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ADVANCES IN PHYTOREMEDIATION AND RHIZOREMEDIATION

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Abstract: In the rapidly developing world, the contamination of soil, water and air become a serious threat for our environment. Answer lies in adaptation of clean and green technology to remediate the polluted sites in a sustainable ways without depleting the natural resources. This process of Phytoremediation and Rhizomediation has proved to help in amending water and soil pollution successfully without any side effect and help in maintaining the health of environment. There are several methods of Phytoremediation and Rhizoremediation with much option to be chosen for specific types of contaminant and medium. These are eco-friendly method which would help to safeguard natural resources without causing any harm or depleting the quality of environment, which offers the possibility to destroy or render harmful various contaminants by using natural biological activity of plants and microbes to degrade organic compounds in to simple inorganic compounds. The paper deals with recent advancement in the field of Phytoremediation and Rhizomediation and their significant role in combating environmental pollution.

Keywords: Phytoremediation, Rhizoremediation, Pollution, Soil, Water.

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INTRODUCION

The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source; however, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising environmental technology known as phytoremediation. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. The term phytoremediation (phyto = plant and remediation = solving a problem) is relatively new, coined in 1991. Phytoremediation, the use of plants to remove, detoxify, or immobilize environmental contaminants, is being pursued as a new

approach for the cleanup of contaminated soils and waters, including groundwater. In recent years, phytoremediation has received much attention as an innovative and cost-effective alternative to the more established treatment methods used at hazardous waste sites. The application of phytoremediation technique results in reduction and/ or removal of contaminants without the need of excavation and hence is a relatively low cost technology compared to the other methods. The technique could provide cost-effective method of remediating soils and groundwater contaminated with metals, radionuclides, and various types of organics, with fewer secondary wastes and less environmental impact than would be generated using traditional remediation methods. Soil and water

contaminated with metals pose a major environmental and human health problem that is still in need of an effective and affordable technological solution. Nonradioactive As, Cd, Cu, Hg, Pb and Zn and radioactive Sr, Cs and U (referred to here as toxic metals) are the most environmentally important metallic pollutants. Microbial bioremediation has been somewhat successful for the degradation of certain organic contaminants, but is ineffective at addressing the challenge of toxic metal contamination, particularly in soil. Although organic molecules can be degraded, toxic metals can only be remediated by removal from soil. Phytoremediation takes advantage of the fact that a living plant can be considered a solar-driven pump, which can extract and concentrate particular elements from the environment. Phytoremediation is becoming possible because of the productive interdisciplinary cooperation of plant biochemists, molecular biologists, soil chemists, agronomists, environmental engineers and federal and state regulators. The metals targeted for phytoremediation include Pb, Cd, Cr, As and various radio-nuclides. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying or composting. The volume of toxic waste produced as a result is generally a fraction of that of many current, more invasive remediation technologies, and the associated costs are much less. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues.

Applicability

Contaminants that have been remediated in laboratory and/or field studies using phytoremediation or plant-assisted bioremediation include:

- Heavy metals (Cd, Cr (VI), Pb, Co, Cu, Pb, Ni, Se, Zn),
- Radionuclides (Cs, Sr, Ur),
- Chlorinated solvents (TCE, PCE),
- Petroleum hydrocarbons (BTEX),
- Polychlorinated biphenyls (PCBs),
- Polynuclear aromatic hydrocarbons (PAHs),

- Chlorinated pesticides,
- Organophosphate insecticides e.g. parathion,
- Explosives (TNT, DNT, TNB, RDX, HMX),
- Nutrients (nitrate, ammonium, phosphate),
- Surfactants.

Site Conditions

Phytoremediation and plant-assisted bioremediation are most effective if soil contamination is limited to within 3 feet of the surface, and if groundwater is within 10 feet of the surface. These technologies are applicable to sites with low to moderate soil contamination over large areas, and to sites with large volumes of groundwater with low levels of contamination that have to be cleaned to low (strict) standards.

PROCESSES OF PHYTO-REMEDICATION

Studies have shown that many plants known as hyper-accumulators have the ability to extract toxic metals from soil and water. Hyper-accumulator plants are able to grow in soils with very high concentration of metals and can accumulate these metals through their root system, to a concentration far greater than that present in the surrounding soil. For an example, the plant Alpine Pennycress (*Thlaspi caerulescens*), accumulates high levels of cadmium and zinc from the soil. The bracken fern (*Pteridium aquilinum*) extracts arsenic from the soil at a much greater rate than other plants. The arsenic is stored in the fern's leaves at as much as 200 times that present in the soil (Ma *et al.*, 2001). Sunflowers were also used to clean up uranium near Chernobyl. These plants can be subsequently removed, processed and disposed. Hyper-accumulator plants differ from non-hyperaccumulators because of a high rate of metal uptake, a rapid root to shoot translocation and storage of high concentration of toxic metals in their leaves and root without suffering toxic effect. The basic mechanism involves absorption of metals onto roots or complexation or metal valence reduction and consequently precipitation of metals within the root zone of plants. Remediation of metal contaminated soils became a goal for many research laboratories in the

world. The use of plants in designing low cost treatment system is still a challenge in environmental managements. Different species of plants have been used in various applications including: *Salix* spp. (hybrid Poplars, Cottonwoods, and Willow), grasses (Rye, Bermuda grass, Sorghum, Fescue, Bullrush), legumes (Clover, Alfalfa, and Cowpeas), aquatic plants (Parrot feather, Duckweed, arrowroot, Cattail, Pondweed), and hyper accumulators for metals (Sunflowers, Indian mustard, and *Thlaspi* spp.). A comparative physiological and molecular analysis of hyper-accumulators and non hyper-accumulators has revealed the presence of different set of gene regulation and expression in hyper-accumulator plants. In hyper-accumulator plants it is due to the over expression of certain constitutive gene that encode for the formation of heavy metal transporters such as the members of ZIP, HMA, MATE, YSL and MTP families (Rasico and Navari-Izzo, 2011). Phytoremediation is based on certain natural processes carried out by plants including:

- Uptake of metals and certain organic compounds *i.e.* moderately water soluble such as BTEX, from soil and water.
- Accumulation or processing of these chemicals via lignification, volatilization, mineralization, metabolization, (transformation into CO₂ and water);
- Use of enzymes to breakdown complex organic molecules into simpler molecules (ultimately CO₂ and water);
- Increasing the carbon and oxygen content of soil around roots (and so promoting microbial/ fungal activity) through release of chemicals (exudates) and decay of root tissue;
- Capture of groundwater (even contaminated groundwater) and utilization for plant processes.

It is due to the unique metabolic diversity and absorption capabilities of plants that they can up take contaminants along with nutrients from the contaminated soil or water. Studies have shown that many plants known as hyper-accumulators have the ability to extract toxic metals from soil

and water. Hyperaccumulator plants are able to grow in soils with very high concentration of metals and can accumulate these metals through their root system, to a concentration far greater than that present in the surrounding soil.

TYPES OF PHYTOREMEDIATION

There are several different types of phytoremediation process. Depending on the primary mechanism, applicability, and type of contaminants, phytoremediation can be broadly categorized as:

- a) *Phytoextraction*: This is the uptake and accumulation of contaminants from the environment into the plant biomass.
- b) *Phytostabilization*: Phytostabilization involves immobilization of metals in soil, for example by binding of contaminants into the soil matrix.
- c) *Phytotransformation or Phytodegradation*: It refers to the uptake of contaminant into plant and their metabolic degradation within the plant tissue.
- d) *Phytostimulation or Rhizodegradation*: It refers to the enhancement of soil microbial activity by the substance released from the plants root. This further increases the biodegradation of contaminants.
- e) *Phytovolatilization*: It involves the uptake of the contaminants through the plant root and finally transformation and volatilization into the atmosphere.
- f) *Rhizofiltration*: Rhizofiltration involves plants root system to absorb, concentrate and precipitate the toxic metals from contaminated water. This system can be used for *ex-situ* treatment of polluted water.

PHYTO-REMEDICATION OF METAL CONTAMINATED SITES

Phytoextraction: Phytoextraction refers to the process whereby plant roots absorb metals from the contaminated soil or water and transport them to their above ground parts such as stem and leaves. Plants, known as hyper-accumulators, absorb metals at a level higher to those toxic to other plants. Example includes

Atriplex, *Brassica*, *Helianthus*, *Kochia*, *Pelargonium*, *Pinus*, *Salicornia*, and *Thlaspi*. At maturity, metal enriched plants are harvested and either incinerated or decomposed safely. Plants have a natural tendency to take up metals. Such as Cu, Co, Fe, Mo, Mn, Ni, and Zn, are essential mineral nutrients for plant growth. The identification of plants capable of hyper-accumulating toxic metals, demonstrates that plants have genetic potential to clean up contaminated site. This technique is mostly used for the removal of heavy metals and radionuclides in soil, water, sediments, and sludge. Phytoextraction is primarily used in the treatment of soil, sediments and sludge. It can be used to a lesser extent for treatment of contaminated water. Prior to the uptake of metals by the plant roots, the metal must be mobilized in the soil. Mobilization process makes the soil bound metal into soluble state, which can be easily taken up by the plants root. The mobilization of 'soil-bound' metal can be accomplished in a number of ways. Metal-chelating molecules can be secreted by plants into the soil to chelate and solubilize soil-bound metal. Some examples of such metal chelators are phytosiderophores (iron-chelating compounds), organic acids or carboxylates. Moreover, these chelators conceal the metal and thus the plant tissues are protected from the toxic effect. Usually, the phytosiderophores are secreted in response to iron deficiency and can, in principle, mobilize Cu, Zn and Mn from soil. Phytosiderophores such as mugineic and deoxymugeneic acids from barley and corn and avenic acid from oats are the most studied plant siderophores (Kinnerseley, 1993). Roots can increase the bioavailability of metal by acidifying the soil environment with protons extruded from them or by plasma membrane bound metal reductases, which may increase metal availability. Once the metal is mobilized it is then taken up by plant roots from the soil solution and exported to the shoots. Transport of toxic metal in plants is not well understood, however some studies have shown that some toxic metals may

be transported to the shoot through complexation with organic acids, mainly citrate.

Advantages: The plant biomass containing the extracted contaminant can be a resource. 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.

Disadvantages: Metal hyper-accumulators are generally slow-growing with a small biomass and shallow root systems. Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass. Hyper-accumulators may accumulate significant amount of metals e.g. *Thlaspi rotundifolium* grown in a lead-zinc mine area contained 8,200 g/g Pb (0.82%) and 17,300 g/g zinc (Zn) (1.73%), and *Armeria maritima* var. *halleri* contained 1,300 g/g Pb, dry weight basis (Reeves and Brooks, 1983). Metals may have a phytotoxic effect (Nandakumar *et al.*, 1995). Phytoextraction studies conducted using hydroponically grown plants, with the contaminant added in solution, and may not reflect actual conditions and results occurring in soil.

Rhizofiltration: Rhizofiltration refers to the use of plant roots to remove toxic metals from aqueous environment. The process involves uptake, accumulation, and precipitation of toxic metals from contaminated water rather than from soil. Rhizofiltration allows in-situ treatment, and minimizes the cost of transport. Plants are grown directly in the contaminated water body or in the form of hydroponics, where the dense root system can efficiently remove metals from aqueous solution. These plants continue to extract contaminant until they are harvested. The plants are then harvested and fresh plants are again grown and harvested until a satisfactory level of contaminant reduction is achieved. Rhizofiltration can be used for the removal of metals from surface water and groundwater, industrial and domestic effluents, acid mine drainage, agricultural runoffs and radionuclide contaminated solutions. Different plant species have been shown to successfully remove toxic

metals such as copper, cadmium, chromium, nickel, lead and zinc from aqueous solutions. Trees have also been successively used for rhizofiltration. The most commonly used trees are willow and poplar tree. These trees act like a pump and can cycle large amount of water per day per tree.

Phytostabilisation: Phyto-stabilisation is the use of certain plant species that can immobilize metals by various mechanisms in soil or water and thus reduces their mobility in water or soil. The basic mechanism involves absorption of metals onto roots or complexation or metal valence reduction and consequently precipitation of metals within the root zone of plants. Such plants should be able to tolerate high levels of metal contaminants, should have high production of root biomass, and have the ability to hold metals in the roots. The target areas for the application of phytostabilisation are usually large barren surfaces caused by mining operations or by aerial deposition of metals from metal smelters. Sometimes soil additives such as organic matter, phosphates, alkalizing agents, and biosolids are added in soil to convert the soluble and exchangeable metals to more geochemically stable solid phase resulting in reduced biological availability of toxic metals. Various studies have shown that phytostabilisation may reduce underground metal leaching by converting metals from a soluble oxidation state to an insoluble oxidation state. Also the higher transpiration rate reduces water percolation through the soil and thus restricts the formation of hazardous leachates. Plant-cover on the surface of the contaminated soil or sludge helps to prevent the exposure of contaminant to wind, water, and direct contact with humans. This also serves as barrier against soil erosion. The method is useful to keep metals in their current location where other methods are not feasible to remove metals from large areas. The method is used at sites with shallow contaminations and where the concentration of metals is relatively low.

Limitations:

- The site should be monitored continuously to make sure that the conditions for stabilization remain unaltered, as the contaminants are left in place.
- If soil additive are used, they should be periodically reapplied to maintain effective immobilization.
- Cannot be successful in areas with high concentration of toxic metals, unless extensive amendments were done (such as additive application).

PHYTOREMEDIATION OF ORGANIC CONTAMINANTS

Phytodegradation: Phytodegradation, also referred as phytotransformation, describes the breakdown and mineralization of organic contaminants by the internal or external metabolic functions of the plants. External metabolic function implies the secretion of enzymes, in the rhizosphere zone, where they hydrolyze and/or degrade complex organic pollutants into simpler molecules that are further incorporated into plant tissue. External degradation by enzymes is essential for contaminants that cannot be taken up by the plants due to their large size and complexity. Various types of plant enzymes have been discovered, that breakdown pesticides, explosives, hydrocarbons, ammunition waste, and other xenobiotic compounds (Table 1). Various plant species have been studied for the phytoremediation of organic contaminants (Table 2). Some plant species studies include; Poplars, Brassica spp., and the tropical tree *Leuceana leucocephala* for dehalogenation; Poplar and other herbaceous plants to detoxify gasoline additives; Rye, Leuceana, and Curcurbita to degrade pesticides; Arabidopsis, Parrot feather, Tobacco, Poplar, Canola, Alfalfa, and Bean for the degradation of explosives; and Rye, Fescue, Poplar, Willow, Pothos, Kandelia, Brugiera, and Californian grasses for detoxifying contaminants derived from petroleum.

Advantages: Contaminant degradation due to enzymes produced by a plant can occur in an environment free of microorganisms. Plants are able to grow in sterile soil and also in soil that has concentration levels that are toxic to microorganisms. Thus, phytodegradation potentially could occur in soils where biodegradation cannot.

Disadvantages: Toxic intermediates or degradation products may form. In a study unrelated to phytoremediation research, PCP was metabolized to the potential mutagen tetrachlorocatechol in wheat plants and cell cultures (Komossa *et al.*, 1995). The presence or identity of metabolites within a plant might be difficult to determine; thus contaminant destruction could be difficult to confirm.

Rhizodegradation: Rhizodegradation also known as phytostimulation or plant-assisted degradation is the term used to describe the degradation of organic contaminants in the soil by the activity of microorganism inhabiting the rhizosphere. Plants root secretes certain organic compounds (root exudates consisting of sugars, alcohol, and organic acids) which provides additional nutrients and stimulates the growth of soil microorganisms. Certain microorganisms are capable of degrading hazardous pollutants such as petroleum hydrocarbons into nontoxic and harmless products. Additionally, the root growth loosens the soil and thus increases the supply of water and air to the microorganism. Plant roots can affect soil conditions by increasing soil aeration and moderating soil moisture content, thereby creating conditions more favorable for biodegradation by indigenous microorganisms. Thus, increased biodegradation could occur even in the absence of root exudates. One study raised the possibility that transpiration due to alfalfa plants drew methane from a saturated methanogenic zone up into the vadose zone where the methane was used by methanotrophs that cometabolically degraded TCE (Narayanan *et al.*, 1995). The chemical and physical effects of the exudates and any associated increase in

microbial populations might change the soil pH or affect the contaminants in other ways.

Advantages

- Contaminant destruction occurs *in situ*.
- Translocation of the compound to the plant or atmosphere is less likely than with other phytoremediation technologies since degradation occurs at the source of the contamination.
- Mineralization of the contaminant can occur.
- Low installation and maintenance cost as compared to other remedial options.

Disadvantages

- Development of an extensive root zone is likely to require substantial time.
- Root depth can be limited due to the physical structure or moisture conditions of the soil.
- The rhizosphere might affect an increase in the initial rate of degradation compared to a non-rhizosphere soil, but the final extent or degree of degradation might be similar in both rhizosphere and non-rhizosphere soil.
- Plant uptake can occur for many of the contaminants that have been studied. Laboratory and field studies need to account for other loss and phytoremediation mechanisms that might complicate the interpretation of rhizodegradation. For example, if plant uptake occurs, phytodegradation or phytovolatilization could occur in addition to rhizodegradation.
- The plants need additional fertilization because of microbial competition for nutrients.
- The exudates might stimulate microorganisms that are not degraders, at the expense of degraders.
- Organic matter from the plants may be used as a carbon source instead of the contaminant, which could decrease the amount of contaminant biodegradation.

In laboratory sediment columns, debris from the salt marsh plant *Spartina alterniflora* decreased the amount of oil biodegradation. This has been explained due to competition for limited oxygen and nutrients between the indigenous oil-

degrading microorganisms and the microorganisms degrading plant organic matter (Molina *et al.*, 1995).

Phytovolatilization: Phytovolatilization is the process whereby plants absorb organic contaminant and release them into the air through the process of transpiration. In the process certain contaminants are broken down and are modified to volatile components. The rate of uptake and transpiration of contaminants

depends strongly on its physical nature. For example, volatile compounds such as BTEX (benzene, toluene, ethylbenzene, and xylene), TCE (trichloroethylene) and MTBE (Methyl tertiary butyl ether) can be easily phytovolatilized into the atmosphere. These compounds when released in the atmosphere are quickly photo degraded. Phytovolatilization has mainly been applied to groundwater, but it can be applied to soil, sediments and sludge.

Table 1. List of important enzymes associated with phytodegradation

Enzyme	Target organic contaminants
Dehalogenase	Chlorinated solvents (perchloroethylene (PCE), TCE and dichloroethylene (DCE))
Cytochrome P450 monooxygenase	Chlorinated solvents (perchloroethylene (PCE), TCE and dichloroethylene (DCE), Xenobiotics (PCBs)
Glutathione s-transferase	Organophosphorus insecticides
Peroxygenases	Xenobiotics
Peroxidases	Polycyclic aromatic hydrocarbon, organochlorines, TNT, Chlorinated solvents, phenolic compounds and dye
Laccases	Chlorinated solvents and phenolic compounds
Tyrosinase	Chlorinated solvents and phenolic compounds
N-glucosyltransferases	Xenobiotics
Nitrilase	Nitrile group containing herbicides e.g. bromoxynil
Nitroreductase	Explosives like trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
N-malonyltransferases	Xenobiotics
O-demethylase	Alachlor, metalachor
O-glucosyltransferases	Xenobiotics
O-malonyltransferases	Xenobiotics
Phosphatase	Pesticides (Organophosphates)
Esterases	Ester containing xenobiotics (triacin and p-nitrophenylaceta), (herbicide e.g. 2,4-D (2,4-di-chlorophenoxy) acetic acid.
Aryl acylamidase	Acylanilide herbicides

Table 2. List of some plants studied for their effects on contaminants (Adapted and modified from Newman and Reynolds, 2004).

Plants studied	Contaminants	Effect studied
Poplar	TCE (trichloroethylene)	Metabolism, volatilization
Poplar	MTBE (methyl-tert-butyl ether)	Volatilization
Poplar	Carbon tetrachloride, Tetrachloroethane	Volatilization
<i>Leuceana</i> , Poplar rhizosphere	TCE	Metabolism
<i>Brassica</i>	2,4-Dichlorophenol	Metabolism
<i>Hellianthus</i>	Benzotriazoles	Metabolism
Herbaceous plants, pine	MTBE	Metabolism
<i>Leuceana</i>	EDB (ethylene dibromide)	Metabolism
Rye	Trifluralin, Lindane	Uptake
<i>Curcubita</i>	DDE (2,2-bis(p-chlorophenyl)-1,1-dichloroethylene)	Uptake
Parrot feather, Canna	Simazine	Uptake
Arabidopsis	TNT	Metabolism
Poplar, Bean, Alfalfa, Canola	HMX (octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine)	Metabolism
Poplar endophytes	TNT, RDX, HMX	Metabolism
Tobacco, poplar	Perchlorate	Metabolism

Table 3. Phytoremediation processes, mechanisms, and plant species used for removal of contaminants
(Mukhopadhyay and Maiti, 2010)

Process	Mechanism	Media	Contaminants	Typical Plants
Rhizodegradation	Degradation by plant rhizosphere microorganisms	Soil, Sediments, Land application of wastewater	Organic contaminants (pesticides, aromatics and polynuclear aromatic hydrocarbons [PAHs])	Phenolics releasers (mulberry, apple, orange); Grasses with fibrous roots (rye, fescue, Bermuda) for contaminants 0-3 ft deep; Phreatophyte trees for 0-10 ft; Aquatic plants for sediments
Rhizofiltration	Rhizosphere accumulation	Water	Metals (Pb, Cd, Zn, Ni, Cu) Radionuclides (137Cs, 90Sr, 238U) Hydrophobic organics	Aquatic Plants: - Emergents (bullrush, cattail, pondweed, arrowroot, duckweed); - Submergents (algae, stonewort, parrot feather, <i>Hydrilla</i>)
Phytoextraction	Hyperaccumulation	Soil, Sediments	Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb, Selenium.	Sunflowers, Indian mustard, Rape seed plants, Barley, Hops, Crucifers, Serpentine plants
Phytodegradation	Degradation in plant	Soil, Groundwater, Landfill leachate, Land application of wastewater	Herbicides (atrazine, alachlor) Aromatics (BTEX) Chlorinated aliphatics (TCE) Nutrients (NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻) Ammunition wastes (TNT, RDX)	Phreatophyte trees (poplar, willow, cottonwood); Grasses (rye, bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)
Phyto-stabilization	Complexation	Soil, Sediments	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) Hydrophobic Organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT, dieldrin)	Phreatophyte trees to transpire large amounts of water for hydraulic control; Grasses with fibrous roots to stabilize soil erosion; Dense root systems are needed to sorb/bind contaminants
Phytovolatilization	Volatilization by leaves	Soil, Groundwater, Sediments	Mercury, Selenium, Tritium	Poplar, Indian mustard, Canola, Tobacco plants.

Advantages

- Contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas.
- Contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation.

Disadvantages

- The contaminant or a hazardous metabolite (such as vinyl chloride formed from TCE) might be released into the atmosphere. One study indicated TCE transpiration, but other studies found no transpiration.

- 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 (Newman *et al.*, 1997).

Harvesting/Disposal of Plant Material

Once plants have accumulated waste materials, plant shoots can be harvested and roots removed, with disposal or subsequent processing methods dependent on the toxicity of the end products of in-plant organic chemical processing and the storage locations and relative concentrations of contaminants within plant tissue. If organic contaminants are degraded to

harmless compounds, disposal may not be required. If significant accumulation takes place only in roots, then only these tissues must be disposed of or processed. The most commonly mentioned process for dealing with metals-enriched plant material is controlled incineration, which results in ash with a high metals content. It is hoped that an economically feasible method of metals recovery from this ash will be developed, further reducing the environmental impacts of this technology. Radioactively-contaminated plant material could be vitrified as with other radioactive wastes. Conventional disposal methods such as land filling may also be possible in some instances. Other methods of plant tissue treatment currently under investigation include:

- Sun, heat, and air drying.
- Composting.
- Pressing and compacting.
- Leaching.

GROUNDWATER REMEDIATION

Rhizofiltration: Surface water rhizofiltration may be conducted *in situ*, with plants being grown directly in the contaminated water body. If groundwater is located within the rhizosphere (root zone), rhizofiltration of groundwater can also be *in situ*. Alternately, rhizofiltration may involve the pumping of contaminated groundwater into troughs filled with the large root systems of appropriate plant species. The large surface areas provided by these root systems allow for efficient absorption of metals from the contaminated groundwater into root tissues. In addition to removal through absorption, metals are also removed from groundwater through precipitation caused by exudates (liquids released from plant tissues). These precipitates are filtered from the groundwater after it passes through the plant troughs and before treated water is removed from the process loop. Roots are harvested, and depending on the species of plant used, shoots may be transplanted to grow new roots. Plants can be replaced in the system to ensure constant operation results. Rhizofiltration using sunflowers has been used in the remediation of radionuclides from surface

water near Chernobyl (strontium and cesium) and in water using a rhizofiltration system. Extracted groundwater, surface water, and waste water can be treated using this technology. Rhizofiltration is generally applicable to low-concentration, high-water-content conditions. This technology does not work well with soil, sediments, or sludge's because the contaminant needs to be in solution in order to be sorbed to the plant system.

Advantages

- Either terrestrial or aquatic plants can be used. Although terrestrial plants require support, such as a floating platform, they generally remove more contaminants than aquatic plants.
- This system can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system).
- An *ex situ* system can be placed anywhere because the treatment does not have to be at the original location of contamination.

Disadvantages

- The pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake.
- The chemical speciation and interaction of all species in the influent have to be understood and accounted for.
- A well-engineered system is required to control influent concentration and flow rate.
- The plants (especially terrestrial plants) may have to be grown in a greenhouse or nursery and then placed in the rhizofiltration system.
- Periodic harvesting and plant disposal are required.
- Metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field.

Phyto-transformation: Surface water remediation via phyto-transformation can be accomplished *in situ* in ponds or wetlands. In addition, groundwater can be remediated using phytotransformation *in situ* if the water table is within the zone tapped by deep-rooted plants such as poplars or *ex situ* by pumping water to

troughs or constructed wetlands containing appropriate plants. In the phyto-transformation process, plants take up organic contaminants and degrade them to less toxic or non-toxic compounds. This technique is being tested on explosives-contaminated groundwater (TNT and RDX).

SOIL REMEDIATION

Phytoextraction: This process involves the removal of metals, radionuclides, and certain organic compounds (i.e., petroleum hydrocarbons) by direct uptake into plant tissue. Implementation of a phytoextraction program involves the planting of one or more species that are hyperaccumulators of the contaminants of concern. Amendments (i.e., fertilizer, water, etc.) may be required, as determined from preliminary field testing, to ensure successful plant growth. Lengths of time before harvesting the plants are also determined from preliminary testing, and after this period of time, plant tissue is removed and, if necessary, a new crop of plants are planted. Although testing has focused on single plants, several species may be used at a site, either at the same time or subsequently, to remove more than one contaminant. Characteristics of plants able to perform phytoextraction include:

- Ability to accumulate and tolerate high concentrations of metals in harvestable tissue;
- Rapid growth rate;
- High biomass production.

Phytostabilization: Phytostabilization, is the use of certain plant species to absorb and precipitate contaminants, generally metals, reducing their bioavailability, and so reducing the potential for human exposure to these contaminants. Phytostabilization is used in the treatment of soil, sediments, and sludge's. This technique can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high metals concentrations in surface soils or physical disturbances to superficial materials. Metal-tolerant species can be used to restore

vegetation to the sites, thereby decreasing the potential migration of contamination through wind erosion and transport of exposed surface soils and leaching of soil contamination to groundwater. Characteristics of plants appropriate for phytostabilization at a particular site include:

- Tolerance to high levels of the contaminant(s) of concern;
- High production of root biomass able to immobilize these contaminants through uptake, precipitation, or reduction;
- Retention of applicable contaminants in roots, as opposed to transfer to shoots, to avoid special handling and disposal of shoots.

Advantages

- Soil removal is unnecessary.
- It has a lower cost and is less disruptive than other more-vigorous soil remedial technologies.
- Re-vegetation enhances ecosystem restoration.
- Disposal of hazardous materials or biomass is not required.

Disadvantages

- The contaminants remain in place. The vegetation and soil may require long-term maintenance to prevent rerelease of the contaminants and future leaching.
- Vegetation may require extensive fertilization or soil modification using amendments.
- Plant uptake of metals and translocation to the above ground portion must be avoided.
- The root zone, root exudates, contaminants, and soil amendments must be monitored to prevent an increase in metal solubility and leaching.
- Phytostabilization might be considered to only be an interim measure.
- Contaminant stabilization might be due primarily to the effects of soil amendments, with plants only contributing to stabilization by decreasing the amount of water moving through the soil and by physically stabilizing the soil against erosion.

Phytoremediation of Organic Pollutants

Methodology: Most phytoremediation work carried out currently is determined by the requirement, which is locally available as well as by the type of contaminants present. These include:

- Plant selection,
- Treatment,
- Pattern of selection,
- Agronomic facilities,
- Ground water capture zone,
- Containment uptake rate and clean-up time required,
- Analysis of failure modes,

Plant selection includes:

- Fast growth rate,
- Easy to maintain,
- Utilize huge quantity of water for transpiration,
- Can transform contaminants to less toxic form.

Treatment: Variation in toxicity and transformation rate varies from plant to plant hence appropriate phytoremediation will be selected to achieve desired result. For known toxic metal or salt, different concentrations of toxicants may be analyzed by the use of hydroponics. Then fate of contaminants will be assessed in plant systems.

Pattern of plantation: Plantation density will be done depending upon the nature of climatic zone, rate of evapo-transpiration, root system, precipitation and absorption and pattern of foliage of the selected plant.

Phytoremediation holds potential for the extraction of metals from soil and has been successfully applied for the restoration of abandoned mining sites. The applications of phytoremediation are extensive including the removal of pesticides, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates. Over the last 20 years, phytoremediation has become increasingly popular as a clean, cost-effective and eco-friendly technology to treat soils contaminated

with lead, uranium, and arsenic. However, one major weakness of phytoremediation is that it requires a long-term dedication as the process is dependent on plant growth, tolerance to toxicity and bioaccumulation capacity. With the help of this technology researches has reported phytoremediation of wastes containing petroleum hydrocarbons such as: benzene, toluene, ethylbenzene, and xylenes and polycyclic aromatic hydrocarbons (PAHs), pentachlorophenol, polychlorinated biphenyls (PCBs), chlorinated aliphatics (trichlore-ethylene, tetra-chloroethylene and 1,1,2,2- tetrachloro-ethane), ammunition wastes (2,4,6-trinitrotoluene or TNT, and RDX), metals (lead, cadmium, zinc, arsenic, chromium, selenium), pesticide wastes and runoff (atrazine, cyanazine, alachlor), radionuclides (cesium-137, strontium-90, and uranium), and nutrient wastes (ammonia, phosphate, and nitrate).

Advantages

- It is economically more cost effective than other traditional processes both *in situ* and *ex situ*.
- No excavation or transport of contaminated soil is required.
- The harvested plant can be disposed by simple incineration or can be used as fuel.
- The plants can be easily maintained and monitored.
- It holds potential for the recovery and re-use of valuable metals *i.e.* phytomining,
- Public acceptance of bioremediation is expected to be high.

Disadvantages

- Long length of time required for remediation Treatment is generally limited to soils at less than 3 feet from the surface and groundwater within 10 feet of the surface.
- Climatic or hydrologic conditions *e.g.* flooding, drought, may restrict the rate of growth of types of plants that can be utilized;
- Ground surface at the site may have to be modified to prevent flooding or erosion;

- Contaminants may still enter the food chain through animals/insects that eat plant material containing contaminants;
- Soil amendments may be required, including chelating agents to facilitate plant uptake by breaking bonds binding contaminants to soil particles.

Limitations

- It usually takes long time for remediation compared with other technologies.
- Contaminant removal by the plants is limited to the surface area and depth occupied by the plants root. Any contaminant below the rooting depth will not be removed.
- It depends on the ability of plant species to extract or degrade the contaminants of concern, adaptation to local climates, root depth and tolerance of plant towards the pollutant.
- The selection of plant is critical as the contaminant may pass into the food chain leading to bio-accumulation in animals and therefore, only the non-fodder crop should be chosen.
- The high solubility of contaminant may results in leaching.

RHIZOREMEDIATION

Rhizoremediation, a specific type of phytoremediation, involves both plants and their associated rhizosphere microbes and can occur naturally or can be practiced deliberately by introducing specific microbes. These microbes are either contaminant degrader and/ or can promote plant growth under stress conditions and offer much potential for rhizoremediation. The process was proposed as the most potential approach for remediation of polycyclic aromatic hydrocarbons in soil. Contaminant removal is facilitated through a rhizosphere effect where plants give off organic compounds through their roots and thereby increase the density and activity of potential contaminant degrading microorganisms in the zone, surrounding the roots. The use of plants in combination with microbes has the advantage of causing an increase in microbial population and metabolic

activity in the rhizosphere. It also can establish an improvement of the physical and chemical properties of contaminated soil, and an increase in contact between the microbes associated with the roots and the contaminants in soil. The release of nutrients by plant root exudates creates a nutrient-rich environment in which microbial activity is enhanced. Plant root exudates contain sugars, organic acids, and amino acids as main components. In turn, rhizosphere organisms also have a large impact on plants, because many microbes isolated from the rhizosphere are described to have root growth-stimulating or growth-inhibiting properties. Rhizoremediation process can be designed to improve in several aspects like bioavailability of contaminant molecules, expression and maintenance of genetically engineered plant-microbial systems and root exudates for the effectiveness of the process. Selection of bacteria, which are able to produce biosurfactants in the rhizosphere of the plants, is an interesting alternative to improve the removal efficiency. Rhizoremediation process can be successfully used for restoration of contaminated sites by choosing right type of plant cultivar with right bacteria or by inoculating efficient bacterial strains on plant seeds. Rhizospheric microbes can degrade the majority of environmental pollutants and degradation process stops when the microbe is deprived of food. These microbes have access to the best food source available in soil, namely root exudates. Researchers have described an enrichment method for the isolation of microbes, which combine the properties of degradation of a selected pollutant and excellent root colonization. They have termed this process rhizoremediation instead of phytoremediation to emphasize the roles of the root exudates and the rhizosphere competent microbe. The high concentration of metals in soils and their uptake by plants harmfully influence the growth, symbiosis and consequently the yields of crops by disintegrating cell organelles and disrupting the membranes, acting as genotoxic substance disrupting the physiological process, such as, photosynthesis or by inactivating the respiration,

protein synthesis and carbohydrate metabolism. *Pseudomonas putida* is a root colonizer of potential interest for the rhizoremediation of pollutants and the biological control of pests. According to hypothesis when a suitable rhizosphere strain is inoculated together with a suitable plant e.g. coating bacteria on plant seed, these well-equipped bacteria might settle on the root together with the normal indigenous population, thereby enhancing the bioremediation process. Pioneer work about degradation of compounds in the rhizosphere was mainly focused for herbicides and pesticides. In the past two decades, a large number of publications on rhizodegradation of various organic toxicants using different plants and/or microbial inoculants have been published. Field contaminated soils that have undergone prolonged periods of ageing generally appear to be much less responsive to rhizodegradation than freshly spiked soil. This has important implications for the applicability of rhizodegradation as well as for the evaluation of data obtained on freshly or only shortly aged, spiked soil material. Other strategies to enhance rhizodegradation (e.g. inoculation of degrader strains) are likely to fail where low bioavailability is the main constraint. Interestingly, microbial treatments appeared to be successful at the laboratory experiments but failed when applied to long term contaminated soil on field experiments. This indicates again the importance of the experimental scale and of bioavailability. In view of the still disappointing and controversial results of traditional inoculation, enhanced rhizodegradation requires more sophisticated approaches. Enhanced degradation capabilities of inoculated microorganisms may be obtained by induction of a nutritional bias towards the inoculated strains. A successful rhizoremediation process could depend on the highly branched root system of the plant species where a large number of bacteria harbor, establishment of primary and secondary metabolism, survival and ecological interactions with other organisms. Plant roots can act as a substitute for the tilling of soil to incorporate additives (nutrients) and to improve aeration in soil. Plants also release a

variety of photosynthesis derived organic compounds (root exudates), which might help in degradation of pollutants. The root exudates consists of water soluble, insoluble, and volatile compounds including sugars, alcohols, amino acids, proteins, organic acids, nucleotides, flavonoids, phenolic compounds and certain enzymes. Normally a symbiotic relationship develops between plant and soil microbes in the rhizosphere, where plants provide nutrients necessary for the microbes to flourish, 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. In addition, 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.

Factors Affecting Phytoremediation and Rhizoremediation

Various factors affects the selection of a plant species to be used for bioremediation such as the ability of plant species to extract or degrade the contaminants of concern, adaptation to local climates, high biomass, growth rate, root depth and structure, compatibility with soils, ease of planting and maintenance, and ability to take up large quantities of water through the roots.

Site characterization, including determination of soil and water chemistry/conditions, climate, and contaminant distribution affects the process. Root contact is a primary limitation on phytoremediation applicability. Remediation with plants requires that the contaminants be in contact with the root zone of the plants. Either the plants must be able to extend roots to the contaminants, or the contaminated media must be moved to within range of the plants. This movement can be accomplished with standard agricultural equipment and practices, such as deep plowing to bring soil from 2 or 3 feet deep to within 8 to 10 inches of the surface for shallow-rooted crops and grasses, or by irrigating trees and grasses with contaminated groundwater or wastewater. Because these activities can generate fugitive dust and volatile organic compound emissions, potential risks may need to be evaluated. Phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies. Excavation and disposal or incineration takes weeks to months to accomplish, while phytoextraction or degradation may need several years. Therefore, for sites that pose acute risks for human and other ecological receptors, phytoremediation may not be the remediation technique of choice. Soil conditions must be appropriate for plant growth and contaminant migration to the plant, yet not allow leaching of the metals. The pH of the soil may need to be adjusted and/or chelating agents may need to be added to increase plant bioavailability and uptake of metals. There are also some limitations to this technology that need to be considered carefully before it is selected for site remediation. These include limited regulatory acceptance, long duration of time required for clean-up, potential contamination of the vegetation and food chain, and difficulty in establishment and maintenance of vegetation at some sites.

Future Perspective and Challenges

Intensive research is required to know the fate of various compounds in the plant metabolic

cycle to ensure that plants droppings and products do not contribute toxic or harmful chemicals into the food chain. When the plants have absorbed and accumulated contaminants, they can be harvested and disposed of or used in alternative processes such as burning for energy production. Plant selection, breeding, and genetic engineering for fast growing, high-biomass hyper-accumulators are active areas of research. The main challenge regarding the use of biomass for bioenergy is the issue of pollution transfer and heavy metal content in the biomass. The most commonly used process for dealing with metals-enriched plant material is controlled incineration, which results in ash with a high metals content. It is hoped that an economically feasible method of metals recovery from this ash will be developed, further reducing the environmental impacts of this technology. There is a lack of knowledge regarding the emissions that may be generated by burning such plants and wood materials. It has been suggested by many investigators that phytoremediation is the ideal technology for mitigating landfill environmental problems including soil and ground water contamination, leachate generation and gas emissions (especially if improper post-closure treatment of landfills or deterioration of the conventional clay landfill capping occur). The plants with high levels of contaminants are potentially interesting for use as material for metal enrichment. The use of energy crops in phytoremediation of contaminated soil is a rapidly developing field and generates biomass that can contribute to the energy supply and can thus play a key role in meeting the targets for use of renewable energy sources. The remedial capacity of plants can be significantly improved by genetic manipulation and plant transformation technologies. The identification of unique genes from hyperaccumulators, and their subsequent transfer to fast-growing species, has proven to be highly beneficial as demonstrated by the recent success of transgenic plants with improved phytoremediation capacity. Management and remediation of contaminated environment may become a major environmental issue. Current

remediation methods for contaminated soil include soil removal and washing, physical stabilization and the use of chemical amendments, all of which are expensive and disruptive having so many side effects. Phytoremediation, making healthy environment is emerging technology, due to its cost effectiveness and eco friendly way to minimize toxic effect.

CONCLUSION

Phytoremediation and Rhizoremediation are an option that offers the possibility to destroy or render harmless various contaminants by using natural bioactivity of plants and microbes to degrade harmful pollutants with the help of selected plants for remediating the specific contaminant at particular sites. This natural process can be easily carried out on site without causing a major disruption of normal activities and threats to natural resources. There is an urgent need to educate and aware local people about the various life forms, their potential applications and tendencies to absorb and remove the contaminants with the help of selected plants/ microbes. Our natural resources are contaminated due to the disposal of hazardous substances. The pollution load may be minimized by using Phytoremediation and Rhizoremediation techniques which are a very safe and need based technique.

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