hyderabad

Annexure 1:
Institutions having expertise in Bioremediation research

ARI	Agharkar Research Institute, Pune
BARC	Bhabha Atomic Research Centre, Trombay
BHR	Bharathiar University, Coimbatore
BHU	Banaras Hindu University, Varanasi
BU	Bangalore University, Bangalore
CAZRI	Central Arid Zone Research Institute, Jodhpur
CIMFR	Central Institute of Mining and Fuel Research, Dhanbad
CRIDA	Central Research Institute for Dry land Agriculture, Hyderabad
DU	University of Delhi, Delhi
GU	Guwahati University, Guwahati
IARI	Indian Agricultural Research Institute
IICT	Indian Institute of Chemical Technology, Hyderabad
IIP	Indian Institute of Petroleum, Dehradun
IISC	Indian Institute of Science, Bangalore
IITB	Indian Institute of Technology, Bombay
IITD	Indian Institute of Technology, Delhi
IITK	Indian Institute of Technology, Kanpur
IITKG	Indian Institute of Technology, Kharagpur
IITM	Indian Institute of Technology, Madras
ILS	Institute of Life Sciences, Bhubaneswar
IMTECH	Institute of Microbial Technology, Chandigarh
ISM	Indian School of Mines, Dhanbad
ITRC	Industrial Toxicology Research Centre, Lucknow
JNTU	Jawaharlal Nehru Technological University, Hyderabad
JNU	Jawaharlal Nehru University, New Delhi
JU	Jadavpur University, Kolkata
KU	Kuvempu University, Karnataka
MLSU	Mohan Lal Sukhadia University, Udaipur
MSU	Maharaja Sayajirao University, Vadodora
NAARM	National Academy of Agricultural Research Management, Hyderabad
NBRI	National Botanical Research Institute, Lucknow
NEERI	National Environmental Engineering Research Institute, Nagpur
NEHU	North Eastern Hill University, Shillong
NEIST	North East Institute of Science and Technology, Jorhat
NGRI	National Geophysical Research Institute, Hyderabad
NIO	National Institute of Oceanography, Goa
OU	Osmania University, Hyderabad
RPRC	Regional Plant Resource Centre, Bhubaneswar
RRL	Regional Research Laboratories, Bhopal, Trivandrum, Bhubaneswar
RU	Ravishanker University, Raipur
TERI	The Energy and Resources Institute, Delhi
TIET	Thapar Institute of Engineering & Technology, Patiala
UH	University of Hyderabad, Hyderabad
UM	University of Madras, Chennai

Annexure 2
Frequently asked questions

Frequently asked questions about bioremediation and some rules of thumb are detailed in this section (Q = Questions, A = Answer).

Q1: What is bioremediation?

A1: It is the branch of biotechnology that uses biological processes and biodiversity for environmental cleanup.

Q 2: How does bioremediation work?

A 2: Bioremediation operates through vegetation to sequester, extract, or degrade hazardous waste present in soils, sediments, groundwater, surface water and air. There are six major mechanisms associated with bioremediation: 1. phytosequestration, 2. rhizodegradation, 3. phytodegradation, 4. phytohydraulics, 5. phytoextraction and 6. Phytovolatilization (Figure 4).

Q 3: What contaminants can be treated with bioremediation?

A 3: Typical organic contaminants (“organics”) such as petroleum hydrocarbons, gas condensates, crude oil, chlorinated compounds, pesticides, and explosive compounds can be remediated using bioremediation. Typical inorganic contaminants (“inorganics”) that can be addressed include salts (salinity), heavy metals, metalloids, and radioactive materials. Extensive databases are available covering a wide range of contaminants treated using bioremediation.

Q 4: Do the plants become contaminated in this process?

A 4: For organic contaminants, the octanol-water partition coefficient (log Kow) typically needs to be between 1 and 3.5 for uptake by plants to occur. (Box 8). For inorganic contaminants, including essential plant nutrients, uptake is specific to the element and plant species. According to the current research, there is little or no accumulation of volatile contaminants in plant roots, wood, stems, leaves, or fruit. Plants may accumulate metals or other toxic materials that reach contaminated levels, but several mechanisms exist that often limit the uptake and/or persistence of nonessential compounds in the plant.

Q 5: Do plants release contaminants into the air? If so, how much and how often?

A 5: Yes; there is an established mechanism known as phytovolatilization whereby volatile chemicals are taken up by a plant and released through leaf surfaces. However, extensive samplings in the field show that minimal amounts of volatile contaminants are emitted from plants. Further, bioavailability of contaminant is the crucial factor for ecotoxicity which is explained in the Figure 70.

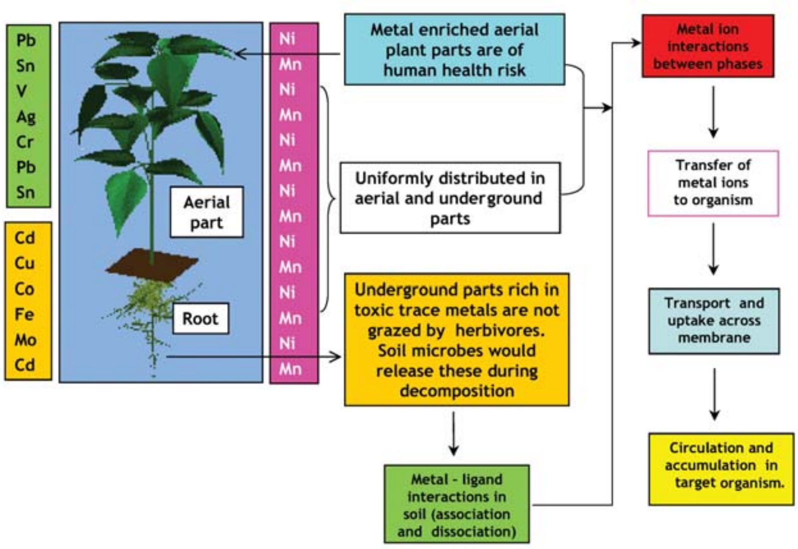

Figure 70. Bioavailability is often used as the key indicator of potential risk that chemicals pose to environmental and human health. The paths are elucidated in this sketch. Bioavailability is defined as the integral sum of a four-component process consisting of: (a) ingestion, (b) bioaccessibility, (c) adsorption and (d) first-pass effect.

Q 6: If fruit and nuts are produced, are they safe for humans and animals?

A 6: Products need to be tested before use. Biofortification is emerging for this concept (see Prasad 2008 for details). One example provided is Selenium and Opuntia (Figures 71-75).

Figure 71. Production of selenium-enriched phytoproducts and therapeutics from *Opuntia sp*. (courtesy Dr Gary Banuelos, USA and Dr. Rainer Schulin, Switzerland)

a b

Figure 72a, b: Ornamental production on contaminated sites

Figure 73 a,b: Phytoproducts from contaminated sites. Tree/shrub biomass from contaminated sites is being used to produce charcoal and in biomass based power plants as feed stock

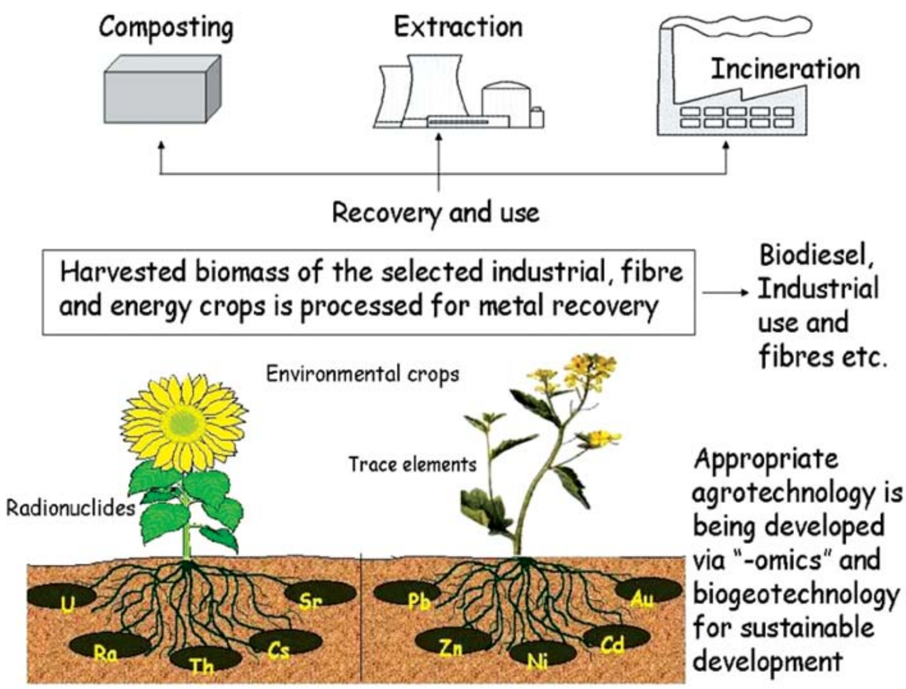

Figure 74: Co-generation of products from plants used in phytoremediation (Gratão *et al* 2005)

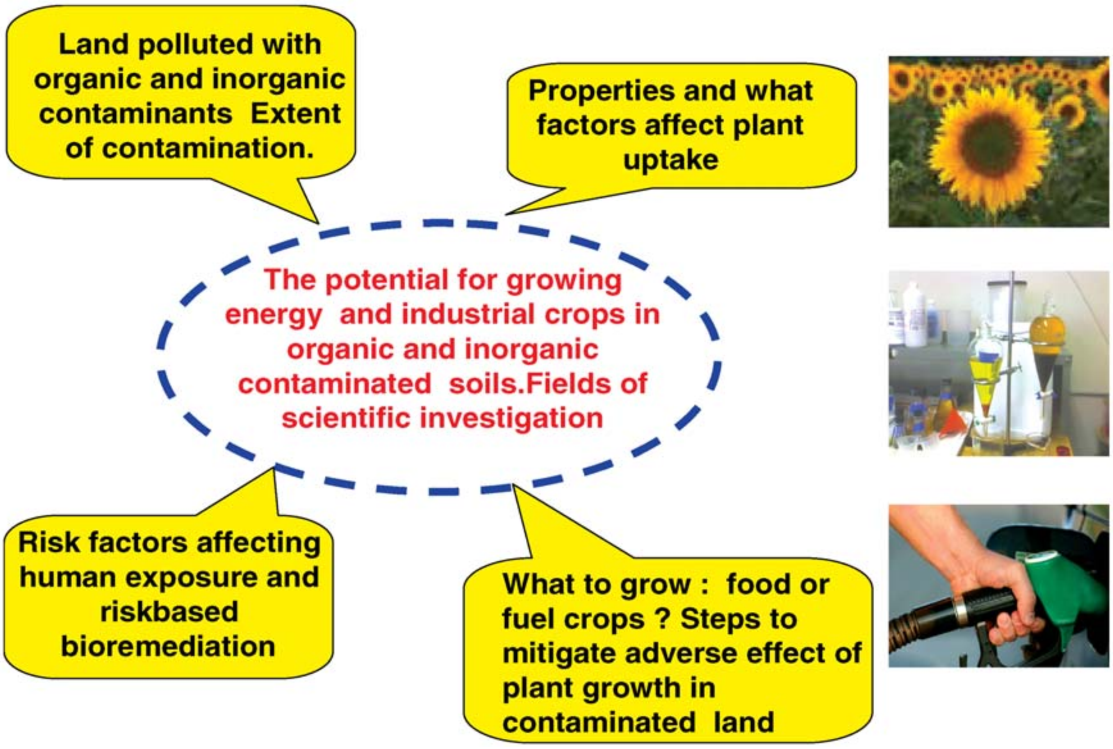

Figure 75: Growing energy and industrial crops on organic and inorganic contaminated soils and conversion to industrial feedstocks including production of liquid fuels.

Q 7: Will bioremediation work on every contaminated site ?

A 7: Success of bioremediation depends upon many factors such as soil conditions, climate, suitable plant species and associated rhizosphere microbes (Anonymous 2009, Prasad *et al* 2010). Therefore, every project is unique and must be custom designed, operated and maintained. Flow charts have been developed for successful projects (Anonymous 2009, ITRC report for details). In the copper mines of Globe and Tucson (Arizona Ranch, Resource Management and Mine Reclamation Arizona, USA), the ecosystem rehabilitation and mine reclamation programme is primarily based on cattle. Herds of cattle are impounded with electric fences on mine tailings, metal contaminated soils by providing fodder and water for varying durations. Cattle stabilized the soil with their hoofs and enriched nutrient status via urination and dung. This process is repeated at regular intervals in cycles. Thus, cattle accelerate rhizosphere development and improve plant root association via enriching soil micro organisms and nutrients. The results obtained with cattle for rhizosphere ecodevelopment are spectacular (Dagget 1997) (Figures 76 a-b; 77 a-f).

a b

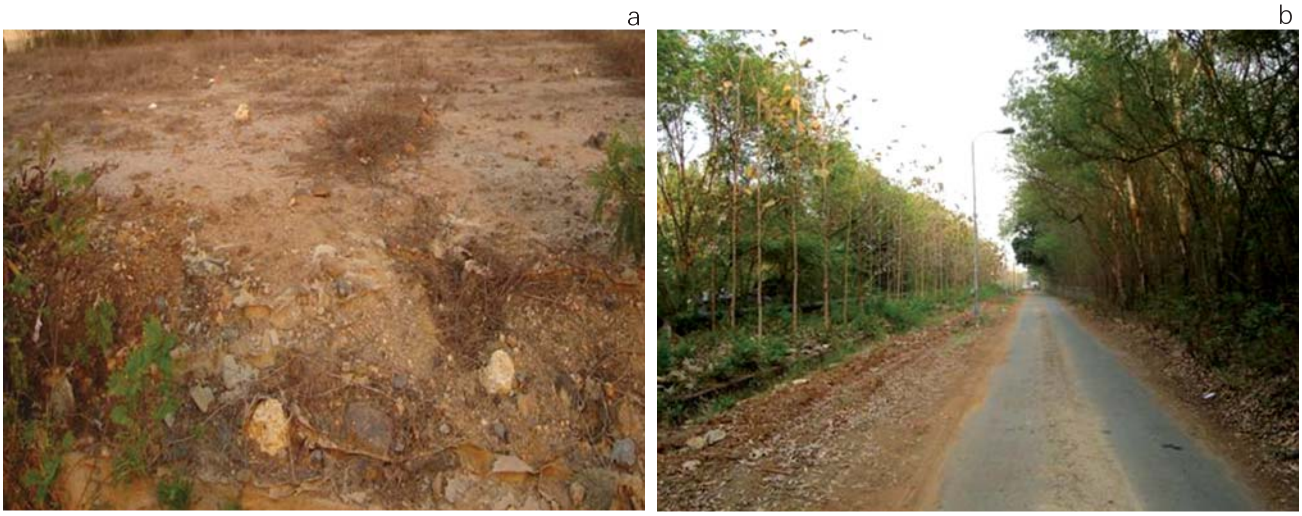

Figure 76 a,b: Contaminated site before and after 5 years of bioremediation

Figure 77 a-f: Cattle accelerated rhizosphere development and improved plant root association. Prior to bioremediation the mine operators used cost prohibitive synthetics and chemical dispersion methods (see Prasad & Freitas 1999).

Q 8: How deep do plant roots grow ?

A 8: Typical rooting depths for selected plants are shown in Figure 19. Generally herbaceous, upland species such as grasses and forbs are 30-60 cm ; however, depths down to 1.5 meter have been reported as within the range of influence under some situations. Furthermore, prairie grasses have root systems that can reach 3-4.5 meters below ground surface. Regardless, in general, 70%–80% of the root structure will be within the top 30-60 cm of soil (including tap-rooted species) with exploratory roots sent deeper and laterally. However, local soil conditions (nutrient content, moisture, compaction, etc.) will dictate the ultimate depth to which any plant will reach. Furthermore, the depth of penetration may progress as the plants grow year over year. For wetland species, typical depths are less than 1 foot due to oxygen limitations. For trees, typical depths are 3-4.5 meters but often require special culturing practices. Typical penetrations can be 90-150 cms per year when planted into a borehole or trench. The maximum practical depth is generally down to about 8 meters below ground surface (bgs) using these practices, although deeper depths can be reached under certain circumstances. The deepest influence of a phytotechnology system was measured at 12 meters below ground surface . A general rule of thumb, however, is that trees will not access deeper than 1.5 meters into the saturated zone.

Q 9: How fast do plants grow? How long do they live ?

A 9: Plant growth rate and longevity depend on species, soil, and climate. "Annual" species grow and die within a single season. Others, such as trees and other herbaceous perennials, continue to grow over years. Fast-growing species such as hybrid poplars and willows can grow 2–3 meters per year in the first few years. However, in general, those species that grow rapidly tend to be shorter lived. In cold countries' plant growth period is very limited. Therefore initial expenses for establishing on-site bioremediation demonstration projects are quite expensive (Figure 78). In India' plant growth period is spread throughout the year and is rich in biodiversity, therefore comparatively initial expenses are relatively less and expected to yield good results (Figure 79).

Figure 78: Green houses for bioremediation in temperate environment

Figure 79: Phytostabilization of Gumgaon manganese mine dump site by NEERI

Q 10: How long does it take for the system to become effective?

A 10: In some cases, the application of bioremediation can have an immediate effect on contaminant concentrations upon planting. In other cases, it may require several seasons before the plant can interact with a contaminated zone at depth. Furthermore, it may depend on whether the plant itself is directly or indirectly involved with remediating the contaminant (i.e., phytodegradation or simply stimulating biodegradation in the rhizosphere-rhizodegradation).

Q 11: What happens in winter when the plants are dormant?

A 11: Water consumption and contaminant uptake essentially stop when plants are dormant. Degradation by microbes and the rhizosphere effect continue but at a reduced rate. Efforts to estimate the rate of remediation should account for the dormant conditions.

Q 12: How long does it take until cleanup is achieved?

A 12: It depends on the criteria set forth in defining the cleanup objectives for the site. Furthermore, it depends on the type, extent, and concentration of contamination, continuing sources, obstructions, soil conditions, hydrologic/groundwater conditions, and other site characteristics, the plant species, growth rate, and climate conditions. Complete restoration will depend on the type of bioremediation applied at the site.

Q 13: Which plant species should be used? How are plants selected for remediation?

A 13: All plant selections must be made based on site-specific conditions. Climate, altitude, soil salinity, nutrient content, fertility, location, depth, concentration of contaminant, commercial availability, plant ability, and plant hardiness are some of the determining elements. A variety of approaches and information resources can be used, including databases, site-specific vegetation surveys, and specifically designed tests to evaluate species. In addition to selecting species for the remediation, end-use considerations can be included in the initial plant selection. Typically, 10%–15% climax species might be included in the initial design (Annexure 3).

Q 14: When should planting be done?

A 14: In tropical climate except summer seasons, all other seasons are favourable for planting. In cold climate, planting is generally done in the early spring (after the last frost) the most desirable period to establish a phytotechnology system. Seeding should be done whenever it is most appropriate for the species, also typically in the early spring. Tree cuttings for propagation should be taken while the source tree is still in winter dormancy and should be maintained dormant (stored under refrigerated conditions) until planted into the ground. In many cases, survivability hinges on the timing of the planting, which should be planned appropriately in the design.

Q 15: How much area should be planted?

A 15: It depends on the extent of contamination and the characteristics of the site. A general rule of thumb for a very preliminary design during the remedy selection phase of a project is a planting density of 7 square meters per tree. Seeding rates for common grass species (ryegrass, fescue, etc.) are typically higher than prairie species. For example, 200 kgs of a fescue/perennial ryegrass seed mix is needed to cover one acre, while only 5-6 kgs of a prairie grass seed mix is needed to cover the same acre. The spacing between potted plants depends on the size of the specimens, but for plants that come in palettes, typically 30-60 cms, greater for larger specimens. A standard landscaping rule of thumb is that 10% of recently planted trees or potted plants will not survive the first year.

Q 16: How much does it cost?
A 16: It depends. Various cost items will need to be considered, such as earthwork, labour, planting stock, planting method, field equipment, heavy machinery (typically farming or forestry equipment), soil amendments, permits, water control infrastructure, utility infrastructure, fencing, security, etc.

Q 17: Isn't this just a "Do something quick and cheap in the field and then walk away" approach?
A 17: No. Like any remediation system, bioremediation requires significant operation, maintenance, and monitoring for several years after planting. Costs can include labour, sampling, analytical, materials, field equipment, utilities, waste handling, and disposal. Once the plantation becomes established, however, the operation and maintenance costs tend to diminish. Furthermore, additional sampling and monitoring will typically be required during the initial phases compared to subsequent years. Bioremediation are generally long-term remedial solutions.

Q 18: What operation and maintenance is required for bioremediation?
A 18: Phytotechnology plantations may require irrigation, fertilization, weed control (mowing, mulching, or spraying), and pest control. At the onset of a planting, which too may be a reccurring operation and maintenance event, some percentage of replanting may be required due to the lack of establishment. As a general rule of thumb, 10%–15% of the initial capital costs should be added as a contingency for replanting.

Q 19: In general how much water is required?
A 19: A general rule of thumb is that during establishment (i.e., before trees have reached a groundwater source) and perhaps throughout the growth of the vegetation (i.e., groundcover systems), plants should receive a total of 25-50 mm of water per week, including both precipitation and supplemental irrigation. Another rule of thumb for a very preliminary design during the remedy selection phase of a project is that a tree plantation uses about 5-6 litres per day per tree, annualized over the year.

Q 20: When should fertilization be done? What fertilizers should be used?
A 20: Soil fertility can be analyzed by a local agriculture extension service using established methods. The formulation of the fertilizer depends on the site-specific soil conditions.

Q 21: What happens if the plants die as a result of a natural catastrophe or infestation?
A 21: If the plants die or are damaged, the beneficial effects are lost or greatly diminished. However, the effect can be temporary, depending on the ability of the vegetation to regrow. Contingency plans should be established for different degrees of loss.

Q 22: If plants have to be harvested, how to decide if the sampled tissue is safe or not?
A 22: Harvested tissues (core tissue sampling of leaves and stems) are to be analyzed for contaminant levels.

Q 23: What is the easiest tissue to sample?
A 23: The above ground tissues such as leaves, needles, stems, branches, and fruits/seeds/nuts are easiest. These are collected simply by pulling or cutting sufficient material from the plant and storing in sealed plastic bags. For most analyses, samples of 20 g dry weight (10–15 average leaves) should be sufficient. As general rules of thumb, to estimate the wet-to-dry weight ratio for field sample collection, green stems typically contain 95% water weight, leaves 90%, fruits 85%, hardwood stems 50%, and nuts and seeds 5%. Once collected, the tissues should be stored on ice for transport to the laboratory.

Q 24: Is the harvested material usable for commercial payback?
A 24: Yes, but it may depend on the use, harvested material, and contaminant. The material may need to be tested.

Q 25: How do we know that bioremediation is working ?
A 25: Bioremediation should be monitored i.e., concentration trends, hydrology, soil effects, etc. (See Figure 8)

The information in this annexure is based on ITRC (Interstate Technology & Regulatory Council) (2009). Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised. PHYTO-3. Washington, D.C.

Annexure 3
Examples of plants applied in phytoremediation function.

For details of experiments, see Glass 1999; McCutcheon and Schnoor 2003, Prasad 2004a,b; 2007a,b; Prasad and Strzalka 2002)

Plant name	**Common name**	**Phytoremediation function**
Agropyron repens	Wheat grass	Stabilization of lead in soil
Agropyron smithii	Wheat grass	Rhizodegradation of hydrocarbons
Agrostis castellana	Bent grass	Metal accumulation
Agrostis tenuis,		
A, capillaries	-	Copper resistance and, metal hyper accumulation
Alisma subcordatum	Water-plantain	Explosives exposure and uptake
Allenrolfea occidentalis	Pickle weed	perchlorate tolerance
Alopecurus myosuroides	-	Multiple resistances to paraquat
Alyssum bertolonii	-	Hyper accumulation, metals
Andropogon gerardii	Big bluestem	Rhizodegradation of polycyclic aromatic hydrocarbons (PAHs)
Arabidopsis halleri	-	Metal tolerance
Armoracia rusticana	Horseradish	Source for peroxidase
Artemisia vulgaris	Mugwort	Cyanide tolerance
Astragalus	-	Extraction of selenium
Athyrium yokoscense	Fern	Metals accumulation
Atriplex hortensis	Garden orach	Phenolic compound metabolism, degradatrion of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbon PAH)
Avena fatua	Wild oat	Perchlorate phytotoxicity
Azalia pinnata	Water velvet	Biosorption of toxic metals
Azolla filiculoides	Water fern	Metals hyperaccumulation
Bacopa monnieri	Water hyssop	Metals accumulation
Bambusa	Bamboo	Useful in wastemanagement
Berkheya coddii		Nickel hyperaccumulation
Beta spp.	Beet	Denitration of glycerol trinitrate (GTN) in phenolic compound metabolism, PCBs, fate of PAH transformation
Bouteloua gracilis	Blue grama	Rhizodegradation of hydrocarbons
Brassica oleracea	Broccoli	Bench tests for metals accumulation
Brassica oleracea	Cabbage	
Brassica oleracea	Cauliflower	
var botrytis	Canola	
Brassica napus	Indian mustard	
Brassica juncea	Rape	
Brassica rapa	Turnip	
Brassica campestris		
Bromus hordeaceus	Blando brome	Degradation of explosives
Bromus inermis	Bromegrass	TNT exposure and phytotoxicity
Bromus hordeaceus		

Buchloe dactyloides	Buffalo grass	Rhizodegradation of hydrocarbons
Cannabis sativa	Hemp	Waste management
Carex praegracilis	Sedge	Phytoirrigation
Carex vulpinoidea		Degradation of Explosives
Ceratophyllum demersum	Coontail	Degradation of organics and toxic metal uptake
Cladium Jamaicense	Sawgrass	Brine concentration
Cynodon dactylon	Bermuda grass	Rhizodegradation of hydrocarbons
Cyperus esculentus	Yellow nutsedge	TNT remediation
Daucus carota	Carrot	Hydrocarbon remediation, phenolic compound metabolism, PCB transformation, PAH transformation by, I, I, I-trichloro-2,2-bis-4' chlorophenyl ethane DDT transformation of trichloroethylene TCE metabolism
Eichhornia crassipes	Water hyacinth	Metals accumulation, biosorption
Eleocharis obtusa	Blunt spike	Explosives transformation of TNT
Eleocharis tuberosa	Water chestnut	Transformation of trinitrotoluene
Eleusine indica	Barnyard grass	Dinitroaniline resistance explosives, accumulation revegetation of wetland banks
Elodea canadensis	Canadian waterweed	Aminodinitroto1uene (ADNT) removal by dehalogenation, uptake and transformation of explosives, halogenated organics , organophosphorus and perchlorate degradation, pesticide transformation and binding
Elymus canadensis	Canada wild rye	Rhizodegradation PAHs
Eremochloa ophiuroides	Centipede grass	Methyl bromide removal
Festuca arundinacea	Tall Fescue	Phytotoxicity to trinitrotoluene
Festuca ovina	Hard Fescue	Phytoirrigation understory
Festuca rubra	Red Fescue	Rhizodegradation of hydrocarbons
Galega orientalis	Goat rue	Rhizodegradation of benzene,toluene, and xylene
Haumaniastrum robertii	-	Hyper accumulation of metals
Hordeum brachyantherum	-	Phytoirrigation, metals hyperaccumulation, polychlorinated biphenyls (PCBs), transformation, nitrobenzene exposure, metal uptake
Hordeum vulgare	Barley	Fluoranthene transformation
Hydrilla verticillata	-	TNT transformation and metals accumulation
Hydrocotyle umbellata	Pennywort	Biosorption of toxic metals
Hygrophila corymbosa	-	Cadmium accumulation
Iris pseudocorus	Yellow iris	Methyl bromide and TNT transformation, Lemna, Spirodela and Wolffia Duckweeds Biosorbents of inorganic and organic pollutants and metals accumulation
Lespedeza cuneata	-	TCE metabolism
Linum usitatissimum	Flax	Waste management

Lolium multiflorum	rye	Phytomanagement
Lolium perenne	Ryegrass	Rhizodegradation of hydrocarbons
Lotus corniculatus	Birds-foot trefoil	Rhizodegradation of petroleum hydrocarbons
Macadamia neurophylla	-	Metal hyperaccumulator
Marsilea drummondii	Nardoo	TNT transformation
Melilotus officinalis	sweet Clover	Rhizodegradation of hydrocarbons
Muhlenbergia rigens	Deergrass	phytoirrigation
Myriophyllum aquaticum	Parrot feather	Explosives sensitivity to and transformation, halocarbon metabolism, halogenated organics transformation, hormesis, organophosphorus degradation, perchlorate degradation
Myriophyllum spicatum	Milfoil	Trinitrotoluene monitoring and transformation
Nelumbo nucifera	Indian lotus	TNT transformation
Nymphaea odorata	fragrant water lily	Trinitrotoluene (TNT) transformation
Panicum coloratum	Kleingrass	Rhizodegradation of PAH
Panicum coloratum	Kleingrass	Atrazine metabolism, Conjugation,herbicide phenol metabolism, root pressures
Panicum virgatum	Switch grass	TNT exposure, rhizodegradation of PAHs
Papaver dubium	Poppy	cyanide tolerance
Paspalum notatum	Bahia grass	TCE metabolism
Pennisetum americanum		Polychlorinated biphenyls (PCBs) transformation
Phalaris arundinacea	Canary grass	Cleanup of explosives and uptake
Phaseolus vulgaris	Bean	Cyanide phytotoxicity, dichlorobenzoate rhizodegradation
Phleum pratense	Timothy grass	Rhizodegradation of 2,4,5 trichlorophenoxyacetic acid
Phragmites sp	Reed	Methyl iodide volatilization
Pistia stratiotes	Water lettuce	Metals accumulation
Plantago major	Plantain	Cyanide tolerance
Poa annua	Bluegrass	Tolerant to inorganic and organic pollutants
Pontederia cordata	Pickerelweed	TNT transformation
Populu stremula	Aspen	Lead extraction, TCE sorption
Potamogeton nodosus	Pondweed	Explosives exposure and uptake
Potamogeton pectinatus	-	explosives phytotoxicity and transformation
Pteris vittata	Brake fern	Arsenic hyper accumulator
Pueraria thunbergiana	Kudzu	Transformation of 1,1,l-trichloro-2, 2-bis-4'chlorophenyl) ethane DDT
Rumex obtusifolius	Dock	Cyanide
Sagittaria latifolia	Arrowhead	Explosives exposure and uptake
Salicornia virginica	Perennial glasswort	Perchlorate tolerance, Brine concentrator
Salix alba	Willow	Cyanide metabolism
Salvinia molesta	Kariba weed	Metals accumulation
Salvinia rotundifolia	Floating moss	TNT transformation
Schizachyrium scoparius	Little bluestem	Rhizodegradation of PAH
Scirpus spp	Bulrush	Used in constructed wetland explosives wastewater treatment
Scirpus validus	-	Brine concentration

Scrophularia nodosa	Figwort	Cyanide tolerance
Senecio glaucus	-	Rhizodegradation of crude oil
Silene vulgaris	Bladder champion	Stabilization of metals
Solanum nigra	Black nightshade	Hairy root cultures
Solidago altissima	golden rod	Degradation of TCE
Solidago hispida	hairy golden rod	Metals hyperaccumulation
Sonchus arvensis	Thistle	Cyanide tolerance
Sorghastrum nutans	Indian grass	Rhizodegradation of PAHs
Sorghum bicolor		Rhizodegradation of petroleum hydrocarbons
Sorghum halepense	Johnson grass	Glutathione transferase
Sorghum vulgare	sudan grass or broom corn	Atrazine metabolism, hexahydro-l ,3,5-trinitro-1 ,3,5-triazine, RDX; phytotoxicity, 217 rhizodegradation of hydrocarbons
Spartina alterniflora	Cordgrass	Saltwater, brine concentration
Spirodela oligorrhiza	giant duckweed	Organic degradation and metals accumulation
Sporobolus virginicus	Coastal dropseed	Brine concentration
Stenotaphrum secundatum	St.Augustine grass	Rhizodegradation of hydrocarbons
Streptanthus polygaloides	-	Nickel hyperaccumulation
Tamarix spp.	Salt cedar	Hydraulic control of arsenic
Taraxacum officinale	Dandelion	Cyanide tolerance
Trifolium pratense	Red clover	Rhizodegradation
Trifolium repens	White Clover	Hydrocarbon rhizodegradation, transformation of PCBs
Trifolium spp	Clover	Methyl bromide removal
Typha angustifolia	-	Degradation of explosives
Typha latifolia	Cat-tail	Biosorption and perchlorate degradation
Vallisneria americana	tape grass	TCE transformation and metals accumulation
Vallisneria spiralis	Eel grass	Metals hyperaccumulation
Vetiveria zizanioides	Vetiver grass	Metal accumulation, integrated land management
Zoysia japonica	Zoysiagrass	Rhizodegradation of PAHs

Annexure 4
Glossary

This is a glossary of terms related to bioremediation. Not exhaustive.

Abiotic: Devoid of life; nonliving.

Acidophile: Organism that grows best under acid conditions (down to a pH of 1).

Adaptation: Change in an organism or population of organisms through which they become more suited to the prevailing environment. Adaptation can be genetic and/or physiological.

Aerobe: An organism that can grow in the presence of air.

Alkalophile: Organism that grows best under alkaline conditions (up to a pH of 10.5).

Allelochemicals: Compounds formed and released by one species with the aim of influencing its surroundings (e.g. other, sensitive plant species and their rhizospheres).

Anaerobe: An organism that grows in the absence of oxygen or air.

Annual: Having a yearly periodicity; living for 1 year.

Anoxic: Literally "without oxygen." An adjective describing a habitat devoid of oxygen.

Anthropogenic: Derived from human activities.

Autotroph: An organism that uses carbon dioxide as its source of carbon for growth.

Bacteria: A group of diverse and ubiquitous prokaryotic single-celled micro organisms.

Bioaugmentation: The addition to the environment of micro organisms that can metabolize and grow on specific organic compounds.

Bioavailability: The availability of chemicals to potentially biodegradative micro organisms.

Biochemical oxygen demand: (BOD) The requirement for molecular oxygen by microbes during oxidation of biological substances in sewage. The BOD test measures the oxygen consumed (in mg/L) over 5 days at 20^{o}C.

Bioconcentration factor: The concentration in aboveground plant parts (on a dry-weight basis) divided by the concentration in the soil.

Biodegradation: The breakdown of organic substances by micro organisms.

Biofilters: Application of bacteria in filters to the decontamination of polluted water and wastes.

Biogeochemical prospecting: Exploration for mineral deposits through analysis of metal concentrations in plants that might indicate underlying ore bodies.

Bioleaching: Specific micro organisms like *Thiobacillus ferrooxidans* and *T. thiooxidans* promote the metals solubilization.

Biomass: The amount of living matter present in a particular habitat.

Biopiling: The material to be treated is piled over an aerated system and nutrients are added to it.

Bioremediation: The process in which living organisms act to degrade or contain contaminant.

Biosorption: Adsortion of metals and other ions of an aqueous solution by the use of biological materials.

Biostimulation: A process that increases activity of micro organisms biodegrading contaminants. For example, addition of nutrients, oxygen, or other electron donors and acceptors.

Biotic: Pertaining to life or living organisms; caused or produced by, or comprising living organisms.

Biotransformation: Alteration of the structure of a compound by a living organism or enzyme.

Bioventing: The process of supplying oxygen *in situ* to oxygen deprived soil microbes by forcing air through unsaturated contaminated soil at low flow rates. This stimulates biodegradation and minimizes stripping volatiles into the atmosphere. Frequently used to remediate soil under structures since it is relatively non-invasive.

Bound residues: Chemical contaminants that are not extractable from plant tissues by conventional methods.

Brownfield: An abandoned, idle, or under-used industrial or commercial facility where expansion or redevelopment is complicated by a real or perceived environmental contamination.

BTEX: Benzene, toluene, ethylbenzene, and xylenes.

Chaff: The dried plant material separated from the seeds.

Chemical oxygen demand (COD): The amount of oxygen in milligrams per litre to oxidize both organic and oxidizable inorganic compounds.

Cometabolism: The biodegradation of a pollutant by an organism while using some other compound(s) for growth and energy. There is little or no benefit to the biodegrading organism, the pollutant just happens to be affected by the growth of the cometabolizing organism.

Community: Any group of organisms belonging to a number of different species that co-occur in the same habitat or area and interact through tropic and spatial relationships; typically characterized by reference to one or more dominant species.

Composting: Nutrients are added to soil that is mixed to increase aeration and activation of indigenous micro organisms.

Consortium: Two or more members of a natural assemblage in which each organism benefits from the other. The group may collectively carry out some process that no single member can accomplish on its own.

Constructed wetlands: Artificial or engineered wetlands used to remediate surface water or waste water.

Creosote: An antifungal wood preservative used frequently to treat telephone poles and railroad ties. Creosote consists of coal tar distillation products, including Phenols and PAHs.

Denitrification: This can sometimes be used to remove nitrate or nitrite from liquid wastes.

Desaturase: Enzyme introducing a carbon–carbon double bond, in this case into a fatty acid in a specific position.

Ecosystem restoration: The process of intentionally altering a site to establish a defined, indigenous ecosystem. The goal of this process is to emulate the structure, function, diversity, and dynamics of the specified ecosystem.

Electron acceptor: Small inorganic or organic compound that is reduced to complete an electron transport chain. Compound that is reduced in a metabolic redox reaction.

Electron donor: Small inorganic or organic compound that is oxidized to initiate an electron transport chain. Compound from which electrons are derived in a metabolic redox reaction.

Enhanced rhizosphere biodegradation: Enhanced biodegradation of contaminants near plant roots where compounds exuded by the roots increase microbial biodegradation activity. Other plant processes such as water uptake by the plant roots can enhance biodegradation by drawing contaminants to the root zone.

Enzymes: Proteins that act as biological catalysts.

Eutrophication: The enrichment of natural waters with inorganic material especially nitrogen and phosphorous such that they support excessive growth of plants and algae. (Compare with Oligotrophic.)

***Ex situ*:** Out of the original position (Excavated).

Exotic: Not native; alien; foreign; an organism or species that has been introduced into an area.

Exudates: Soluble organic matter released from the roots of plants.

Fermentation: An energy yielding metabolism that involves a series of oxidation-reduction reactions in which the substrate and terminal electron acceptor are organic compounds.

Fibrous root: A root system that has numerous fine roots dispersed throughout the soil.

Forb: Any nonwoody plant having broad leaves; a nongrass species.

Fungi: A group of diverse and widespread unicellular and multicellular eukaryotic organisms. Some species are important in the decomposition of plant litter.

Geobotanical prospecting: The visual study of plants as indicators of the underlying hydrogeologic and geologic conditions.

Geobotany: The use of plants to investigate the underlying geology, especially related to metal ores.

genetically modified organism (GMO): An organism with some specific gene(s) introduced or removed artificially.

Halophilic: Organisms whose requirement for salt exceeds that of other organisms.

Halophyte: A salt-resistant plant; one that will grow in saline soil. Salt cedar is an example.

Heterotroph: Any organism that requires exogenous organic material for growth and reproduction.

Humification/fixation: The incorporation of contaminants into biomass in soil.

Hydraulic pumping: Plant roots grow to the water table, take up water and prevent the migration of polluted water.

Hydrologic control: The use of plants to rapidly uptake large volumes of water to contain or control the migration of subsurface water. Synonym: Phytohydraulics.

Hyperaccumulators: Metallophytes that accumulate an exceptionally high level of a metal, to a specified concentration or to a specified multiple of the concentration found in other nearby plants. Alpine pennycress is an example.

***In situ*:** In place, without excavation.

Inoculation: The introduction of a micro organism into a host organism.

Invading: The movement or encroachment of organisms from one area into another.

Isoenzyme: An enzyme that occurs in more than one form in a given species. Sometimes called an isozyme.

Landfarming: Soil is organized in piles and is periodically turned over by agricultural practices to stimulate the degradation by indigenous micro organisms.

Litter: A surface layer of decaying detritus covering the ground.

Macropores: Openings in the soil matrix caused by worms, burrowing animals, old root channels or soil properties that allow the relatively free flow of water and contaminants through soil methods (covalent bonding, polymerization, or lignification within the plant).

Medium: Any material that supports growth of an organism.

Mesophile: An organism whose optimum growth range is 20-45 ^{o}C.

Metallophytes: Plants that can only grow in metal-rich soils.

Metal-tolerant plants: Plants that can grow in metal rich soils without accumulating the metals.

Methanogen: Bacteria that anaerobically oxidize hydrogen to methane and water using carbon dioxide as the electron acceptor. These occur in anaerobic muds, ponds, and sewage sludge.

Microclimate: The climate of the immediate surroundings or habitat.

Microcosm: A community or other unit that is representative of a larger unity. (Miniature ecosystem)

Microflora: All of the micro organisms associated with location or environment.

Micronutrient: Chemical element necessary for growth found in small amounts, usually <100 mg kg^{-1} in a plant. These elements consist of B, Cl, Cu, Fe, Mn, Mo, and Zn.

Micro organisms: Includes bacteria, algae, fungi, and viruses.

Mineralization: The breakdown of organic matter to inorganic materials (such as carbon dioxide and water) by bacteria and fungi.

Minimal medium: Culture medium that lacks certain growth factors so that it will support growth of only certain types of micro organisms.

Most probable number (MPN): A method for estimating the concentration of micro organisms in a sample. A given volume of liquid or suspension is inoculated into each of (typically) 5 tubes containing growth media. Decreasing volumes are inoculated into successive sets of 5 tubes. After an incubation period the tubes are scored for growth or lack of growth. Those tubes in which growth occurred are assumed to have contained at least one VIABLE organism in the inoculant. The concentration of viable micro organisms in the original liquid or suspension is calculated using a statistical table.

Mycelium: (plural, mycelia) Mass of hyphae that form the vegetative body of many fungal organisms.

Mycobacterium: A genus of aerobic bacteria found in soil and water that are capable of biodegrading multi-ring compounds such as PAHs.

Mycorrhiza: A mutually beneficial association between a fungus and the root of a plant. These occur in a wide range of plants including trees, shrubs, and herbaceous plants.

NAPL: Non-aqueous phase liquid. This can be lighter than water (LNAPL), or more dense than water (DNAPL).

Natural attenuation: Use of natural processes to contain the spread of contamination from chemical spills and reduce the concentration and amount of pollutants at contaminated sites. Natural attenuation-also referred to as intrinsic remediation, bioattenuation, or intrinsic bioremediation is an *in situ* treatment method. This means that environmental contaminants are left in place while natural attenuation works on them. Natural attenuation is often used as one part of a site cleanup that also includes the control or removal of the source of the contamination.

Nitrate respiration: (dissimilatory nitrate reduction) The use of nitrate as a terminal electron acceptor for anaerobic respiration. This process occurs under anaerobic or microaerophilic conditions. Not all bacteria are capable of this form of metabolism and the nitrate may not be reduced completely to nitrogen gas (stopping at nitrite, for example). When the nitrate is reduced to gaseous forms the process is called.

Nitrification: The oxidation of ammonia to nitrite and then nitrate by bacterial species such as Nitrosomonas and Nitrobacter, respectively. This process is strictly aerobic.

Nitrogen fixation: The reduction of gaseous nitrogen to ammonia or other inorganic or organic compound by micro organisms.

Nodule: A small knot on a stem or root or leaf, especially one containing nitrogen-fixing bacteria.

Obligate: Any state or condition that is an essential attribute of a given organism. For example, an obligate aerobe can grow only under aerobic conditions.

Oligotrophic: Bodies of water poor in those nutrients that support growth of aerobic photosynthetic organisms. (Compare with eutrophication).

Organic pump: Uptake of large quantities of water by plant (trees) roots and translocation into the atmosphere to reduce a flow of water. Used to keep contaminated groundwater from reaching a body of water, or to keep surface water from seeping into a capped landfill and forming leachate.

Oxidase: An enzyme that catalyses a reaction in which electrons are removed from a substrate and donated directly to molecular oxygen.

Oxygenase: An enzyme that catalyses a reaction in which one (monooxygenase) or both (dioxygenase) atoms of molecular oxygen are incorporated into a molecule of substrate. Oxygenases catalyze the first step in degradation of strait-chained and aromatic hydrocarbons.

Pesticides: Compounds toxic to pests.

Phenol: Carbolic acid (C_6H_5OH). Phenols and substituted phenols.

Phreatophyte: A deep-rooted plant that obtains water from the water table.

Phytoaccumulation: The uptake and concentration of contaminants (metals or organics) within the roots or aboveground portion of plants. See Phytoextraction.

Phytocapping: Plants consume water from the rainfall and reduce leaching and pollutant movement.

Phytodegradation: The breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants. Synonym: Phytotransformation OR A process in which plants are able to degrade (break down) organic pollutants through their metabolic processes.

Phytoextraction coefficient: The ratio of metal concentration in the plant (g metal/g dry weight tissue) to the initial soil concentration of the metal (g metal/g dry weight soil), for phytoextraction of metals.

Phytoextraction: The uptake of contaminants by plant roots and translocation into the aboveground portion of the plants, where it is generally removed by harvesting the plants. This technology is most often applied to metal-contaminated soil or water. See also: Phytoaccumulation. OR Use of plants to extract contaminants (such as metals) from the environment (especially soil). When the plants are saturated with contaminants, they are harvested.

Phytoinvestigation: The examination of plants at a site for information about contaminant presence, distribution, and concentration.

Phytomining: Use of plants to extract inorganic substances of economic value (precious metals, etc.)

Phytoremediation cap (or cover): A cap consisting of soil and plants, designed to minimize infiltration of water and to aid in the degradation of underlying waste OR The direct use of living green plants for *in situ* risk reduction for contaminated soil, sludges, sediments, and groundwater, through contaminant removal, degradation, or containment. Synonyms: Green remediation, Botanoremediation.

Phytoremediation: Use of plants to extract, sequestrate or decontaminate terrestrial or aquatic environments.

Phytosorption: Adsorption of pollutants by plant roots and leaves and prevention of the pollutant movement.

Phytostabilisation: Process in which plants are exploited to prevent migration of environmental contaminants to sites where they may pose a danger to human health.

Phytotransformation: The uptake and transformation (metabolism) or volatilization of organic.

Phytovolatilization: Plants take up the pollutants along with water, pollutants pass through xylem and are released from foliage.

Plasmid: Extra DNA in a cell that is usually dispensable, but may confer an advantage to the cell, such as the ability to biodegrade certain compounds or resistance to antibiotics.

Propagule: Any part of an organism, produced sexually or asexually, that is capable of giving rise to a new individual. (Or) The minimum number of individuals of a species required for colonization of a new or isolated habitat.

Psychrophile: An organism with an optimum growth temperature less than 20 °C.

Recalcitrant: Resistant to biodegradation.

Redox potential: The oxidation-reduction potential of an environment. Measures the tendency of the environment to be reducing (donate electrons) or oxidizing (accept electrons).

Reductive dechlorination: Removal of Cl as Cl- from an organic compound by reducing the carbon atom from C-Cl to C-H.

Respiration: Energy yielding metabolism in which oxygen is the terminal electron acceptor for substrate oxidation.

Rhizodegradation: The breakdown of a contaminant in soil through microbial activity that is enhanced by the presence of the root zone. Synonyms: Plant-assisted degradation, Plant-assisted bioremediation, Plant-aided *in situ* biodegradation, Enhanced rhizosphere biodegradation.

Rhizofiltration: Plant roots growing in polluted water precipitate and concentrate metals.

Rhizome: A creeping stem lying usually horizontally at or under the surface of the soil and differing from a root in having scale leaves, bearing leaves or aerial shoots near its tips, and producing roots from its undersurface.

Rhizoplane: The surface of plant roots.

Rhizoremediation: Exploitation of micro organisms within the root zone of plants to remove contaminants from the environment. Sexual pheromone: a compound for chemical communication between females and males within one species.

Rhizosphere bioremediation: The microbial transformations of organic contaminants by bacteria, fungi, and protozoans within the biologically-rich zone of the immediate vicinity around plant roots, OR Soil in the area surrounding plant roots that is influenced by the plant root. Typically a few millimeters or at most centimeters from the plant root. Important because this area is higher in nutrients and thus has a higher and more active microbial population.

Root concentration factor (RCF): The concentration in the roots divided by concentration in external solution, for non-ionized organic compounds taken up by plants with nonwoody stems.

Root exudates: Chemical compounds such as sugars or amino acids that are released by roots.

Root turnover: The rapid decay of fine roots in the soil profile by endogenous respiration.

Runoff: That part of precipitation that is not held in the soil but drains away freely.

Salvage: The saving, storage, and use of plant material which would otherwise be lost to disturbance.

Seed coat: The outer layer of the seed.

Siderochromes: Compounds produced by micro organisms that are involved with

Siderophores: See siderochromes.

Spores: (bacterial endospores) A metabolically dormant state of bacteria in which they are more resistant to heat, chemicals, etc. (Compare with Vegetative.)

Surfactant: A natural or synthetic chemical that promotes the wetting, solubilization, and emulsification of various types of organic chemicals.

Thermophile: Any organism that has an optimum growth temperature to the soil surface.

Transgenic plant: GMO, plant with some specific gene(s) introduced or removed artificially.

Transpiration stream concentration factor (TSCF): The concentration in the transpiration stream divided by the concentration in the external solution, for organic compounds.

Turgor: The swollen condition of a cell caused by internal water pressure.

Vadose zone: Unsaturated zone of soil above the groundwater.

Vegetative cap (or cover): A long-term, self-sustaining cap of plants growing in and/or over materials that pose environmental risk; a vegetative cover reduces that risk to an acceptable level and requires minimal maintenance. Two specialized types of vegetative caps are the Evapotranspiration Cap and the Phytoremediation Cap.

Vegetative soil stabilization: The holding together of soil by plant roots to decrease wind or water erosion or dispersion of soil.

Vegetative: Cells with an active metabolism. Not dormant or Spores.

Waterbars: A transverse levee designed to reduce erosion by slowing and diverting water flow.

Weathering: All physical and chemical changes produced by

White rot fungi: Fungi that decompose all components of wood. Important because they produce enzymes that are capable of acting on and biodegrading a wide variety of compounds, including many pollutants.

Xenobiotic: Compound foreign to biological systems. Often refers to human-made compounds that are resistant or recalcitrant to biodegradation and decomposition.

Xerophile: Organism adapted to grow at low water potential, i.e., very dry habitats.

Annexure 5
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About the Author

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He is the author, co-author, editor, or co-editor for eight books (John Wiley and Sons Inc.New York; CRC Press, Boca Raton; Springer-Verlag Heidelberg; Narosa Publishing House, New Delhi; Kluwer Academic Publishers, Dordrecht; Marcel Dekker, New York).

Published 216 articles, 134 research papers in peer reviewed journals and 82 book chapters and conference proceedings in the broad area of Environmental botany and heavy metal stress in plants.

He received the B.Sc. (1973) and M.Sc. (1975) degrees from Andhra University, India, and the Ph.D. degree (1979) in botany from the University of Lucknow, India.

He made outstanding contributions to "Plant-Metal interactions" from molecules to ecosystem. Cadmium inducible proteins in *Scenedesmus quadricauda* were reported first time from India (Curr. Sci. 58: 1380-381, 1989). Also discovered cadmium-induced HSP cognate in *Zea mays* L. and highlighted their role as molecular chaperones (Biochem. Arch. 9: 25-32, 1993). This new concept is accepted as a potential metal detoxification mechanism (Neumann *et al*, 1994, Planta, 194, 360-367; Hall, 2002, J Exp. Botany 52, 1-11). The author's work on Phytoremediation of trace metals received worldwide recognition and secured membership in COST action 859 on Phytotechnologies [2004-2009] supported by European Science Foundation (the only member from India).

Academic distinctions

Pitamber Pant National Environment Fellow of 2007, awarded by MoEF, GOI

Visiting Professor, Biology Faculty, Botany Department, al-Farabi Kazakh National University, Almaty Republic of Kazakhstan - 2006 May & 2007 May
Visiting Professor – CERAR, Univ. South Australia, Mawson Lakes, Australia Nov-Dec 2005
Visiting Professor, Finnish Academy, Dept. of Biology, Univ. of Oulu , Finland 2003
Visiting Fellow – SIDA- Sweden, 2000 , Dept. Botany, Stockholm University
Visiting Professor – FCT- Portugal, 1998-2010 Dept Botanica, Univ. de Coimbra (annual visits)
UGC Indo-Polish Cultural Exchange Visiting Fellow, Jagiellonian Univ. Krakow Poland -1996
NSERC, Canada, Foreign researcher awardee, Univ du Québec, INRS-Eau - 1994
Elected Fellow, Linnean Society of London, UK, 1998
Elected Fellow, National Institute of Ecology, New Delhi, 1998

Service to International journals as member of the editorial boards

Acta Physiologiae Plantarum, Associate Editor, Springer (tenure completed)
Environmental and Experimental Botany, Elsevier (tenure completed)
Biological Diversity and Conservation, Turkey
European Journal of Mineral Processing and Environmental Protection ,Turkey
The Scientific World-Online multidisciplinary journal
Bioremediation, Biodiversity and Bioavailability, GSB, UK
Functional Plant Science and Biotechnology, GSB, UK
Medicinal and Aromatic Plant Science and Biotechnology , GSB, UK
Terrestrial and Aquatic Environmental Toxicology, GSB, UK
The Asian and Australasian Journal of Plant Science and Biotechnology, GSB, UK