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PHYTOREMEDIATION – AN OVERVIEW REVIEW

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ABSTRACT

Phytoremediation, the use of green plants to clean up polluted soil and water resources has received much attention in the last few years. Phytoremediation offers owners and managers of metal contaminated sites is an innovative and cost effective option to address recalcitrant environmental contaminants. Although not a new concept, phytoremediation is currently being re-examined as an environmentally friendly, cost-effective means of reducing metal contaminated soil. Genetic engineering approaches are currently being used to optimize the metabolic and physiological process that enable plants to phytoremediate, sites contaminated with heavy metals. Genetic manipulation of environmentally important plants can produce elite plant lines with enhanced remediation abilities. Recent research results include over expression of genes whose protein products are involved in metal up take, transport and sequestration, have opened up new possibilities in phytoremediation. This review article provides a critical review of the recent progress towards the development of transgenic plants with improved phytoremediation capabilities and their potential use in environmental clean up.

INTRODUCTION

Environmental bio-technology is a new discipline which integrates living materials, mainly plants, and very small animals like earth worms, micro organisms to address the problems of environmental management and sustainable development Phytoremediation is a word formed from the Greek prefix "Phyto" means plant and suffix "remedium" meaning to clean (or) restore (Cunningham et.al.,1996) The term actually refers to advise collection of plant based technologies that use either naturally occurring (or) genetically engineered plants for cleaning contaminated environments (Flathman and lanza, 1998), Phytoremediation consists of four different plant based technologies, each having a different mechanism of action for the remediation of metal polluted soil, sediment or water. These include phytoextraction, where plants absorb metals from soil and translocate them to harvestable shoots where they accumulate. Rhizofiltration involves the use of plant to clean various aquatic environments. Phytostabilization, where plants are used to stabilize rather than clean contaminated soil. Phytovolatalization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization. Some plants tolerate and accumulate high concentrations of metal in their tissues but not at the level required to be called as hyper accumulators. Researches in environmental biotechnology promise to enhance the phytoremediation efficiency by a known phytoremediator plant. A genetic combination

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of fast-growing, high biomass yielding, and high tolerance and hyper accumulation of toxic metals in plant shoot is best suited for the purpose of phytoremediation. It should also be easily cultivated and harvested. The novel bioremediation system called "Symbiotic Engineering" involves advantages of both the bacterium rhizobium and the leguminous plants using many useful genes like ATPCS, MTL4, IRT1, may provide another valuable bioremediation tool. The Introduction of genes can be readily achieved for many plant species using direct DNA methods of gene transfer. Transformation of required gene for the production of transgenic plant using molecular techniques is a novel technology.

MATERIALS AND METHODS

Phytoremediation is a method which green plants use for cleaning up contaminated hazardous wastes sites. Phytoremediation has applied Ex-situ and In-Situ, continually and induces to clean up contaminated terrain of toxic metals. The following are the steps involved in the phytoremediation process

1. Identification of area.
2. Chemical analysis of the soil before application of the phytoremediation.
3. Sowing the plant phytoaccumulators.
4. Usage of agricultural and technical measures and Inspection of vegetative development.
5. Picking and drying the plants.
6. Chemical analysis of the soil near the root after finished phytoremediation.
7. Chemical analysis of green leaves of plants.
8. Determination of co-efficient (Concentration factors) of plants.

The material is dried in shadow and draft without sunlight presence. After sample preparation content of heavy metal is determined by Atomic Absorption Spectroscopy. For enhancing the phytoremediation process the following steps are considered.

- To enhance the speed and quantity of metal uptakes by plants, some researchers are advocating the use of various chemicals like acidifying agents (Blaylocke and Huang, 2000), fertilizer salts (Lasat *et al*. 1997, 1998) and chelating materials (Blay lock *et al*. 1997).
- Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Marschenev, 1995).
- Acidifying agents are also used to increase the availability of radio active elements in the soil for plant uptake.

Working process of phytoremediation

Plant roots take contaminants from the ground into the plant body. The plant root zone is referred to as the rhizosphere; this is where the action occurs. This soil supports large populations of diverse micro organisms. This is due to chemicals exuded by plants roots which provide carbon and energy for microbial growth. This combination of plants and micro organisms used to increase the biodegradation by compounds.

Proper plant selection

As a plant based technology, the success of phytoextraction is inherently dependent upon proper plant selection. Plants used for the phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue (Blay lock *et al.* 1994) Researches initially envisioned using hyper accumulators (Salt and Kramer, 2000) to clean metal polluted sites. At present, there are nearly 400 known hyper accumulators but majority are not appropriate for phytoextraction, because of their slow growth and small size. Several researches are screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Banuelus *et al.* 1997).

Types of Vegetation Used

Some of the plants used in phytoremediation are:

- Alfalfa
- Hybrid Poplar Trees
- Blue-green Algae
- Duck Weeds
- Arrowroot
- Sudan Grass
- Rye Grass
- Bermuda Grass
- Alpine Bluegrass
- Yellow or White Water Lilies
- Sunflower
- Vetiver grass
- Poplar tree
- Brake fern
- Carrot
- Periwinkle
- Switch Grass
- White reddish

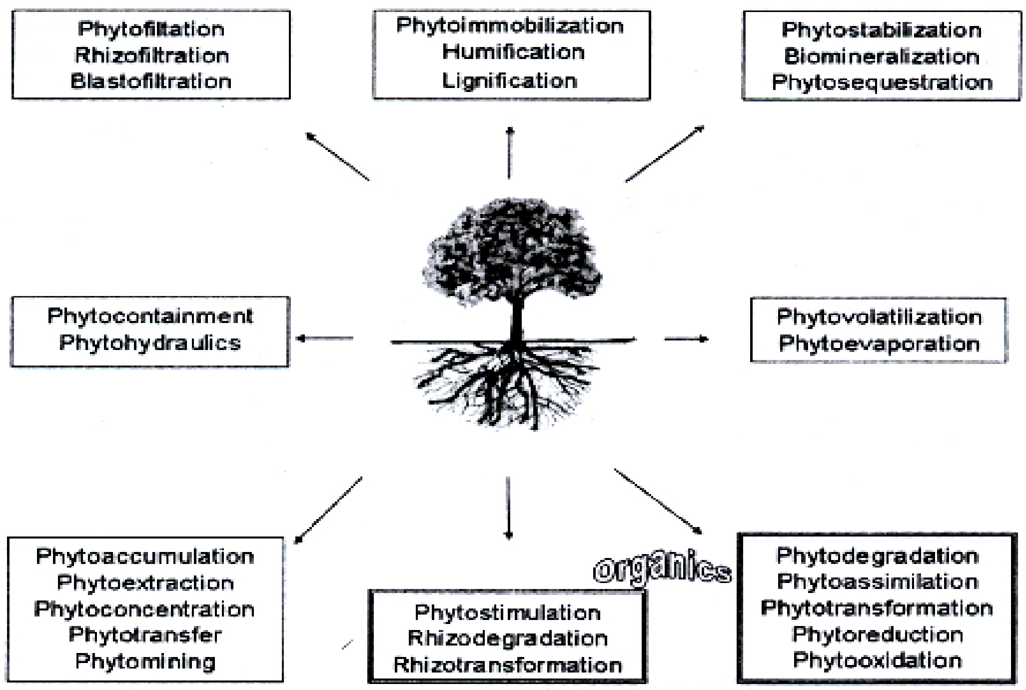

Fig. 1 Various processes of phytoremediation

Some known hyper accumulator species of different metals.

Metal and Plant Species	Concentration of metal accuumulated (mg/kg)
A. Nickel (Ni)	200 - 31,000
Thalaspi spp. (Brassicaceae)	1280 – 29,400
Alyssium spp (do)	11,600
Berkheya codil (Asteraceae)	16,600
Pentacacalia spp (do)	25,540
Psychotria corinota (Rubiaceae)	
B. Zinc (Zn)	
Thalaspi caerulescene (Brassicaceae)	43,710
Thalaspi rotundifolium (do)	18,500
Dichopetalum gelonioids (do)	30,000
C. Cadmium (Cd)	
Thalaspi caerulescenace (Brassicaceae)	2,130
D. Lead (Pb)	
Minuartia verna (caryophyllaeceae)	20,000
Agrostis tenius (Poaeceae)	13,490
Vetiveria zizaniodes	>1,500
E. Cobalt (Co)	
Crotolaria Cobalticola (Fabaceae)	30,100
Haumaniastum robertii (Lamiuceae)	10,232
F. Copper (Cu)	
Ipomea alpine (convolvulaceae)	12,300
G. Arsenic (As)	
Pteris rittate (Fern)	-

DISCUSSION

Soil & water contaminated with toxic metals pose major environmental and human health problems. According to EEA (European Environment Agency) estimation 1.4 million areas are contaminated (Puschenreither and Wenzel, 2003). Environmental biotechnology has given rise to an allied discipline called "bio-engineering". It is a 'Green' (or) 'Soft" and alternative to the "hard" and costly Civil Engineering works for the environmental restoration and reconstruction.

In modern times, not only the biological organisms are used but their genetic materials (DNA) too. Modern biotechnology has made wonders and has revolutionized and heralded a new era in the field of environmental management. With the new biotechnological tools (recombinant DNA technology, genetic engineering) working at genetic or molecular level scientists can change the genetic make up of organisms in which characteristics are not found naturally.

Plant Enzymes implicated in Phytodegradation and phytotransformation of organic compounds

Enzymes	Contaminated degraded / transformed into Less toxic forms

PHYTOREMEDIATION DESIGNS

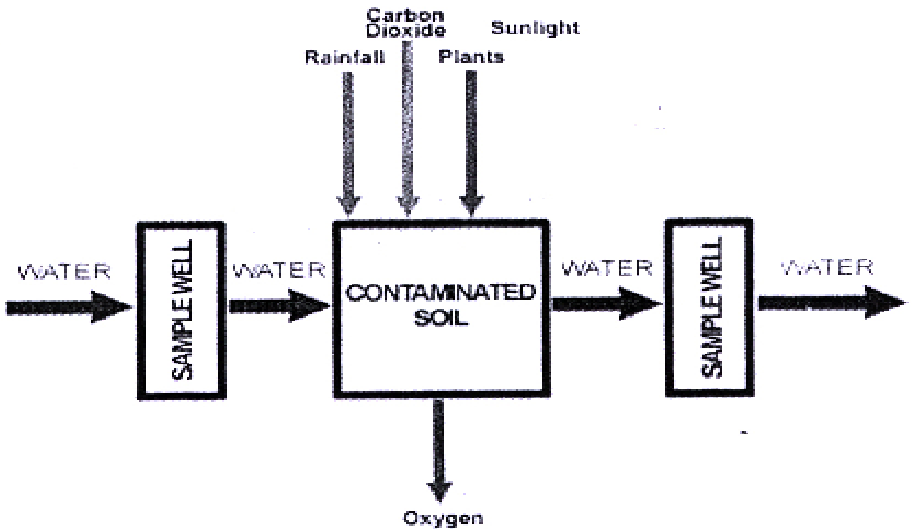

Fig. 2 A typical process diagram of phytoremediation

Fig. 3 Ditoxification of xenobiotics in plant cell

Phosphatase	Organo Phosphates
Aromatic dehalogenases	Chlorinated aromatic Compounds (DDT, PCH's)
Cytochrome 450,	PCB's
peroxidases,	
Peroxygenases,	
Glutathione, S-transferase,	
O-glucosyltransferases,	
O-malonyltransferases	Xenobiotics
B-cyanoalanine synthase	Cyanide

In the last few years, several commercial companies practicing phytoremediation for environmental clean up in USA and Europe. Important among them are phytotech (USA), phyto works (USA), earth care (USA), bioplanta (Germany), picco plant (Germany), plant techno (Italy,), Slater (UK), aquaphyte remediation (Canada).

PHYTOREMEDIATION MARKETS IN THE U.S. IN 2005

Phytoremediation works carried out	Values in million dollars
Removal of heavy metal from Contaminated soils.	70-100
Removal of heavy metal from Contaminated ground water	1-3
Removal of heavy metal from	

Contaminated waste water	1-2
Removal of heavy metal from Radio nuclides	40-80
Removal of organics fro Contaminated ground water	65-115
Others	214-370 million dollars

Role of Environmental biotechnology and genetic Engineering in improving efficiency of Phytoremediation.

Several genes that are involved in metal uptake, translocation, sequestration and bioaccumulation has now been identified. Transfer of these genes into candidate plant will result in developing "Transgenic plants" with enhanced ability for metal uptake and accumulation for the removal from environment. Environmental biotechnology is a tool to accumulate the phytoremediation process through over expression of those genes responsible for the sequestration of heavy metals and radio nuclide in plants or through "gene-transfer". Any appropriate genes of foreign origin been transferred in plants like Arabidopsis thaliana, Nicotiana tobaccum, Brassica junceae, Brassica olevaceae, Varbotrytis, Lycopersicon Esculentum etc., to enhance the phytoremediation efficiency of these plants.

Many hyper accumulator plants are rare, with small population occurring in remote places (or) have restricted distribution. They often have slow growth rate and produce small biomass. Such hyper accumulator species may provide suitable genes involved in metal uptake, translocation, and sequestration for enhancing the phytoremediation. If genes from highly metal tolerant and hyper accumulator plants are transferred to high biomass yielding and growing cultivars, this can do miracle.

Important Achievements

- Transfer of human MT-2 gene to tobacco (*Nicotiana tobaccum*) resulted in transgenic plant with enhanced cadmium (cd) tolerance and accumulation.
- Transfer of Pea MT gene in Arabidopsis thaliana resulted in enhanced copper (Cu) accumulation in the transgenic Arabidopsis Thaliana.
- Transfer of Yeast CUP1 gene in Cauliflower (*Brassica capitata*) resulted in 16-fold higher accumulation of cd in transgenic Cauliflower (Eaper *et al*. 2006).
- Transfer MT gene in *Nicotiana* spp., *Brassica* Spp., *Arabidopsis thaliana* resulted in highly tolerant to Cd and other metals.
- Somatic cell hybrid produced between *Brassica junceae* (a high biomass yielding plant) and Thalaspi caeruiescens (a known Zn and Ni hyper accumulator) showed increased tolerance to Pb and Ni, Zn and the total amount of lead phytoextracted was much greater because of high biomass produced (Dushenkov *et al*. 200).

Advantages

i) The cost of the phytoremediation is lower than that of traditional process both in-situ and ex-situ
ii) The plants can be easily monitored.
iii) The possibility of the recovery and re-use of valuable metals.
iv) It is the least harmful method because it uses naturally occurring organisms and preserves the natural state of the environment.

Limitations

i) With plant based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the ground water.
ii) The survival of the plant is affected by the toxicity of the contaminated land and general conditions of the soil.
iii) Possible bio accumulations of contaminants which then passed into the food-chain from primary level consumers up wards.

Future research direction

Further use of molecular biology expertise to greater understanding of phytoremediation at the genetic and molecular level research objectives in these areas.

i) Probing of the Bio-path ways involved in contaminant degradation and sequestration.
ii) Identifying the specific genes involved in phytoremediation process.
iii) Investigating cell signaling path ways that affect the genetic expression plant and microbial enzymes.
iv) Studying the molecular ecology of root-microbial interactions.
v) Analyzing and identifying root exudates.

Now it has become possible to:

- Create economically valuable and ecologically adapted crops called transgenic crops with desired characters.
- Create hyper-accumulator plants which can tolerate and bio accumulate high levels of toxic metals.

- Grow thousands of replicas of economically important plants in shorter time by tissue culture.

CONCLUSION

A phytoremediation is amenable to a variety of organic and in organic compounds may be applied either in-situ or ex-situ. Phytoremediation is considered to be an innovative technology and hope fully by increasing our knowledge and understanding of this intricate clean up method, it will provide a cost effective, environment friendly alternative to conventional cleanup methods.

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