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Lesson: Role of Plant Biotechnology in Environment

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Introduction

Plants are indispensable to our life as they provide us food, oxygen and also help in preserving the environment. The major applications of using plant biotechnology to improve our environment include:

- Use of plants to remove toxic chemicals by phytoremediation
- Use of plant biotechnology to remove aluminium toxicity from the soil. The scientists have introduced a citric acid producing gene in bacteria which reacts with aluminium and prevents its escape into the roots
- Production of bioplastics
- Produce crops resistant to insecticides and pesticides

We will be dealing with these applications in detail in this chapter.

Phytoremediation

Phytoremediation (*Phyto*-plant and *Remedium*-restoring balance) is the use of plants to clean up the environment. In this process plants are utilized to remove, destroy or sequester hazardous chemicals from the environment. It has numerous advantages over traditional methods of waste management. It is a very cost effective method, has aesthetic advantages and has long term applicability. The plants can be monitored easily and there is a possibility of recovery and re-utilization of valuable metals (phytomining). It appears to be a potentially less harmful method of remediation as it preserves the environment in a natural state and uses natural organisms. Bioremediation or use of living organisms for environment clean up, is now being stated as to be among "the top ten technologies for the improvement of human health in developing countries" (Daar et al., 2002). Limitations of phytoremediation include the potential of introduction of contaminant or its metabolite into the food chain leading to bioaccumulation of contaminants. Also it is limited to the surface area and depth occupied by the roots. Sometimes slow growth and low biomass needs long term dedication. Contaminants can leach out into groundwater. Toxicity might also be encountered in establishment and maintenance of plants at waste sites. Long clean up times are required to achieve regulatory action levels.

There are various types of phytoremediation processes:

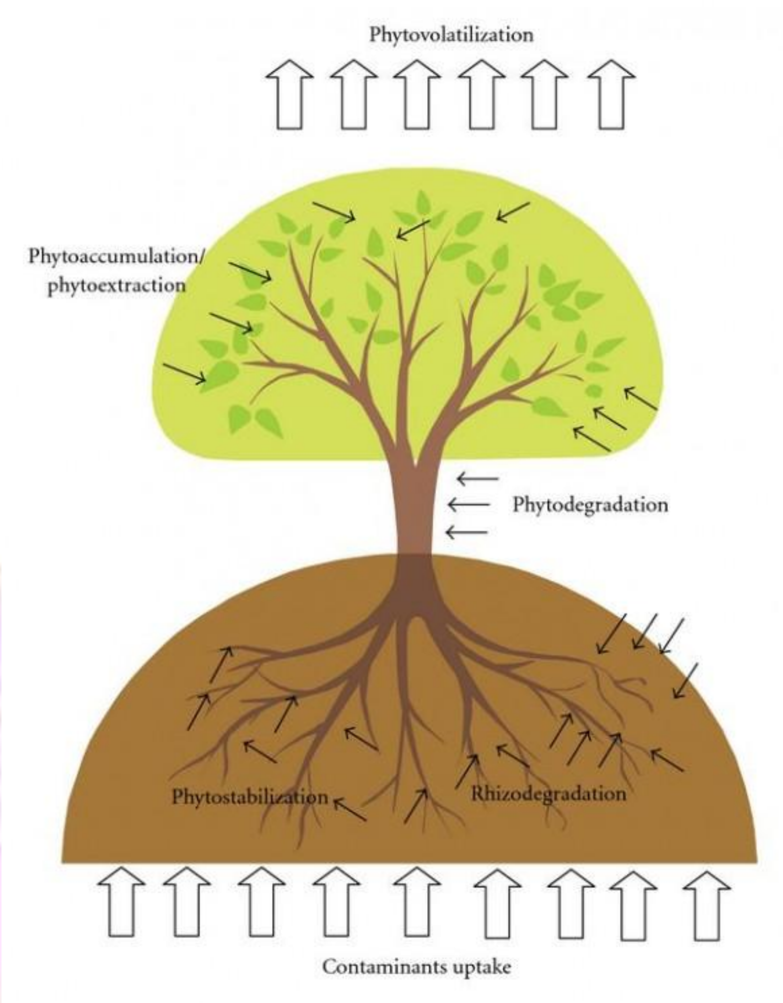

Figure: Various processes of phytoremediation: contaminants in soil as well as groundwater could be uptaken by plant roots (phytoextraction), be sequestered (phytostablization), degraded into harmless compounds (phytodegradation), volatilized in atmosphere (phytovolatilization) or could be degraded by soil microbes (rhizodegradation).

Source: http://whenitrains.commons.gc.cuny.edu/files/2013/08/Phytoremediation-580x747.jpg (CC)

Visit link for animations:
http://www.webapps.cee.vt.edu/ewr/environmental/teach/gwprimer/phyto/phyto.html

- **Phytoextraction (Phytoaccumulation)**:
  It is a process where the pollutants or waste materials are taken up or absorbed by the plant roots and stored in above ground and harvestable part of

biomass. It is a very advantageous process as it is environment friendly and doesn't affect soil quality. Traditional methods of heavy metal cleanup disrupt soil structure and reduce productivity of soil. It is also a cost effective method of waste cleanup. Also, it is important to remove these plants after sometime so that they do not pollute the environment.

The above ground parts of plants can be harvested and burnt to gain energy and recycle metals from ash. This process has been extensively used for remediation of heavy metals like Pb, Cd, Zn, Ni, Cu using plants like Indian mustard (*Brassica juncea*), sunflower (*Helianthus spp*.) and *Thlaspi carule*scens. Effective phytoextraction requires hyperaccumulator plants i.e., those plants that are capable of accumulating over 100 times more metal concentrations as compared to non-accumulator plants. For example, In 1990s Nickel hyperaccumulator *Berkheya coddii* was utilized to decontaminate land near the Rustenburg smelter (South Africa). Arsenic has been cleaned up using Sunflower (*Helianthus annuus*) or Bracken fern, a hyperaccumulator. Bracken stores arsenic in its leaves as much as 200 times as compared to soil. Lead has been phytoextracted using Indian Mustard, Ragweed or Hemp Dogbane.

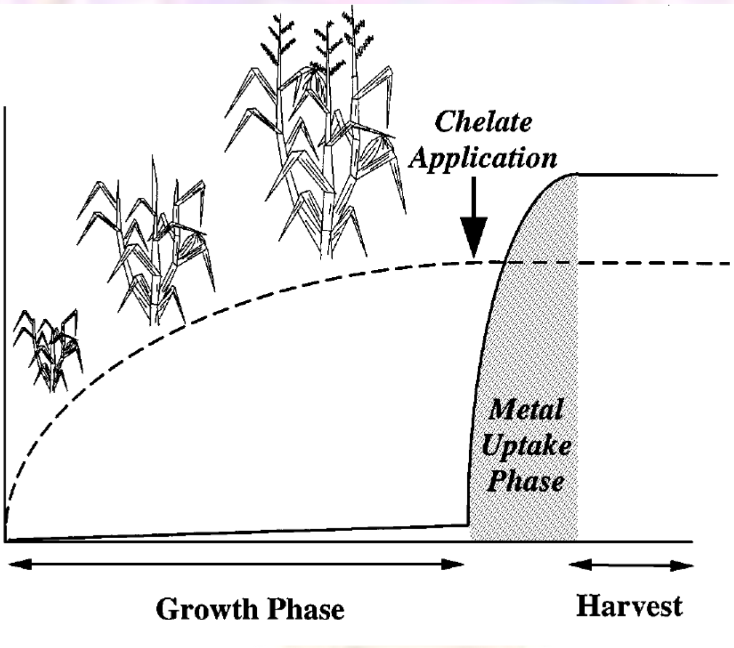

Figure: Chelate assisted phytoremediation. Metal concentration is the solid line and shoot biomass is represented by dashed line.

Source: http://www.personal.psu.edu/users//d/g/dgh5037/extEssay.html (CC)

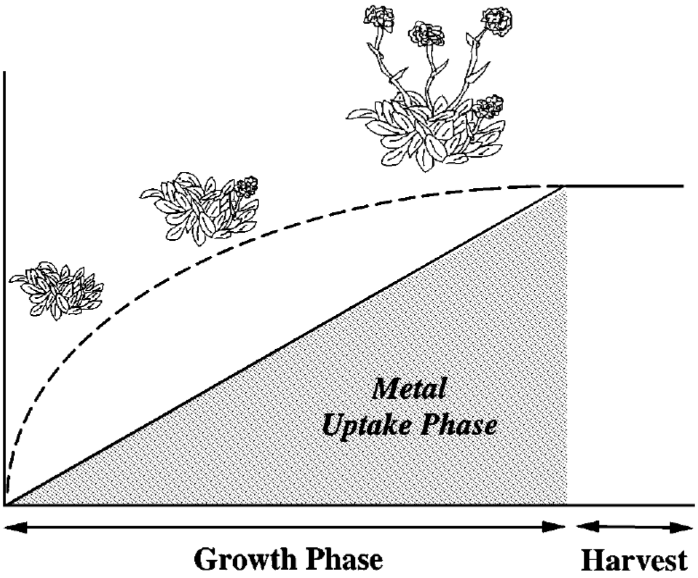

Figure: The process of phytoextraction. Metal concentration is the solid line and shoot biomass is represented by dashed line.

Source: http://www.personal.psu.edu/users//d/g/dgh5037/extEssay.html (CC)

Specific metal ion-tolerant species	
Ar	*Helianthus annus*
Cd	*Salix viminalis*
Zn	*Thlaspi caerulescens*
Ni	*Berkheya coddii*
Pb	*Brassica juncea*
NaCl	*Hordeum vulgare*

Figure: Metal ion tolerant plant species

Source: http://www.personal.psu.edu/users//d/g/dgh5037/extEssay.html (CC)

- **Phytotransformation**

  It is a process that results in chemical transformation of environmental contaminants due to plant metabolism. Phreatophytic trees (Salix family including Willow, cottonwood), grasses (reed canary grass, Bermuda grass, rye, fescue, switchgrass), and legumes (clover, alfalfa, cowpeas) carry out phytotransformation.

  - Firstly the contaminants are taken up by the plant roots and the plant enzymes increase the polarity of these by adding certain functional groups like –OH (hydroxyl groups) (Phase I metabolism).

- This is followed by Phase II metabolism where small molecules like glucose and hydrophilic amino acids are added to xenobiotics to further increase their polar character (conjugation).
- This is followed by sequestration of xenobiotics within the plant (Phase III metabolism). Xenobiotics polymerize in a lignin like manner and are sequestered in the plant. Thus they are stored safely and functioning of plant is not disturbed.

This has been used for the remediation of various herbicides, chlorinated aliphatics and aromatics, ammunitions (TNT, RDX), etc. Here the plants act in a manner analogous to human liver cleaning up xenobiotic compounds hence a "Green liver model" has been proposed. It is often used interchangeably with the term "Phytodegradation" which involves enzymatic breakdown of organic pollutants by both internal as well as secreted plant enzymes.

- **Phytostablization**

  It is a process used for long term containment of pollutants. It focuses on sequestering pollutants in soil near the roots rather than in plant tissues, thus reducing their bio availability. The contaminants are rendered immobile, insoluble and less toxic through adsorption and accumulation by the roots and their exudates, or precipitation within the root zone. Phreatophytic trees for hydraulic control and grasses with fibrous roots for soil erosion control have been extensively used. It is being utilized for containment of heavy metals like Pb, Cd, Zn, As, Cu, Se, U and hydrophobic organics that are not biodegradable.

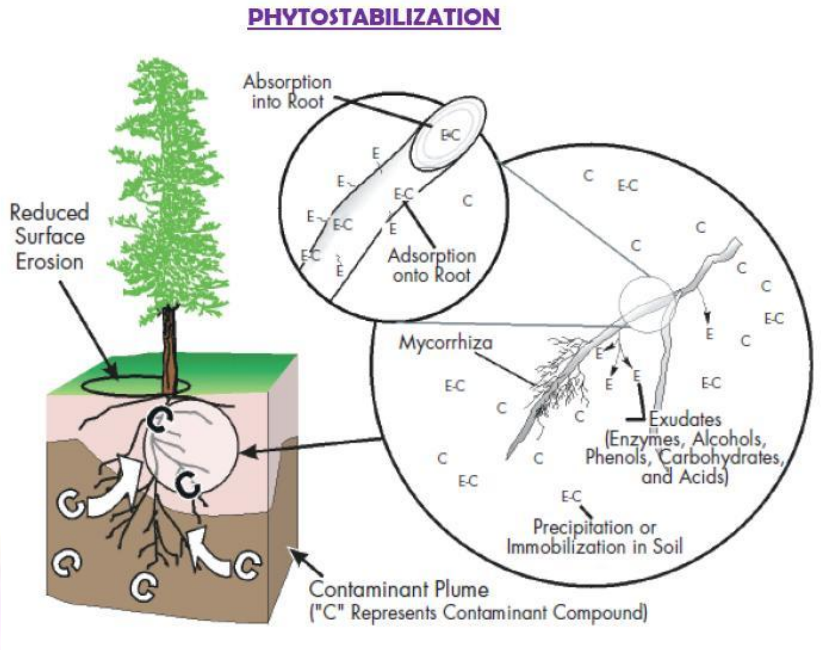

Figure: Phytostablization

Source:http://www.biologyonline.org/js/tiny_mce/plugins/imagemanager/files/boa001 (CC)

- **Rhizodegradation (Phytostimulation)**

  It is also referred to as enhanced rhizosphere degradation. Plant roots stimulate soil microbial activity for the degradation of contaminants in the soil root zone or rhizosphere. Roots stimulate microbial activity in various ways:

  i) they oxygenate rhizosphere leading to enhanced aerobic transformations,

  ii) increase bio availability of organic carbon,

  iii) root exudates have carbohydrates, amino acids, sugars, enzymes, etc. that enrich microbes,

  iv) mycorrhizae fungi growing within rhizosphere degrade organic pollutants

  v) roots provide an ideal habitat for increased microbe populations. This process has found applicability in the containment of pollutants like pesticides, aromatics, polynuclear aromatic hydrocarbons (PAHs) from soil.

- **Phytovolatilization**

  Pollutants are uptaken by the plant roots to the leaves and are volatilized through plant stomata, the sites for gaseous exchange (plant transpiration). It is being used for containment of volatile organic compounds (e.g., MTBE), selenium, arsenic and mercury using *Brassica juncea;* wetlands plants; phreatophytic trees for groundwater capture**.**

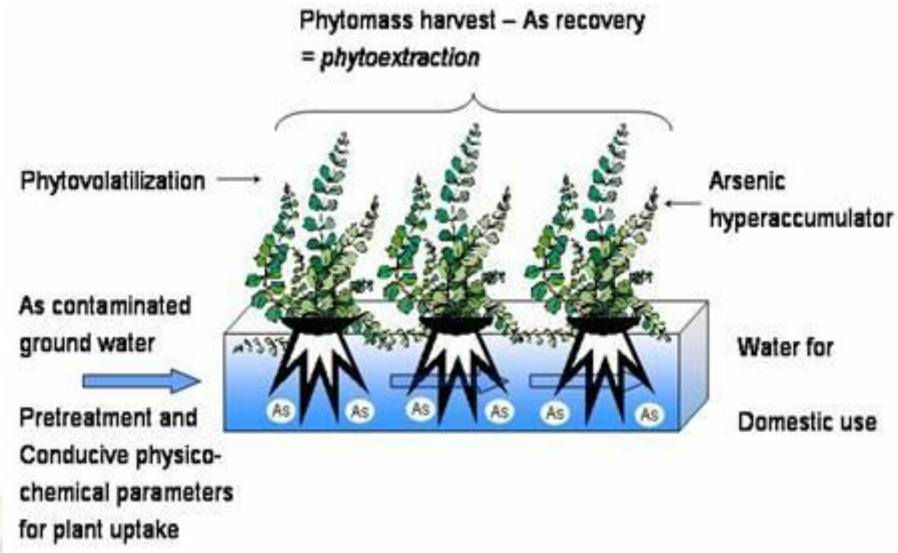

Figure: The representation of phytovolatilization

Source: http://bioremediation123.wikispaces.com/Phytovolatilization (CC)

- **Rhizofiltration**

  It is process where mass of plant roots filter ground water thereby removing pollutants and toxic substances, which remain adsorbed to or absorbed by the roots. It is used for remediation of ground water rather than soil reclamation. Here firstly the plants are grown in clean water to accumulate a large root system. After a sizeable root system develops, polluted water is supplied to the plants to acclimatise them. This is followed by planting them in polluted area where the root system uptake polluted water. Once the roots become saturated with pollutants they are harvested and disposed safely. Aquatic plants, emergents (bullrush, coontail), submergents (*Hydrilla* spp., algae, stonewort) has been used for the bioremediation of metals like Pb, Cd, Cu and Zn, and also for radionuclides and hydrophobic organics. Roots of Sunflower (*Helianthus annuus*) have been found to rhizofilter heavy metals like Pb, Cu, Zn and Cr.

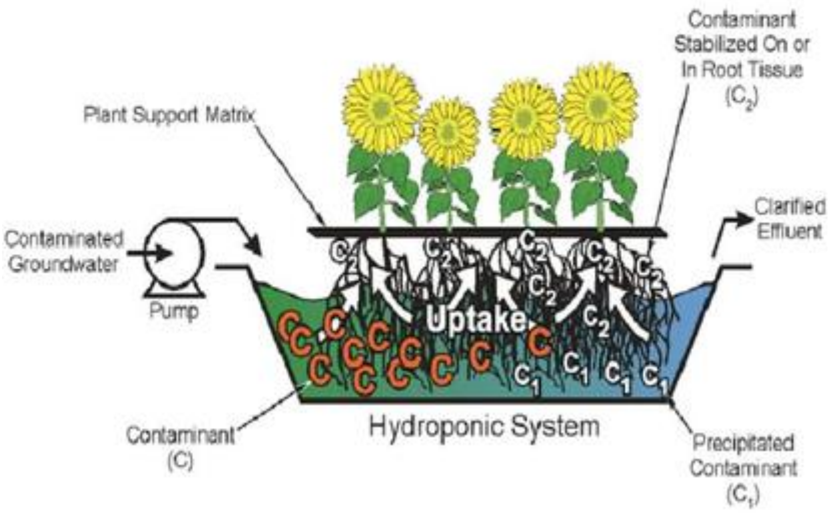

Figure: The process of Rhizofiltration

Source: http://irisforfisherywater.weebly.com/ (CC)

Few common pollutant accumulating plants found by remediation researchers are Seapink thrift (*Armeria maririma*), Ragweed (*Ambrosia artemisiifolia*), Indian mustard (*Brassica juncea*), Rape, Rutabaga, Turnip (*Brassica napus*), Flowering/ornamental kale and cabbage, Broccoli (*Brassica oleracea*), Blue/sheep fescue (*Festuca ovina*), Sunflower (*Helianthus annuus*), Pennycress (*Thalspi rotundifolium*), Wheat (scout) (*Triticum aestivum*) and Corn (*Zea mays*)(Paz-Alberto and Sigua, 2013). Table ahead lists few advantages and limitations of various sub processes phytoremediation.

Table: Advantages and limitations of sub processes of phytoremediation

Advantage	Limitation
Phytoextraction	
The plant must be able to produce abundant biomass in short time. E.g. in green house experiments, gold was harvested from plants (Anderson et al., 1998).	Metal hyperaccumulators are generally slow-growing and bio-productivity is rather small and shallow root systems. Phytomass after process must be disposed off properly.
Phytostabilization	
It circumvents the removal of soil, low cost and is less disruptive and enhances ecosystem restoration/re-vegetation.	Often requires extensive fertilization or soil modification using amendments, long-term maintenance is needed to prevent leaching.
Phytovolatilization	
Contaminant/Pollutant will be transformed in to less-toxic forms. E.g. elemental mercury and dimethyl selenite gas. Atmospheric processes such as photochemical degradation for rapid decontamination/transformation.	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.
Phytofiltration/rhizofiltration	
It can be either in situ (floating rafts on ponds) or *ex situ* (an engineered tank system); terrestrial or aquatic.	pH of the medium to be monitored continually for optimizing uptake of metals; chemical speciation and interactions of all species in the influent need be understood; functions like a bioreactor and intensive maintenance is needed.

Source: http://www.scielo.br/img/revistas/bjpp/v17n1/a05tab02.gif (CC)

Table: List of few chemicals phytoremediated through various sub processes of phytoremediation

Sub process of phytoremediation	Chemicals Treated
Phytoaccumulation/extraction	Cd, Cr, Pb, Ni, Zn, radionuclides, BTEX*, pentachlorophenol, short chained aliphatic compounds
Phytodegradation/transformation	Nitrobenzene, nitroethane, nitrotoluene, atrazine, chlorinated solvents (chloroform, carbon tetrachloride, etc.)
Phytostabilization	Heavy metals in ponds, phenols and chlorinated solvents
Phytostimulation	Polycyclicaromatic hydrocarbon, BTEX, PCB#, tetrachloroethane
Phytovolatilization	Chlorinated solvents, Hg, Se.
Phytofiltration	Heavy metals, organics and radionucleides.

*BTEX = benzene, toluene, ethyl benzene, xylenes; #PCB = Polychlorinated biphenyl

Source: www.academicjournals.org/journal/AJB; *Nwoko, 2010. (CC)*

Transgenics and Phytoremediation

Biotechnology finds its applications in phytoremediation as well. Plant based technologies for bioremediation suffers from certain limitations like slow time scale, toxicity to the plants and basic lack of metabolic pathways to clean up organic compounds as plants are themselves autotrophs. This often results in slow and incomplete remediation. Genetic engineering is nowadays being employed to enhance the natural bioremediation capabilities of plants by introducing newer genes in plants. Transgenic plants refer to those genetically modified plants in which functional foreign gene(s) have been inserted in their genome. The inserted gene may be from an unrelated plant or from a completely different species. These plants are thus genetically modified. These transgenic plants express certain genes from organisms like certain bacteria and mammals for enhanced plant tolerance, uptake and metabolism of heavy metals or organics. Newer advancements in biotechnology, like finding of greater number of catabolic genes, inclusion of new plant varieties and innovation of newer transformation technologies, are likely to ameliorate phytoremediation performances of transgenic plants and help to curb soil pollution (figure 4). Nonetheless, cleaning will be more efficient with these '**biotech mops'**.

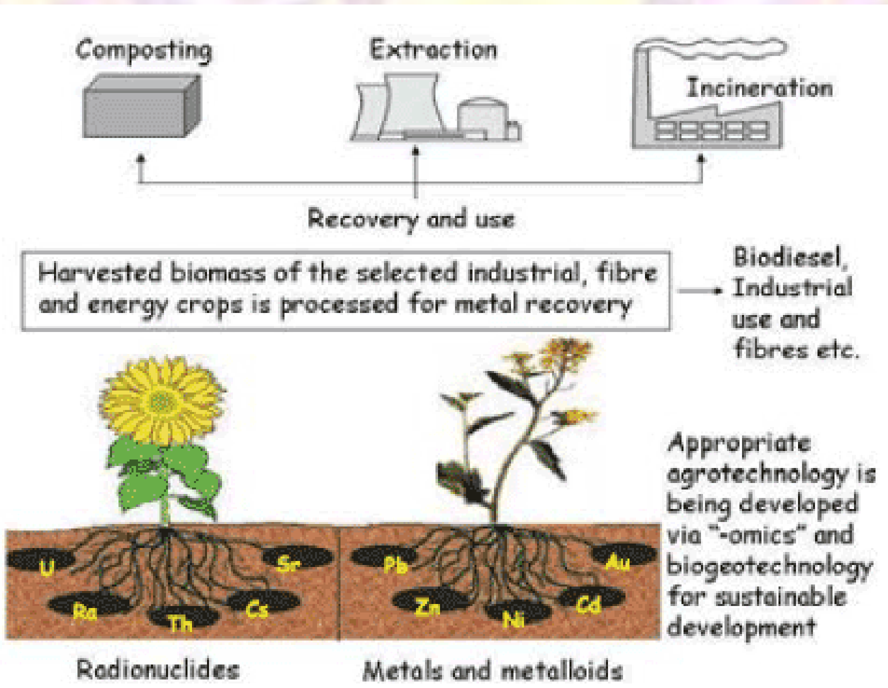

Figure: Transgenic plants could be employed for improved phytoremediation of pollutants like metals, metalloids and radionuclides
Source: http://www.scielo.br/img/revistas/bjpp/v17n1/a05fig01.gif (CC)

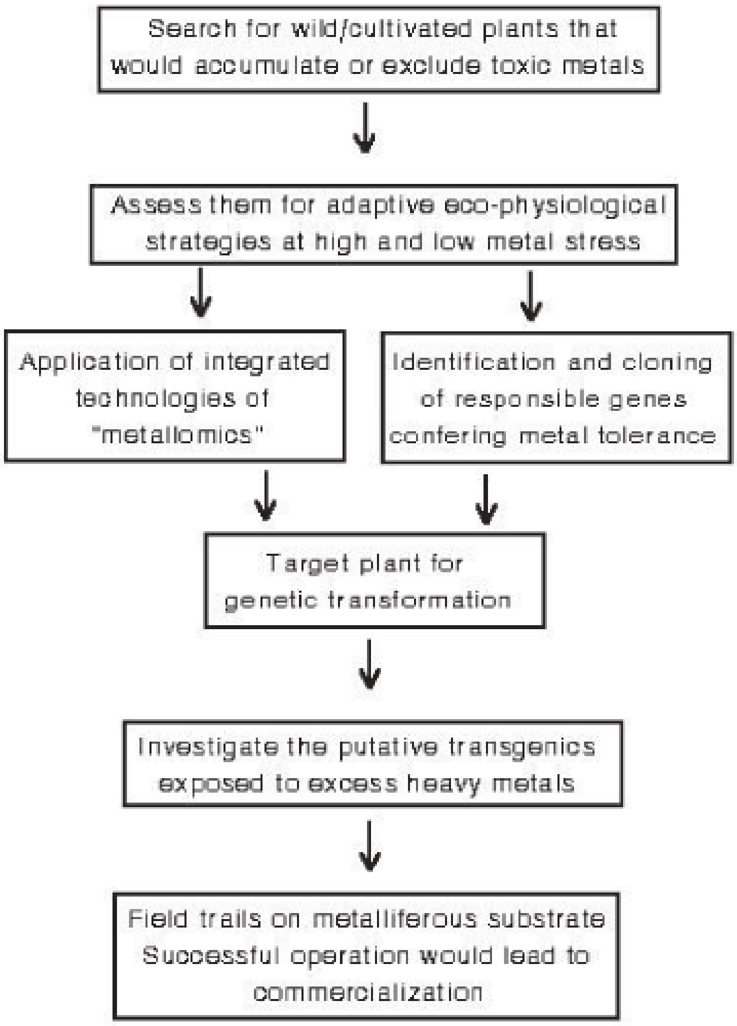

Figure: Flowchart for enhancing phytoremediation potential of plants through biotechnology
Source: http://www.scielo.br/img/revistas/bjpp/v17n1/a05fig02.gif (CC)

Phytoremediation of heavy metals

- **Mercury (Hg)** is a very toxic pollutant and many of the organomercurial compounds can even accumulate in the food chain. Certain bacteria can convert mercury into different forms, through *merA* gene coding for mercuric ion reductase and *merB* gene coding for organomercurial lyase. By constructing transgenic plants expressing these bacterial genes improved resistance to toxic effects Hg has been reported. Further, upon expression of *merB* in the endoplasmic reticulum results improved. Also it is known that chloroplast is the primary target site for Hg poisoning. Using chloroplast genome engineering high expression levels was achieved of *merA* and *merB* in the chloroplasts leading to improved resistance. According to endosymbiont hypothesis of chloroplast origin, it is believed that plastids originated from ancient bacteria. Thus bacterial gene expression in plastids leads to high expression levels without gene silencing. Also plastids follow maternal inheritance and thus there is no possibility of transgenic escape through pollen. Transgenic tobacco plants that express *merA* and *merB* in their chloroplast genome show greater levels of resistance to phenyl mercurial acetate, demonstrating efficient transportation of organic mercury and volatilization of elemental mercury. In addition to these further advances studies are under trial to upgrade phytoremediation technologies of mercury using transgenic tobacco plants.

- **Selenium (Se)** as a micronutrient is generally found in soils but it is also known to induce toxicity at high concentrations. Since selenium resembles sulphur, plants readily uptake and assimilate selenate using transporters and enzymes for sulphur and then volatize it. Toxicity also results from this similarity as Se gets incorporated in various proteins instead of Sulphur. For Se phytoremediation Selenium hyper accumulating or volatilizing plants are being employed. Various transgenic approaches are being employed with great success to further increase plant selenium accumulation, tolerance, and volatilization using information from selenium hyperaccumulators: overexpression and upregulation of genes related to sulphur/selenium metabolism and vaporization, pathways related to selenocysteine methylation, and transformation to innocuous elemental selenium from selenocystiene. Usage of a plethora of transgenic plants for laboratory and field trials hold promise for future use, demonstrating higher levels of selenium accumulation and volatilization.

- **Arsenic** is another major global pollutant. Transgenic plants with improved capabilities for Arsenic phytoremediation offers a cost effective strategy for arsenic cleanup. *E. coli* gene *ArsC* results in reduction of arsenic and formation of a complex with glutathione (GSH) or phytochelatins through conjugation.

Also GSH is overproduced by expression of glutamyl cysteine synthetase enzyme for subsequent conjugation. It can also be translocated to leaves through an efflux homolog of *ArsB* and successively vaporized through methylation of arsenic to trimethylarsine gas by addition of the *ArsM* gene in the genome of transgenic plants. Such genes can be used to construct a transgenic plant that can effectively absorb arsenic and accumulate it in its vacuoles for efficient phytoremediation.

The use of biotechnology to redesign plants for heavy metal accumulation and concealment is a very efficient system for enhanced phytoremediation. Transgenic plants with genes like metal chelator, metal transporter, metallothionein (MT), and phytochelatin (PC) are being engineered for enhanced metal uptake and sequestration. Table 3 enlists few examples of transgenic plants for metal tolerance/phytoremediation (from Eapen & D’Souza, 2005)

Table: Few transgenic plants for metal tolerance/phytoremediation

Gene transferred	**Origin**	**Target plant species**	**Effect**
MT2 gene	Human	*Nicotiana tabacum*, oil seed rape	Cd tolerance
MT1 gene	Mouse	*Nicotiana tabacum*	Cd tolerance
MTA gene	Pea	*Arabidopsis*	Cu accumulation
CUP-1 gene	Yeast	*Brassica oleracea*	Cd accumulation
CUP-1 gene	Yeast	*Nicotiana tabacum*	Cu accumulation
γ-Glutamylcysteine synthetase	*E. coli*	*Brassica juncea*	Cd tolerance
Glutathione synthetase	Rice	*Brassica juncea*	Cd tolerance
Cysteine synthetase	Rice	*Nicotiana tabacum*	Cd tolerance
CAX-2 (vacuolar	*Arabidopsis*	*Nicotiana*	Accumulation of

transporters)		*tabacum*	Cd, Ca and Mn
At MHX	*Arabidopsis*	*Nicotiana tabacum*	Mg and Zn tolerance
Nt CBP4	Tobacco	*Nicotiana tabacum*	Ni tolerance and Pb accumulation
FRE-1 and FRE-2	Yeast	*Nicotiana tabacum*	More Fe content
Glutathione-s-Transferase	Tobacco	*Arabidopsis*	Al, Cu, Na tolerance
Citrate synthase	Bacteria	*Arabidopsis*	Al tolerance
Nicotinamine amino transferase (NAAT)	Barley	Rice	Grew in iron deficient soils
Ferretin	Soybean	*Nicotiana tabacum*	Increased iron accumulation
Ferretin	Soybean	Rice	Increased iron accumulation
Zn transporters ZAT (At MTPI)	*Arabidopsis*	*Arabidopsis*	Zn accumulation
Arsenate reductase γ-glutamylcysteine synthetase	Bacteria	*Brassica juncea*	As tolerance
Znt A-heavy metal transporters	*E. coli*	*Arabidopsis*	Cd and Pb resistance
Selenocysteine methyl transferase	*A. bisculatus*	*A. thaliana*	Resistance to selenite
ACC-deaminase	Bacteria		Many metal tolerance
YCF1	Yeast	*Arabidopsis*	Cd and Pb tolerance
Se-cys lyase	Mouse	*Arabidopsis*	Se tolerance and accumulation
Phytochelatin synthase (Ta PCS)	Wheat	*Nicotiana glauca*	Pb accumulation

Phytoremediation of explosives, herbicides and organic compounds

- **Explosives** are important category of pollutants present at military sites polluting both land and groundwater. Explosives like 2, 4, 6-trinitrotoluene (TNT), hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine and glyceroltrinitrate could be phytoremediated through transgenic plants by introduction and expression of bacterial nitroreductases and cytochrome p450 genes. Such transgenic plants demonstrate significantly increased levels of tolerance, uptake, and detoxification of the target explosive. Also many studies have been done in *Arabidopsis* plants. Also introduction of *pnrA* gene from *Psuedomonas putida*, (superbug) encoding nitroreductase into rapidly growing Aspen tree has indicated encouraging results for effective phytoremediation.

  RDX, most widely used military explosive could be phytoremediated using transgenic plants. *Arabidopsis thaliana* plants have been genetically manipulated to express bacterial gene, *XplA*, which encodes a RDX-degrading fused flavodoxin-cytochrome P450-like enzyme. In laboratory using pure cultures of *Rhodococcus rhodochrous* strain 11Y, which is the donor for the above mentioned gene and is isolated from RDX polluted areas, demonstrated around 30%-mineralization of radiolabelled RDX. The bacterium degrades RDX by denitrification, followed by ring cleavage and the release of small aliphatic metabolites. Liquid cultures of *A. th*aliana expressing *XplA* were capable of 32–100% removal of RDX, while only less than 10% was removed by non-transgenic plants. This suggests that transgenic plants were competent of efficient phytoremediation of RDX.

- **Landmines**

  Approximately nearly about sixty to seventy million active landmines are present all over the world, claiming millions of lives every day, and threatening the subsistence of much more people by hampering their access to humanitarian aid, land and water resources. Transgenic plants are being developed to apprise people of the presence of landmines in a field. Development of *Arabidopsis* plants whose roots are capable of changing colour upon contact with degradation products of landmines are underway and are under laboratory and field trials. Studies are being done where plants can transmit such signals to their leaves and subsequently result in human readable changes for the development of pragmatic explosives detection systems.

- **Herbicides** are another category of serious pollutants but at the same time play vital roles in agriculture. Development of herbicide tolerant transgenic plants for enhanced phytoremediation is underway. Cytochrome-450 (CYP) has been introduced into rice for removal of atrazine, a herbicide. γ- glutathione synthetase gene has been introduced into poplar for efficient removal of alachlor. Expression of bacterial gene atrazine chlorohydrolase (*atzZ*) and genes for increasing root mass bacterial 1- aminocyclopropane-1-carboxylate deaminase offers another strategy for developing transgenic plants for remediation. Cytochromes P450 (CYP) enzymes belong to heme proteins super family and are involved in Phase I metabolism and clearance of numerous xenobiotics, such as therapeutic drugs, substances of abuse, herbicides, and industrial contaminants (figure). They are responsible for safe clearance of around 80% of marketed drugs by conversion into relatively hydrophilic compounds in the liver. CYP enzymes are being used as biocatalysts for remediation owing to their broad substrate specificity.

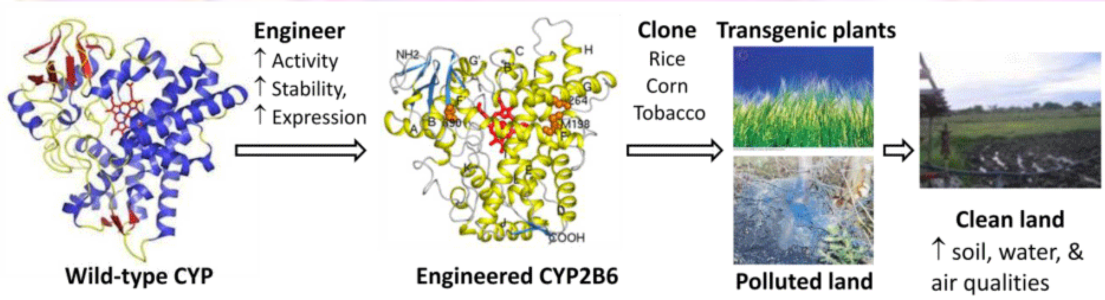

Figure: Genetic manipulation of CYP enzymes for overexpression and better activity and stability and cloning of engineered CYP enzymes (e.g. CYP2B6) in plants. This is then followed by growth of transgenic plants in contaminated areas which has the potential to detoxify the lands.

Source: http://omicsonline.org/2157-7463/images/2157-7463-3-127-g001.gif (CC)

TNT: A case study

For the purpose of phytoremediation genetically modified tobacco plants have been developed by the addition of a bacterial gene for pentaerythritol tetranitrate (PETN) reductase, an enzyme employed in the degradation of nitrate esters that are nitroaromatic explosives, from *Enterobacter cloacae* isolated from explosives contaminated lands. The enzyme PETN reductase, a flavonitroreductase, catalyzes the degradation of TNT by either addition of hydride groups or by consecutive reduction of nitro groups, which results in denitrification and, finally cleaning up of TNT. Such genetically modified tobacco seeds demonstrated capability to germinate and further growth even at high concentration of TNT which was detrimental to non transgenic seeds. Many other nitroreductases, like *NfsA* and *NfsB* from the bacterium *Escherichia coli*, *PnrA* from *Pseudomonas putida*, and *Nfs*I from *E. cloacae*, along with flavonitroreductases, are capable of consecutively reducing TNT's nitro groups to hydroxylamino and amino derivatives, possibly leading to release of ammonium by a Bamberger like rearrangement. It was reported by Hanninck et al., 2007 that genetically modified tobacco that expressed nitroreductase *NfsI* from *E. cloacae* demonstrated high rates of removal of TNT from solutions (100% removal from 0.25mM solution in 72h) when compared to wild type counterparts that were able to clear only negligible quantity of TNT. These transgenic tobacco plants also demonstrated higher tolerance to increased concentrations of TNT (upto 0.5nM) which were lethal to non transgenic varieties. These transgenic plants also reduced TNT to 4-hydoxylamino-2, 6-dinitrotoluene and further conjugated to macromolecules of the plant at much greater levels when compared to non transgenic counterparts. In addition to these studies, Kurumata et al. 2005, showed the transformation of *Arabidopsis thaliana* by the addition of gene for the enzyme nitroreductase, *NfsA*, from *E.coli* capable of acting against a variety of nitroaromatic molecules. A twenty times higher nitroreductase activity and 7-8 times higher TNT uptake was reported in transgenic plants that expressed the gene for *E.coli* nitroreductase. Moreover, such plants exhibited growth even at inhibiting concentrations of TNT (0.1 mM) and also in planta reduced TNT to 4-amino-2, 6-dinitrotoluene which was not observed in non transgenic counter parts. Such studies clearly demonstrate the potential of transgenic plants expressing bacterial catabolic genes for the phytoremediation of dangerous explosives like TNT.

- **Small organic compounds** including solvents having chlorine in their structures for example, CCl_4 (carbon tetra chloride) and CH_2Cl_3 (trichloroethylene) are important industrial contaminants. Transgenic tobacco plants expressing cytochrome P450 like human CYP2E1 demonstrate increased levels of metabolism of trichloroethylene and also other compounds like vinyl chloride, benzene, toluene, and chloroform. Further, expression of catabolic genes from the bacterium *Xanthobacte*r such as *dhlAB* which degrades 1, 2-dichlorethane conferred improved rates of eviction of the same from the plants. In addition to these, overexpression of extracellular enzymes such as laccases and peroxidases is being employed for the phytoremediation of small organic compounds.

Plant related micro-organisms

Certain pollutants like polychlorinated biphenyls (PCBs) are difficult to remediate owing to their chemical nature. Transgenic plants that render these PCBs more accessible to the rhizosphere bacteria for degradation have been developed. These transgenic plants express gene for the first multi-component enzyme (biphenyl 2, 3 deoxygenase) involved in PCB degradation pathway and thus bring about their degradation liberating metabolites for further transformation by bacteria present in the rhizosphere. Also certain transgenic plants harbour *bphC* gene, a 2, 3-dihydroxybiphenyl deoxygenase that can cleave toxic dihydroxybiphenyls.

Endophyte assisted phytoremediation using microbes that live within plants offers another useful approach. These plants show ameliorated growth patterns, increased stress tolerance and increased capabilities of degradation of contaminants. Inoculating plants with transgenic bacteria, an endophyte capable of degrading a pollutant can result in effective remediation of contaminants.

Ozone tolerant plants

Pollutants, like high concentration of ozone is detrimental for the plants resulting in reduced vitality and damping off. Ethylene, a senescence-inducing plant growth hormone, plays a major role in such damage to the plants. Identification of the enzymes involved in ozone induced ethylene overproduction in plants have paved for genetic engineering to make tolerant transgenic plants. By genetic manipulation of genes for these enzymes (knock down and knock outs), efforts are underway for the generation of transgenic plants exhibiting enhanced tolerance to ozone.

Biotechnology for plant diagnosis

Many damaging environmental factors affect plants leading to stunted growth and withering. However identification of these causal factors remains a challenge and even tougher is the visual inspection of damaged plants. Further it is vital to identify damaged plants early. Therefore, intense efforts are underway to develop sensitive and authentic methods for diagnosis of plants, based on measurement of changes in the expression levels of a genome, transcriptome or proteome using a DNA, RNA or protein microarrays, respectively. Using these methods scientists are trying to diagnose and identify plants affected by ozone, insufficient water supplies, or wounds.

Biofuels and biotechnology

Fossil fuels are at present the most common source of energy. Combustion of these fossil fuels to meet energy requirement worldwide is now a very serious environmental threat as it is the leading cause of global pollution. Serious efforts are required to develop alternative renewable energy sources which do not pollute our environment. Biofuels are alternative fuels derived from plants and their products. Bioethanol and biodiesel are the main two types of biofuels. Bioethanol is produced by fermentation of cellulose, starch or sugars generally from crops like corn, maize and sugarcane. Biodiesel is produced from oil crops like soybean, rapeseed and palm. They are being used as liquid fuels and additives of petrol and diesel for transportation. They have several advantages over non-renewable fossil fuels. The CO_2 produced from combustion of biofuels is initially extracted from the atmosphere during biomass production, resulting in zero net greenhouse gas emissions, thus they are carbon neutral. The addition of biofuels to petrol and diesel oxygenates the fuel mixture resulting in complete combustion, thus limiting the release of volatile compounds. Ethanol also eliminates the requirement of lead addition to the fuel mixture. Further biofuels are biodegradable and non toxic.

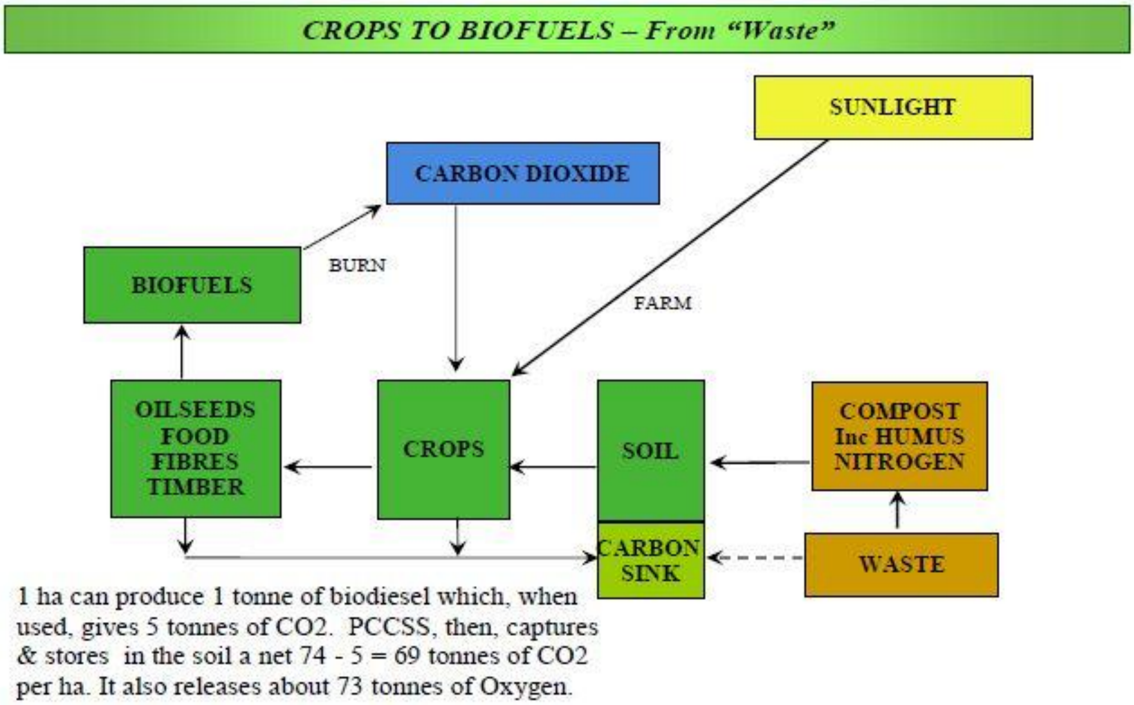

Figure: The flowchart of biofuel production from crops

Source: http://www.mdpi.com/1996-1073/2/4/1192 (CC)

The US Department for Agriculture (USDA) has estimated that one billion dry tons of biomass per year is required to replace 30% of transportation fuels with biofuels. Plant biotechnology aims to substantially increase crop yield, and develop crops with a suitable traits for energy production. By introducing bacterial photosynthetic genes in plants, efficiency of light capture during photosynthesis and thus plant growth can be enhanced. Some transgenic plants (e.g. tobacco, rice and *Arabidopsis*) overexpressing seduheptulose-1,7 bisphosphatase (SBPase), ictB and cytochrome c_6 genes showed positive effects on photosynthetic efficiency and growth characteristics (Ruan et al.,2012). Jing et al. 2004, showed over-expression of a glutamine synthesis gene (GS1) in poplar trees significantly increased tree height. In addition other strategies include extending the growth phase of plants, by reducing seed dormancy, or by preventing or delaying flowering, as plants devote a large proportion of their energy to making reproductive structures which could be harnessed into vegetative growth. Lignin has a negative impact on biofuel production. Poplars expressing cinnamyl alcohol dehydrogenase (CAD) or caffeate/5-hydroxy-ferulate O-methyltransferase (COMT) antisense transgenes showed altered lignin characteristics. Kraft pulping of tree trunks showed that the reduced-CAD lines had improved characteristics, allowing easier delignification, using smaller amounts of chemicals, while yielding more high-quality pulp (Pilate et al., 2002).

Globally, abiotic stress is the major cause of reduced crop yields. Also plant pests and pathogens reduce plant productivity. Therefore one of the major goals of plant biotechnology is the development of plants with enhanced resistance to stress, pests and pathogens. Transgenic rice over-expressing the glutamine synthase gene (GS2) showed increased tolerance to high soil salinity.

Insecticide and herbicide resistant plants

Plants are regularly sprayed with insecticides to curb losses of crops due to insects. These insecticides are serious environmental pollutants that could be harmful to other members of local biosphere as well as humans. *Bacillus thuringiensis* during sporulation forms intracellular crystalline bodies that have an insecticidal protein called the δ-endotoxin. This toxin is highly poisonous to insects and is also relatively selective. The δ-endotoxin protein of the bacterium is an inactive precursor, which upon digestion by the insect gets cleaved by proteases forming shorter toxic proteins. They damage the gut epithelial cells so the insect starves to death as it unable to feed. The gene for δ-endotoxin has been cloned in various crops including maize, cotton and tobacco. The first attempts to genetically engineer maize crops (Bt maize) to produce their own insecticide was made in 1993 when the gene for δ-endotoxin CryIA (b) was introduced in them. Significant levels of resistance to corn borers were achieved. But there is a possibility that cloned gene can escape from the plant and gets established in a weed species. One possible solution is to target the gene to chloroplasts. A transgene in chloroplasts reduces the chance of the foreign gene escaping to other species. This has been achieved in transgenic tobacco plants using chloroplast transformation.

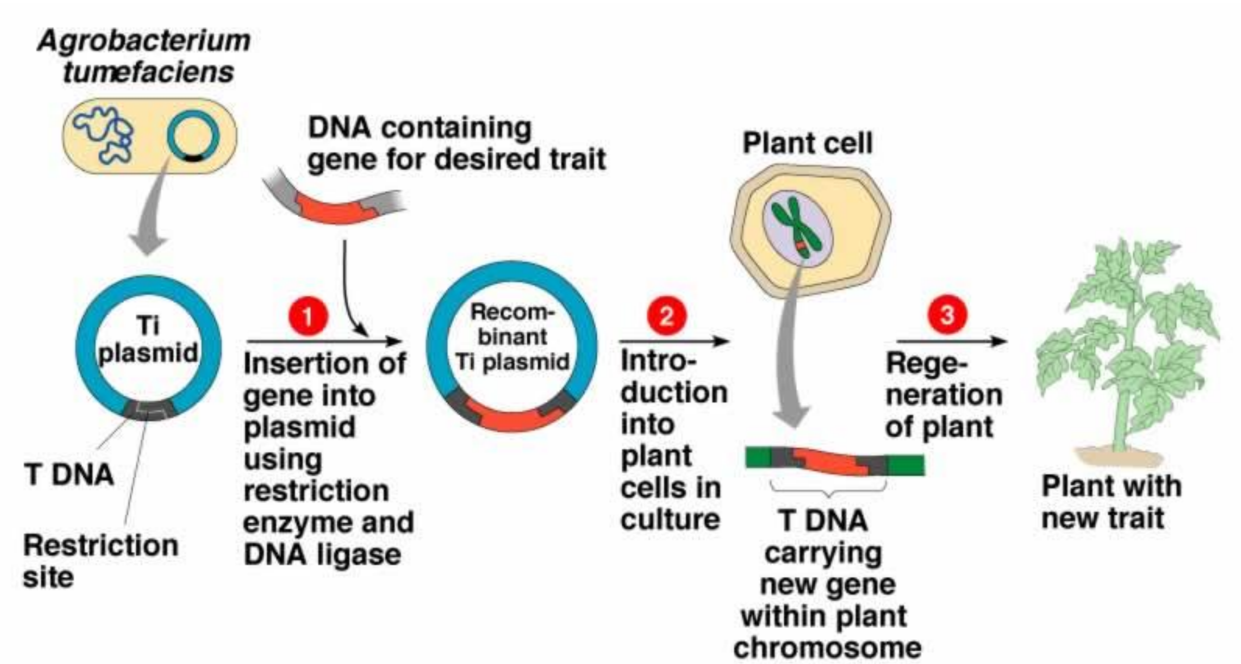

Figure: Diagram representing the construction of a transgenic plant

Source: http://www.mediahex.com/Transgenic_Organism (CC)

Herbicide glyphosate or round up is a widely used herbicide but it kills both crop plants as well as weeds. Plants have genes for the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS), that transforms shikimate and phosphoenol pyruvate (PEP) into enolpyruvylshikimate-3-phosphate, an essential precursor for synthesis of the aromatic amino acids. Glyphosate acts as a competitive inhibitor of EPSPS, competes with PEP for binding to the enzyme surface, thereby inhibiting synthesis of enolpyruvylshikimate-3-phosphate and thus aromatic amino acids. Without these amino acids, the plant succumbs to death. The genetically modified (GM) glyphosate-resistant crops carry in their genome a gene derived from *Agrobacterium tumefaciens* encoding the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which is insensitive to the inhibitory effect of glyphosate. Varieties of Roundup Ready crops have been produced in recent years, and few of them, particularly soybean and maize, are grown routinely in the USA and other parts of the world. New generation of improved round up ready crops are also being developed by techniques of multigene shuffling and chloroplast transformations.

Bioplastics

Plastics are very serious environmental pollutants mostly derived from fossil fuels and produce greenhouse gases. Bioplastics are plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, pea starch or microbes, and are generally biodegradable. They can be made up of a variety of materials including:

starches, cellulose, or other biopolymers. They are used as packaging materials, dining utensils, food packaging, and insulation.

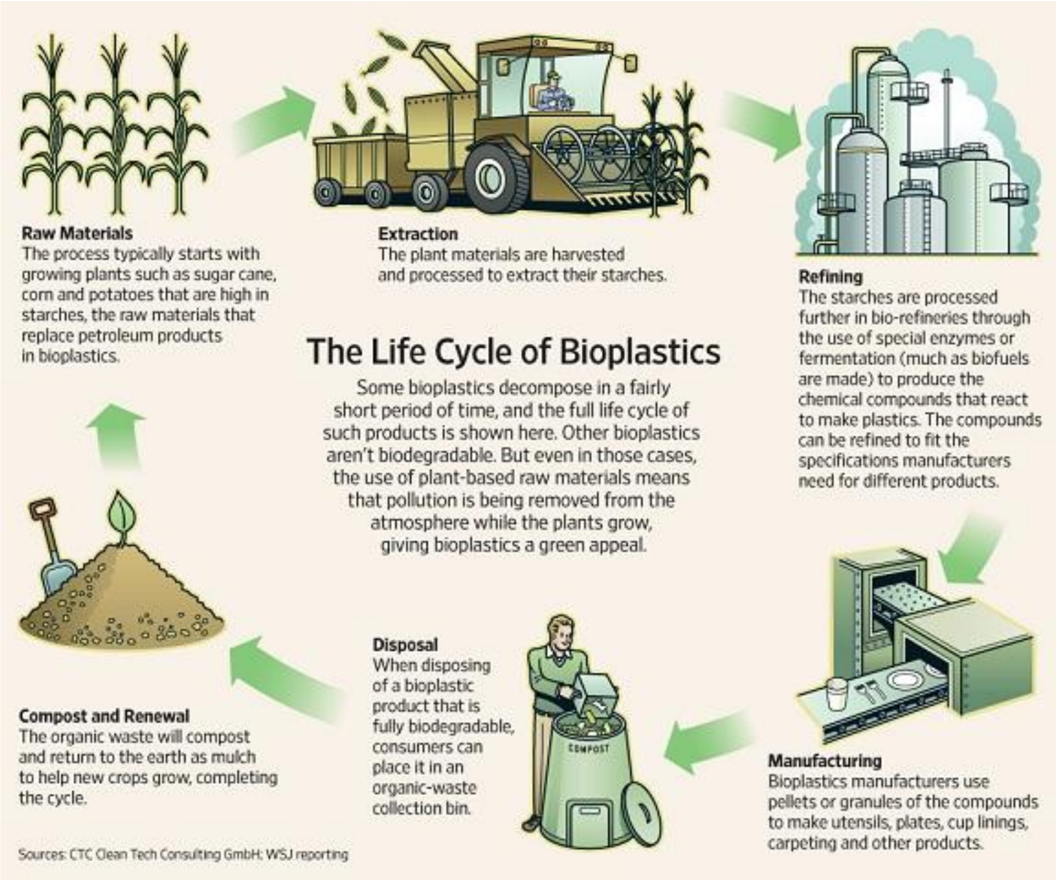

Figure: The life cycle of bioplastics

Source: http://www.natural-biodegrade.com/bioplastics/ (CC)

Common Biodegradable plastics or Bioplastics are polyhydroxyalkanoates (PHAs) which are basically linear polyesters. They are produced by many microbes as lipid reserves and source of intracellular carbon and energy. PHAs are commercially being produced by microbial fermentation. Various experimental studies are underway for the production of bioplastics using transgenic plants. Polyhydroxy butyrate (PHB) production is a 3 stage pathway involving three key enzymes: 3-Ketothiolase (phaA), Acetoacetyl-CoA reductase (phaB) and PHB synthase (phaC). Genes encoding these enzymes have been isolated from *Alcaligenes eutrophus* and cloned in *Arabidopsis* (Poirier et al., 1995). The cytoplasm of plant cell contains 3-Ketothiolase. Therefore only two genes (phaB and phaC) coding acetoacetyl CoA reductase and PHB synthase were transferred. Low levels of expression were obtained. Then PHB was expressed in plastids where all three genes (phaA, PhaB, PhaC) of PHB synthesis was separately fused with a coding sequence of transit peptide bound to N- terminal fragment of Rubisco (ribulose 1,5- bisphosphate carboxylase oxygenase) subunit protein. They were then directed to chloroplast under CaMV 35S promoter. Firstly, transgenic

Arabidopsis plants with each gene construct were developed. This was followed by a series of sexual crossings between the individual transformants. The transgenic plants developed now yielded good quantity of bioplastics without any adverse effect on the growth and fertility of plants. Although excellent progress has been made in recombinant hosts for the production of bioplastics, the barriers to obtaining high quantities of PHA at low cost still remain to be solved. The commercially viable production of PHA in crops, however, appears to be a realistic goal for the future. However, economically viable production of PHA in crops appears to be a realistic goal for the future (Suriyamongkol et al. 2007).

Professor Anand Mohan Chakrabarty an Indian American scientist using Plasmid gene transfers developed a genetically engineered organism (***Psuedomonas putida***) capable of degrading oil one to two orders of magnitude faster than previously known oil eating microbes. This bacterium is a **superbug** and can be used for bioremediation of oil spills. The new microbe, which Chakrabarty named "multi-plasmid hydrocarbon-degrading *Pseudomonas*," is capable of digesting about two-thirds of the hydrocarbons found in a typical oil spill. He got the first patent for a genetically modified organism (GMO).

Ameliorating phytoremediation using biotechnology and genetic manipulation of plants might overcome the hazard of toxic pollutants which contaminating the food chain. Newer advancements through the introduction and overexpression of natural or genetically engineered genes involved in catabolic pathways, like mammalian cytochrome P450, bacterial nitroreductases and fungal peroxidases, for the concomitant removal of a plethora of contaminants generally present in polluted areas, might prove beneficial. Also, the addition of more than one transgenes associated with various catabolic pathways and phytoremediation processes, like phytoextraction and metabolism, further point towards of the success of biotechnology for efficient environment cleaning. But risk of horizontal gene transfer to related varieties of plants possess a significant limitation of using biotechnology for phytoremediation. Thus, scientists are working on next generation of genetically modified transgenic plants by techniques like addition of newer genes in chloroplast genome or usage of conditionally lethal genes that do not allow such gene transfers. Also transgenic plants could be developed for enhanced biofuel production and insecticide resistance. Future holds great promise for biotechnology associated environmental clean up.

Summary

Phytoremediation is the use of plants to cleanup the environment of its contaminants. It is cost effective and provides with an opportunity of phytomining. For metals, plants show the potential for phytoextraction (uptake of metals into above-ground part and harvestable biomass), filtration of metals using water root systems or stabilization of waste through erosion control (phytostabilization). Biotechnology finds its applications in phytoremediation using transgenic plants. Heavy metals like Ag, Se and Hg could be phytoremediated using transgenics. Also herbicides, explosives and small organic compounds can be cleaned of environment using transgenics. Biofuel production could be enhanced using genetic engineering of plants. Insecticide and herbicide resistant plants could be generated. Bioplastics are also emerging as source of biodegradable environment friendly plastics.

Exercise

Q.1: What is phytoremediation?

Q.2: List few processes of phytoremediation.

Q.3: How do transgenic plants help in remediation?

Q.4: How are herbicides remediated from the environment?

Q.5: What do you mean by superbug?

Q.6: What are bioplastics?

Glossary

Bioplastics: Bioplastics are plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, pea starch or microbes.

Green liver model: A model that proposes functioning of plants analogous to human liver for clearance of xenobiotics.

Hyperaccumulator: A plant capable of growing in areas rich in metals, absorbing them through its roots, and concentrating them in extremely high levels in its tissues.

Phytoremediation: The use of plant biomass or sometimes microbes to clean the environment of contaminants.

Phytoming: Recovery and reutilization of Metals from plants.

Phytoextraction (Phytoaccumulation): It is a process where the pollutants or waste materials are up taken or absorbed by the plant roots and stored in above part and harvestable part of biomass.

Phytotransformation: It is a process that results in chemical transformation of environmental contaminants due to plant metabolism.

Phytodegradation: It involves enzymatic breakdown of organic pollutants by both internal as well as secreted plant enzymes.

Transgenic plants: Plants whose genetic material has been altered by insertion of foreign DNA using techniques of gene manipulation.

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