* Phyoremediation

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*Introduction

* Rapid growth of population, mining and industrialization significantly contributed to extensive soil, air, and water contamination.
* Many industrial wastes contain toxic chemicals, organic compounds,
  * Petrochemicals,
  * Polyaromatic hydrocarbons (PAHs),
  * Dyes,
  * Pesticides,
  * Explosives and
  * Inorganic compounds (heavy metals, nitrates, sulfates and chlorides)
* These are harmful to various forms of life.
* Some of organic pollutants are toxic and difficult to degrade compounds.
* Because of their insolubility, they accumulate in sediments and thus become a serious problem
* Therefore it becomes necessary to remediate these contaminated sites.

* Chemical methods
  * Removal of these contaminants, physico-chemical methods
    * Reverse-osmosis,
    * Membrane filtration etc.
  * These treatment methods are costly and difficult to adapt in case of soil contaminated sites.
* Biological methods of remediation (bioremediation) technologies become suitable in such cases.
  * It uses microorganisms to degrade and detoxify pollutants causing environmental contamination.
    * This technology is applied for treatment of wastes.
    * The bioremediation can be carried out at the site of contamination (in situ) or the wastes are transported at some suitable site for treatment (ex situ).
  * Exploration of plants for remediation: an emerging cost-effective approach.

Way outs

*Definition

* "Remediate" = to solve a problem and
* "bioremediate" = to use biological organisms to solve an environmental problem.

* Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site.

* Acc. to Environmental Protection Agency (EPA): “*treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances*”.

* ***Phytotechnologies*** are defined as the use of ***plants*** to remediate, treat, stabilize, or control contaminated substrates, and phytoremediation is one of these, specifically dedicated to the removal or destruction of the contaminant.

* The other names used for bioremediation are
  * Biotreatment,
  * Bioreclamation and
  * Biorestoration.
* ***Xenobiotics*** broadly refer to the ***unnatural***, ***foreign*** and ***synthetic*** chemicals
  * Pesticide,
  * Herbicide &
  * Other organic compounds.

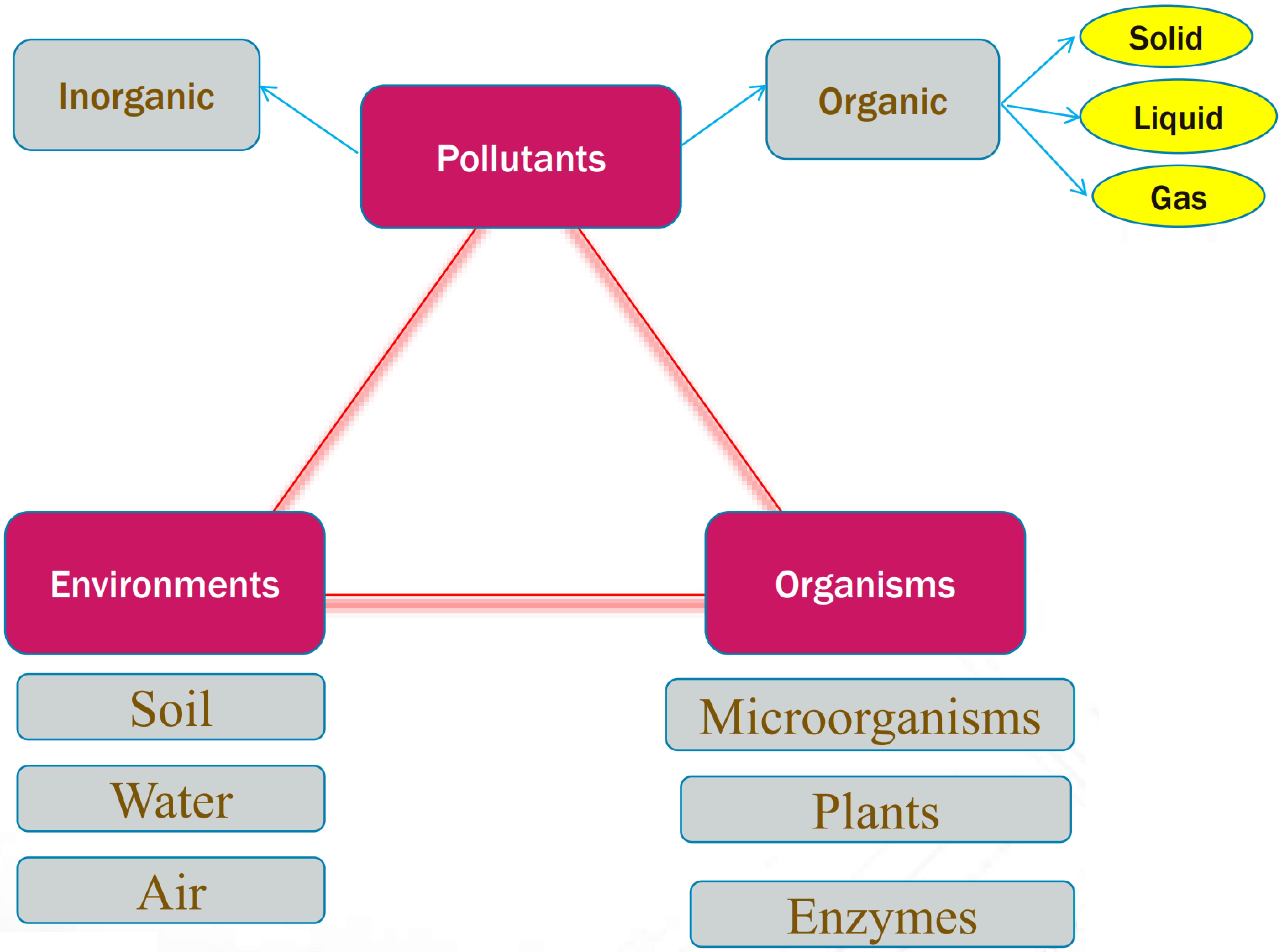
Other names

What is Bioremediation?

* Bioremediation is,
  * The biological degrading processes for the treatment of contaminated
    * soils,
    * groundwater and/or
    * Sediments
* Relying on microorganisms including
  * bacteria and/or
  * fungi
* To use the contaminant(s) as a food source with resulting degradation of the contaminant.

* Bioremediators

* Microorganisms used to perform the function of bioremediation are known as ***bioremediators***.
* Bioremediation is one of the most ***economic remedial techniques*** presently available for treating most <u>*organic fuel based contaminants:*</u>
  * coal tars and liquors,
  * petroleum and
  * other carcinogenic hydrocarbons:
    * benzene and
    * naphthalene, and
    * some inorganics.



*Triple Corners Process

Sources of contamination

* Sources:
  * Industrial effluents.
  * Deposited from flue and exhaust gases.
  * Old industrial sites.
  * Disused mines
  * Run-off from waste pipes and landfills
  * Excess application of herbicides and pesticides
  * Accidental spills
* Nature:
  * ***Volatile component***: Component will evaporate, air pollution,
  * ***Water soluble***: May dissolve in river, lakes or ground water: It will be mobile in the soil and contaminate water. It might degraded by microbial population.
  * ***Insoluble (Hydrophobic)***: Difficult to metabolize by microorganisms but some compounds will be dissolved in the cells lipid membranes.
  * ***Inert***: It will accumulate in the organism and become the part o f food chain. It may bind to soil components and remain fixed within soil.
  * ***Metals***: Non bio-degradable but can be absorbed by microorganisms and allow their concentration and disposal.
  * ***Xenobiotics***: Copounds released into environment but are not normally found like insecticide (DDT) and herbicide (lindane)

* Fate of Pollutants

* Low levels: May not appear a problem
* Some ***organisms*** in the environment may ***concentrate*** the compound to level considerable ***higher*** than those of surrounding environments.
* The toxic level of the compound may be reached and accumulation will be compounded of the organism is a part of the ***food chain***.
* The increase in successive organisms in the food chain is known as: ***Biomagnification***.
* DDT in Grebes are aquatic diving birds: At top level of food chain on a lake created with 10,000 times DDT level than control (0.01 – 0.02 ppm control)

Biomagnification

*Terms

* ***Mineralization***: Partial and complete decomposition of the chemical compounds in organic matter (CO_2, H_2O) and inorganic matter.
* ***Persistent***: Organic matter undergo degradation under circumstances.
* ***Recalcitrant***: Compounds are ***not*** degraded under any conditions.
* ***Bioaccumulation***: Increase of a compound in an organism compared with the level in the environment.
* ***Biomagnification***: Increase of a pollutant in tissues of successive organisms of a food chain.
* ***Bioconcentation Factor***: Concentration of a pollutant from the environment, and the factor is the concentration in an organism compared with that in the environment.

Synthetic compounds

* Many synthetic organic (xenobiotic) compounds have been produced and found into the environment.
  * Most commonly found are the ***pesticides*** (biocides), ***herbicides***, and ***preservatives***.
  * Most ***herbicides*** are released into the environment by ***direct use*** although some may be released during manufacture and spills do occur.
  * ***Polychlorobiphenyls*** (***PCBs***) are used in hydraulic fluids, plasticizers, adhesives and lubricants.
  * ***Flame retardants*** and ***dielectric*** fluids in the transformers are released during production, from spoilage and disposal.
  * ***Chlorinated compound*** (trichloroethane, carbon tetra chloride and pentachlorophenol): used as solvents and wood treatment .
  * ***Dioxins*** and ***dibenzofurans*** can be formed during combustion of poly-aromatic hydrocarbons (PAHs) and when heating plant oils.

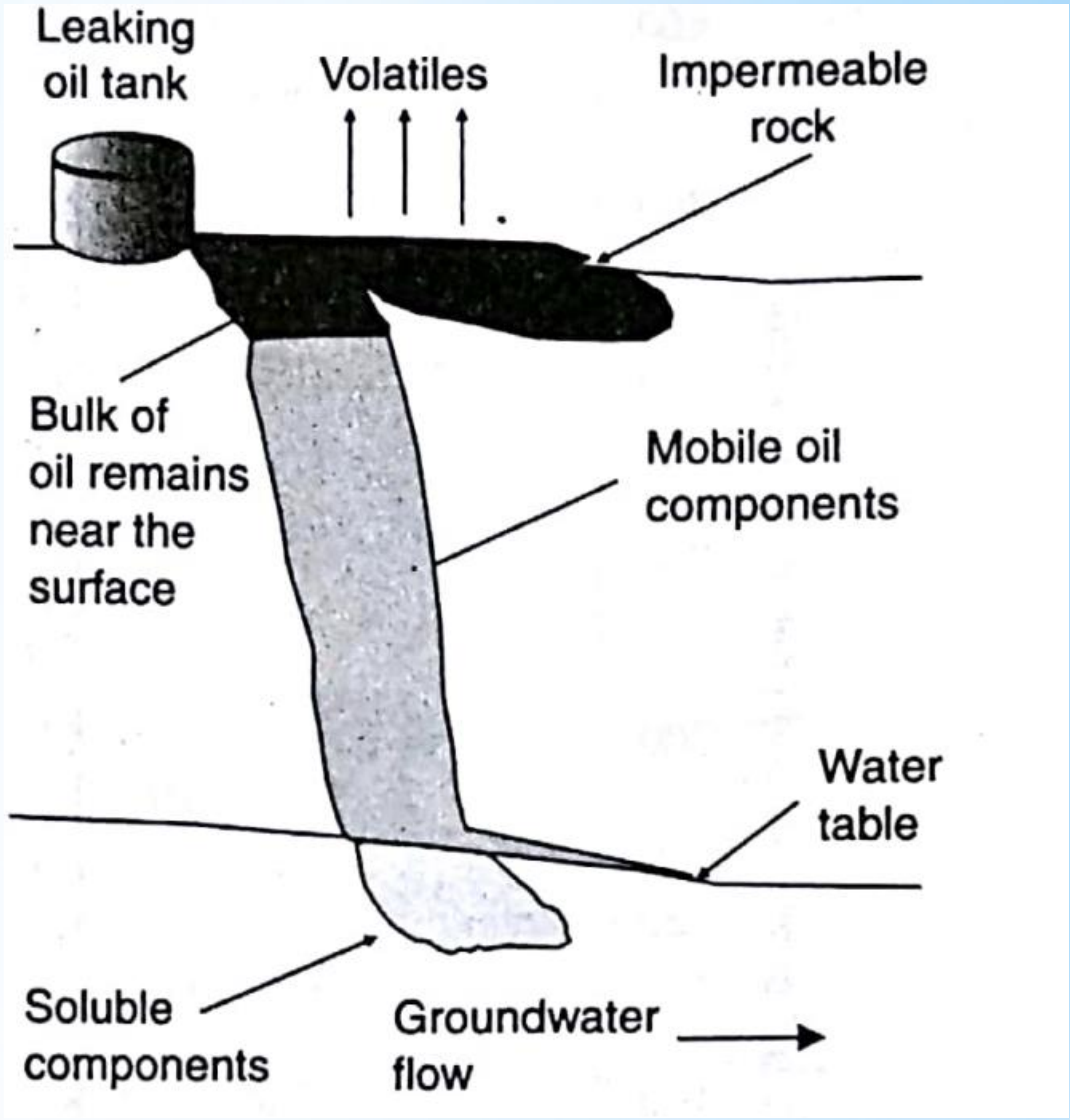
Petrochemical compounds

- ***Crude oils***: extremely complex and variable mixture of compounds.
  - Accumulated underground as a result of anaerobic degradation of organisms over a long time.
  - Under high temperature and pressure, the organic material has been converted to natural gas, liquid, crude oil, shale oils and tars.
  - At underground temperature, shale oils and tar don't flow, but crude oil is liquid.
  - This crude oil will escape to surface, while the volatiles evaporate, forming tar bed.
  - The majority of the compounds in crude oils: hydrocarbons (low molecular weight gas methane to high-molecular-weight tars and bitumen).
- Oils with high proportions of low-molecular-weight material are known as ***light-oil***: flow easily while '***heavy oils***' are reverse.
- Hydrocarbons, crude oils contain heterocyclic compounds containing sulphur, nitrogen and oxygen and some heavy metals.
- When crude oil is refined: most PAHs are converted into monocyclic aromatic compounds,

* Crude oils and products released to the land

* ***Crude oils*** accumulated underground , can ***reach*** the ***surface*** in not contained by impermeable rock.
* The main ***source*** of crude oils and oil products (petrol and diesel) released on land ***comes from disposal of waste motor oils***, the ***leaking of storage tanks*** and ***other spoilages*** and ***accidents*** during transport.
* The ***petroleum leaks*** are important because they contain ***BTEX***: they are mobile and can contaminate ground water.
* The ***volatile*** component will be ***lost*** at atmosphere if the leak is on or very ***near the surface***, but if the leak is ***below the soil level*** the mobile components can ***migrate down*** through the soil to the water table.
* Any compound that are water soluble can also migrate down through the soil and into the ground water.
* The ***higher-molecular-weight components*** are mostly immiscible with water and may either ***move slowly*** through the soil or remain on or near the surface, depending on the soil structure.
* ***Insoluble*** components may also be ***absorbed*** very ***tightly*** on to the soil particle: They ***do migrate*** through the soil and reach the water table and form layer on the surface of the water and spread out in this manner.

* ***Oil taker wrecks***: large quantities are released.
* The considerable amount of oil is released in the ***process of cleaning the oil tanks*** oil tankers.
* These can be ***decreased***: International Convention for the Prevention of Pollution from ships.
* The ***cleaning waste should be retained*** and pumped out when the ship is next to port.
* But ***not*** all the tankers conform to the agreement.
* ***Toxicity*** of some metals have been ***set*** for metal levels in drinking water (national and international standards).
* ***Metal released*** from some ***industrial*** processes often far ***exceeded*** the levels set for the ***drinking water values***, but these wastes are usually greatly ***diluted*** when added to ***water ways*** and sewages systems.
* ***Metals*** released can not be ***biodegraded***. They can be ***absorbed*** to the soil particle, run off into rivers or lakes and leach into the ground water.
* Metals can occur in different forms: known as speciation.
* ***Cadmium***: ***divalent*** form is ***soluble*** and mobile, but can complex with oxides and oxide compounds.



Crude oil and product release in to the sea

- Physical or chemical methods:
  - Immobilization,
  - Removal (dig and dump)
  - Solvent treatment
- Bioremediation:
  - Cheaper
  - Also deals with lower concentrations of contaminants.
- Strategies:
  - Use of indigenous microbial population
  - Encourage the indigenous population
  - Bioaugmentation: the addition of adapted or designed inoculants
  - Biostimulation: the addition of rate limiting nutrients
  - Addition of genetically modified organisms
  - Phytoremediation

Bioremediation strategies

* Greatly affected by ***unstable*** climatic and environmental factors from moisture to temperature.
* For examples, pH in soil is slightly acidic; petroleum hydrocarbon degrading bacteria do not work well < 10º C.
* These microbes are usually thermophilic anaerobic.
* Fertilizers are needed. Seeding or bioaugmentation could be useful too.
* They contain ***monooxygenases*** and ***dehydrogenases*** to break down organic matters including most toxic substances

Use of Bacteria In Bioremediation

* *Candida* can degrade formaldehyde.
* *Gibeberella* can degrade cyanide.
* Slurry-phase bioremediation is useful too but only for small amounts of contaminated soil.
* Composting can be used to degrade household wastes.

White rot fungi

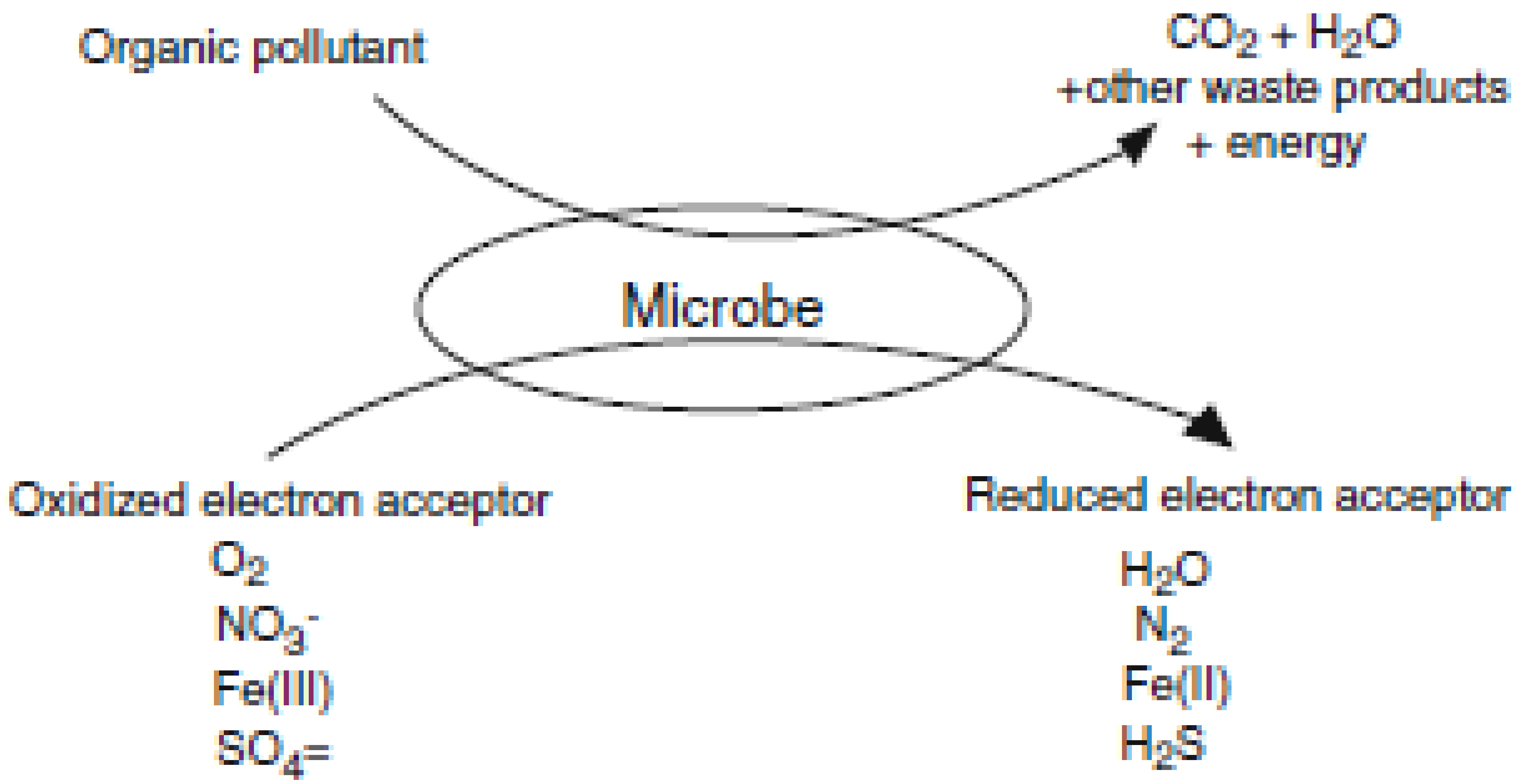
* They can degrade organic pollutants in soil and effluent and decolorize kraft black liquor, e.g. *Phanerochaete chrysosporium* can produce aromatic mixtures with its ***lignolytic system***.
* Pentachlorophenol, dichlorodiphenyltrichloroethane (e.g. DDT)
* Even TNT (trinitrotoluene) can be degraded by white rot fungi.

Use of fungi in bioremediation

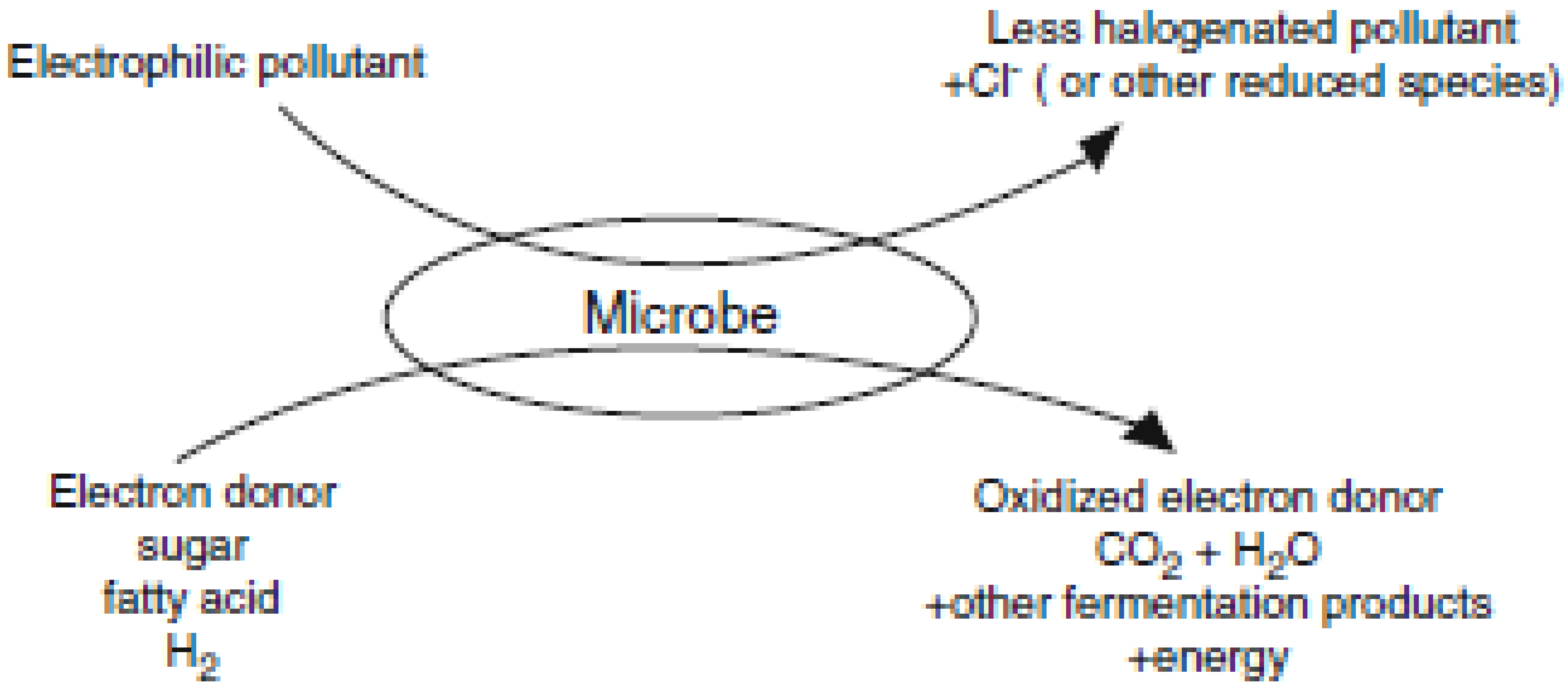
Redox Clean-up reactions

* Anaerobic or aerobic metabolism involve ***oxidation*** and ***reduction*** reactions or ***Redox*** reactions for detoxification.
* Oxygen could be reduced to water and oxidize organic compounds.
* Anaerobic reaction can use nitrate.
* In return, ***biomass*** is gained for bacterial or fungal growth.
* In many cases, combined efforts are needed, indigenous microbes found naturally in polluted sites are useful.

General schemes to biodegrade organic pollutants.



Oxidative Biodegradation



Reductive Biodegradation

* It is applied in the ***eastern*** part of world for the extraction of minerals, enhancement of agriculture and waste management.
* Indigenous microorganisms are a group of innate microbial consortium that ***inhabits*** the ***soil*** and the ***surfaces*** of all living things inside and outside.
* Based on the collection sites, the process of collection and isolation methods are different as they may vary from place to place.
* Degradation of organic compound by indigenous microbes ***without*** any artificial enhancement is termed as an "***intrinsic bioremediation***" and this is one of the best remedial actions for soil contamination.

Indigenous Microorganisms (IMO's)

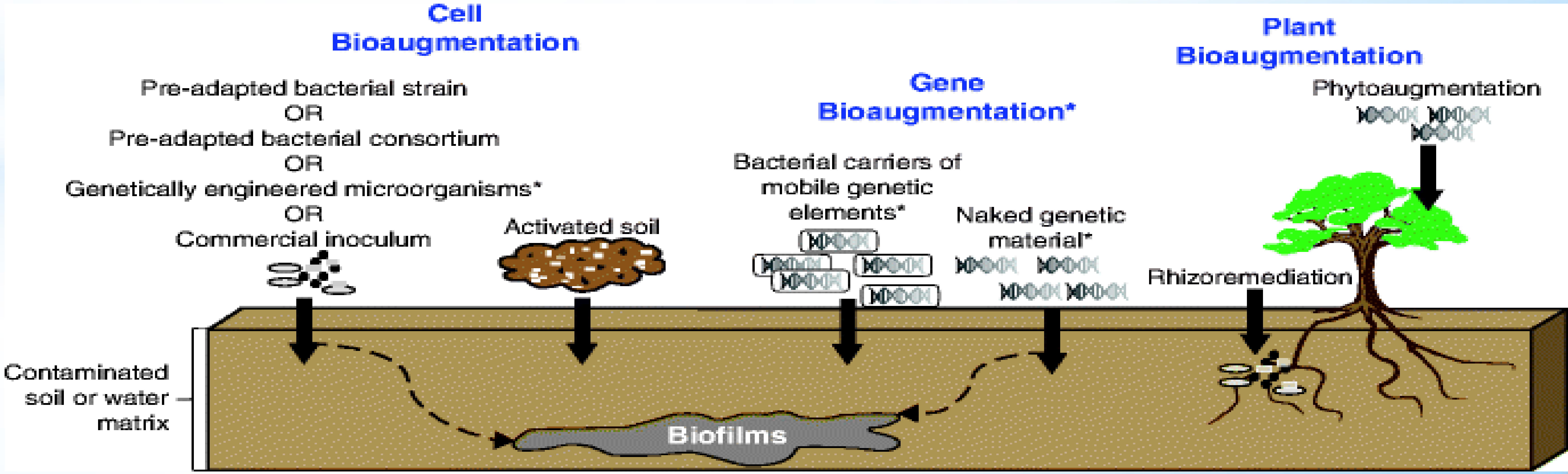
* Oil spills are treated as a widespread problem that poses a ***great threat*** to any ecosystem.
* Crude oil is composed of a wide ***range*** of different compounds
* It is ***difficult*** for the indigenous population to cope with this broad variety of substrates,
* ***Oil-degrading microorganisms*** could be ***added*** to supplement the indigenous population.
* Bioremediation has two complementary approaches:
  * ***Bioaugmentation***: the ***addition*** of oil-degrading ***bacteria*** boosts biodegradation rates.
  * ***Biostimulation***: the growth of indigenous hydrocarbon degraders is stimulated by the ***addition of nutrients*** (mainly N and P) or other growth limiting nutrients.
* A new concept in bioaugmentation, known as "***autochthonous bioaugmentation***" (ABA).

Bioremediation of oil spills

* Bioaugmentation: ***Exclusively uses microorganisms indigenous to the sites*** (soil, sand, and water) slated for decontamination.
* The ***success*** depends on the establishment and maintenance of
    * Physical,
    * Chemical and
    * Biological conditions that favour enhanced oil biodegradation rates in the marine environment.
* Natural Biodegradation as a Remedial Action Option Interim Guidance (1994): illustrated the potential strategy for the successful remediation of polluted marine environments (http://www.dnr.wi.gov/files/pdf/pubs/rr/rr515.pdf).
* Effectiveness of autochthonous bioaugmentation together with biostimulation versus biostimulation- only strategies for the successful remediation of polluted marine environments.

Bioaugmentation

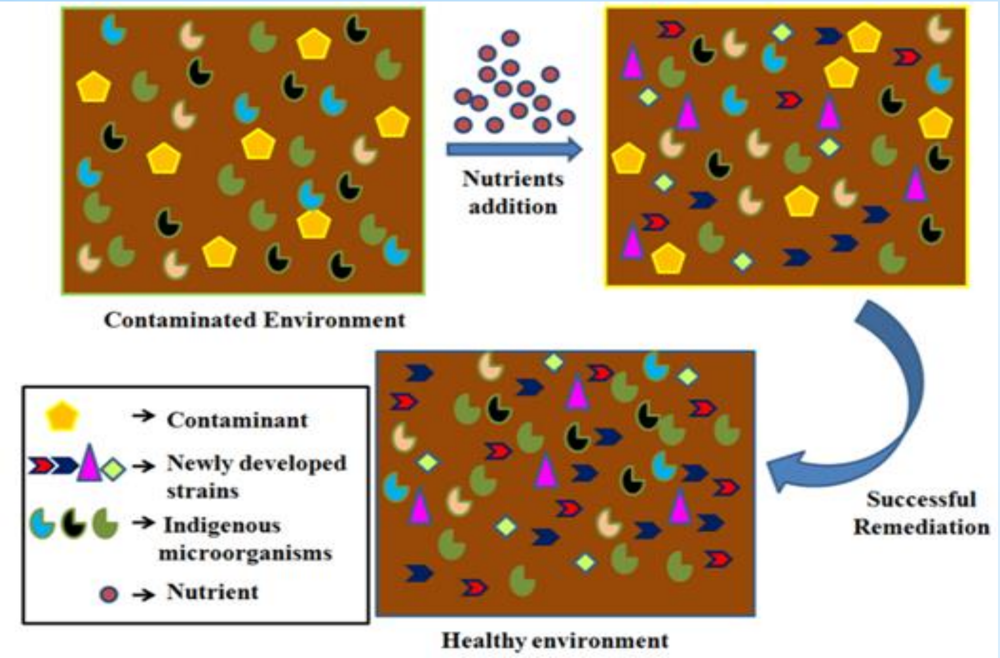
* ***Ozonation*** is effective in removing contaminants such as polyhydrocarbons (PHCs) in diesel contaminated soils.
* Ozone has strong ***oxidizing activity***: It reacts with organic compounds to form oxidized products which ***are more water soluble*** and ***bioavailable*** than parental compounds leading to a better biodegradation.
* ***External introduction*** of microorganisms into ozonized soil does ***not*** show promising result and caused a ***rapid decline*** of cell number and/or activity of inoculated cells
* ***Already-acclimatized indigenous microorganisms*** is an alternative to achieve successful remediation.
* ***Pre-Ozonation*** and ***subsequent bioremediation*** technology is ***best way*** for better degradation of ***polyhydrocarbons*** in the contaminated soils.



* The success depends mainly on the ***adaptation of the microbial consortia*** to the site that needs to be decontaminated.
* The success of the process also relies on the ability of the newly introduced microbial consortia to ***compete*** with the indigenous microorganisms, predators and various abiotic factors.
* ***Other parameters*** that control the rate of bioaugmentation occurring in soil.
* Some of the noted parameters or factors are:
  * pH,
  * temperature,
  * moisture,
  * organic matter content,
  * aeration,
  * nutrient content and
  * soil type.
* The existence of a ***variety of adaptation*** strategies in microorganisms:
  * The ability to ***modify the cellular membrane*** to maintain the necessary biological functions.
  * The production of surface-active compounds such as ***biosurfactants***.
  * The use of ***efflux pumps*** to ***decrease*** the concentration of ***toxic*** compounds inside the cells

Factors influencing *Bioaugmentation*

* It refers to the addition of rate limiting nutrients like phosphorus, nitrogen, oxygen, electron donors to severely polluted sites.
* It stimulates the existing bacteria to degrade the hazardous and toxic contaminants.
* Role of rate limiting nutrients:
  * They improve the degradation potential of the inhabitant microorganisms efficiently as it significantly accelerates the decontamination rate.
  * Biostimulation is considered to be the most efficient method for remediation of hydrocarbons.
  * Petroleum contaminated sites: less efficient and metabolically poor microbial population can be remediated significantly by the addition of some of the rate limiting nutrients.
  * Easy availability of carbon (C) source: just addition of few of the other rate limiting nutrients except carbon boosts up the petroleum degradation rate significantly.



Biostimulation: an efficient strategy of bioremediation

* The major contaminants are
  * Petroleum hydrocarbons,
  * Sulphate and
  * Polyester polyurethanes.
* Sulphate contamination: It requires amendment of electron-donor that will enhance sulphate reduction and thus remediate the same.
* Polyester polyurethanes:
  * PUs are insulating and packaging foams, fibers, fabrics, and synthetic leather goods.
  * These polymers contain intra-molecular bonds: These bonds act as a site for microbial attack.
  * Biostimulation: The best approach in those cases where microbial population gets acclimatized due to exposure to hydrocarbons at contaminated sites.
  * The adapted populations showed higher remediation rates in comparison to those with no contamination exposure history.

Application of biostimulation:

Nutrients used	Target pollutants
Animal manure and sewage sludge	Atrazine and alachlor
Activated sludge	Atrazine and simazine
Plant residues, ground seed, or commercial meal	Alchlor, metolachlor, atrazine and trifluralin
Cellulose, straw and compost	Atrazine
Cornmeal, ryegrass and poultry litter	Cyanazine and fluometuron
Dairy manure	Atrazine
Maize straw	Methabenzthiazuron
Ammonium nitrate, potassium nitrate and ammonium phosphate	Atrazine
Phosphorus	2,6 Di chloro benzonitrile and atrazine
Nitrate	(R)-mecoprop
Nitrate and phosphorus	Isoproturon
Tryptic soy broth	Dichlofop
Mannitol	Atrazine

*Biostimulation in polluted environments

* Advantages:
  * The primary advantage: It is done by native microorganisms that are well suited to the environment and are already well distributed spatially.
  * Secondly: It is an eco-friendly and cost effective technique which can be performed anywhere.
  * Lastly: It helps in the degradation of contaminants internally which prevents any kind of disturbances to the environment.
* Disadvantages.
  * Firstly, it is its dependency on environmental factors that controls its potentiality.
  * Secondly, when the ***contaminants*** are firmly ***engrossed*** to the ***soil particles*** or the contaminant is ***non biodegradable***, then biostimulation cannot be executed.
  * Thirdly, biostimulation is extremely ***site specific*** and requires immense ***scientific observation***.

Advantages and disadvantages of biostimulation

* Phytoremediation

* Different plant physiological processes are exploited to remove or destroy the contaminants from the contaminated sites.
* A range of processes mediated by plants or algae is useful in treating environmental problems.
* Phytoremediation can occur through the following routes.
  * Phytoextraction
  * Phytostabilization
  * Phytotransformation
  * Phytostimulation
  * Phytovolatilization
  * Rhizofiltration

Phytoextraction and Phytostabilization

* Phytoextraction:
  * Certain plants have ability to ***take up*** pollutants from soil, water bodies, and others.
  * The contaminants are ***absorbed*** through ***root system*** and transported to ***leaves*** or ***stem***.
  * Some plants can accumulate pollutants in large quantity till the time they are harvested.
  * Compared to the volume of contaminated site, plant matter that extracts the pollutants is small and therefore easy for disposal.
  * The phytoextraction process is being globally employed.
  * After the process, the cleaned soil can support ***other vegetation***.
  * 'Mining with plants' or phytomining is also being experimented.
  * ***Willow*** plants were used for phytoextraction purpose.
* Phytostabilization:
  * Phytostabilization focuses on long-term ***stabilization*** and ***containment*** of the pollutant.
  * The pollutants are ***adsorbed*** or accumulated in ***root zone*** of the plant system, where they are ***precipitated*** and ***stabilized***.
  * The pollutants are ***restricted*** to ***roots*** only and therefore are not available to animals or human beings.
  * In mining regions, the ***mine tailings are covered by vegetation*** for stabilization of the pollutants.

* ***Phytotransformation***:
  * This process involves ***chemical modification of the pollutant*** as a direct result of plant metabolism, (their ***inactivation***, or ***degradation***)
  * In phytotransformation process, toxic compounds like explosives, pesticides are transformed to ***nontoxic*** compounds by some plants (Cannas).
  * There is ***no complete degradation*** of the pollutants in phytotransformation.
  * ***Microorganisms*** present in ***rhizosphere*** may degrade the organic compounds.
  * ***Plants*** primarily reduce toxicity of the pollutants.
* ***Phytostimulation***:
  * The ***microorganisms*** associated with ***plant roots*** enhance the soil microbial activity for the degradation of contaminants.
  * This process is essentially a rhizodegradation of pollutants.
  * Some aquatic plants support growth of microorganisms and stimulate them to degrade the chemical compounds.
  * Atrazine was studied to be degraded via phytostimulation by the plant hornwort (Rupassara et al. (2002).

* Phytotransformation and Phytostimulation

* ***Phytovolatilization***:
  * The pollutants are first ***transformed*** to ***volatile compounds*** which are released into the air.
* ***Rhizofiltration***:
  * This process involves ***filtering*** water through a ***mass*** of ***roots*** to ***remove*** toxic substances or excess nutrients.
  * The pollutants remain ***absorbed*** in or ***adsorbed*** by the roots.
  * Plants capable of ***absorbing*** pollutants are ***cultivated*** and used to minimize concentration of pollutants.
  * It is likely that the ***pollutants*** are ***incorporated*** in ***plant*** biomass and ***returned*** to environment with death or harvesting of plants.

*Phytovolatilization and Rhizofiltration

* One of the major applications of phytoremediation is to ***treat*** polluted soil or water bodies.
* This process has been demonstrated for ***treatment*** of ***metal mining wastes*** that contaminate soil and water.
* Phytoremediation has been shown to be studied globally for pollutants like ***pesticides, nitroexplosives, metals, crude oil***, and related others.
* Some of the plants, such as ***mustard***, ***hemp***, and ***alpine pennycress*** have been found to be successful in accumulation of pollutants occurring in high concentration at contaminated sites.
* ***Phytoremediation*** has been demonstrated as an efficient process in restoration ***metal mining sites, dumping places*** of polychlorinated biphenyls (***PCBs***).
* This technology is becoming a desirable fruitful option in last twenty years for remediation of sites contaminated with heavy, toxic metals.

APPLICATIONS OF PHYTOREMEDIATION

* Environmental pollution with ***xenobiotics*** is a global problem and the development of phytoremediation technologies for ***the plant-based cleanup*** of contaminated soils is therefore of significant interest.
* Meager data are available on actual use of plants for remediation of wastewaters containing pollutants.

Plant Species Studied for Phytoremediation of Organic Chemopollutants.

Organic Chemopollutant	**Plant Species Studied for Phytoremediation**	**References**
Atrazine	Corn and sorghum	Shimabukuro (1968)
Atrazine	Sorghum	Lamoureux et al. (1973)
Atrazine	*Panicum dichotomiflorum*	De Prado et al. (1995)
Atrazine	Poplar	Burken and Schnoor (1997)
Nitroexplosive TNT	Switch grass, brome grass	Peterson et al. (1998)
Nitroexplosive RDX	*Myriophyllum aquaticum*, *Catharanthus roseus*	Bhadra et al. (2001)
Atrazine	Corn	Cherifi et al. (2001)
Nitroexplosive TNT	*Abutilon avicennae* (Indian mallow)	Chang et al. (2004)
Herbicide atrazine	*Vetiveria zizanioides* L.	Vaishampayan (2004)
Antibiotic tetracycline	*Pistia* sp.	Gujarathi (2005)
Tannery waste water	*Pharagmites australis*	Calheiros et al. (2007)
Phenol	*Brassica napus* (hairy roots)	Coniglio et al. (2008)
RDX	Constructed wetland	Low et al. (2008)
Nitroexplosive TNT	*Vetiveria* sp.	Das et al. (2010)
Textile dye malachite green	*Blumea malcolmii* Hook. f. (cell culture)	Kagalkar et al. (2011)
Naphthalene	*Eichhornia crassipes*	Nesterenko-Malkovskaya et al. (2012)
Textile effluent	*Leucaena leucocephala*	Jayanthi et al. (2014)

* PHYTOREMEDIATION OF ORGANIC POLLUTANTS

* Recombinant bacteria:
  * by genetic ***engineering*** techniques or
  * by ***natural*** genetic exchange between bacteria.
* GEM in bioremediation have received a great deal of ***attention***, but mostly confined to the laboratory environment.
  * Due to ***regulatory risk*** assessment concerns.
  * The ***uncertainty*** of their ***practical impact*** and delivery under ***field*** conditions.
* There are at least ***four principal approaches***
  * (1) Modification of ***enzyme*** specificity and affinity,
  * (2) ***Pathway*** construction and regulation,
  * (3) ***Bioprocess*** development, monitoring, and control, and
  * (4) ***Bioaffinity bioreporter sensor applications*** for chemical sensing, toxicity reduction, and end point analysis.

genetically modified/ manupulated organisms

* The genetically engineered micro organism (GEMs) enhance the ability of degrading the contaminants.
* It is also a part of “bioaugmentation”.
* These organism acts on various xenobiotics.

GMOs	Xenobiotic
Pseudomonas olevorans	Alkane
Pseudomonas diminuta	parathion
P. cepacia	2, 4, 5-Trichlorophenol
Alcaligenes sp	2, 4-dichlorophenoxy acetic acid
Acinetobacter sp	4- chlorobenzene
P. putida	4-ethylbenzoate
C. testosteroni VP44	o-, p-monochlorobiphenyls
E. coli JM109(pSHF1003)	PCB, benzene, toluene
P. pseudoalcaligenes	TCE, toluene, benzene
E. coli FM5/pKY287	TCE, toluene
Deinococcus radiodurans	toluene and ionic mercury

Gene	Source	Target plant	Phenotype	Reference
SMT	*A. bisulcatus*	*B. juncea*	Se volatization asb DMSe and DMDSe from media with 200 μM SeO_4– increased 3 times (10% of Se evapotrated as DMDSe)	LeDuc et al., 2004
CAX2	*A. thaliana*	*N. tabacum*	2.8, 2.5 and 1.3 times higher biomass when grown, respectively, on media with 3 μM Cd^{2+}, 500 μM Mn^{2+} and 150 μM Zn^{2+}, then in roots 1.5 and 1.3 times higher Cd and Zn levels. Amount of metal accumulated per plant growing on media with 3 μM Cd^{2+}, 500 μM Mn^{2+} and 150 μM Zn^{2+} was higher 3.4, 2.3 and 1.9times, respectively	Korenkov et al., 2007b
YCF1	*S. cerevisiae*	*A. thaliana*	2.2 and 1.8 times higher biomass when grown on media with 60 μM Cd^{2+} and 900 μM Pb^{2+}, respectively. Shoots accumulated 1.5 times higher metal levels from media with 70 μM Cd^{2+} or 750 μM Pb^{2+}	Song et al., 2003
gshI	*E. coli*	*B. Juncea*	2.1 times longer roots in media with 200 μM Cd^{2+}. By 90% higher shoot Cd levels when grown in media with 50 μM Cd^{2+}. When grown on polluted soilb, shoots showed 1.5, 2.0, 2.0 and 3.1 times higher Cd, Zn, Cu and Pb levels, respectively	Zhu et al., 1999a Bennett et al., 2003
OAS-TL	*S. oleracea*	*N. tabacum*	On medium with 300 μM Cd^{2+}: 2.5 times higher biomass, 2.8 times longer roots. On medium with 500 μM Ni^{2+} 1.3 times higher biomass, 4.2 times longer roots. On medium with 250 μM SeO_4^- 1.5 times higher biomass, 1.8 times longer roots. Always higher chlorophyl content. When grown in media with 100 μM Cd^{2+}, the Cd levels 1.4 times increased in shoots and 4 times reduced in roots.	Kawashima et al., 2004
TaPCS1	*T. aestivum*	*N. glauca*	1.6 times longer roots on media with 800 μM Pb^{2+} or 50 μM Cd^{2+}. Shoots of transformed line NgTP1 accumulated from polluted soil. respectively, 6.0, 3.3, 4.8, 18.2 and 2.6 times more Pb, Cd, Zn, Cu and Ni.	Gisbert et al., 2003 Martínez et al., 2006
merP	*Bacillus megaterium*	*A. thaliana*	Capable of germination and growth on media with 12.5 μM Hg^{2+} accumulating 5.35 μg Hg^{2+}g of fresh seedling weight.	Hsieh et al., 2009

*Modified plants and genes for phytoremediation

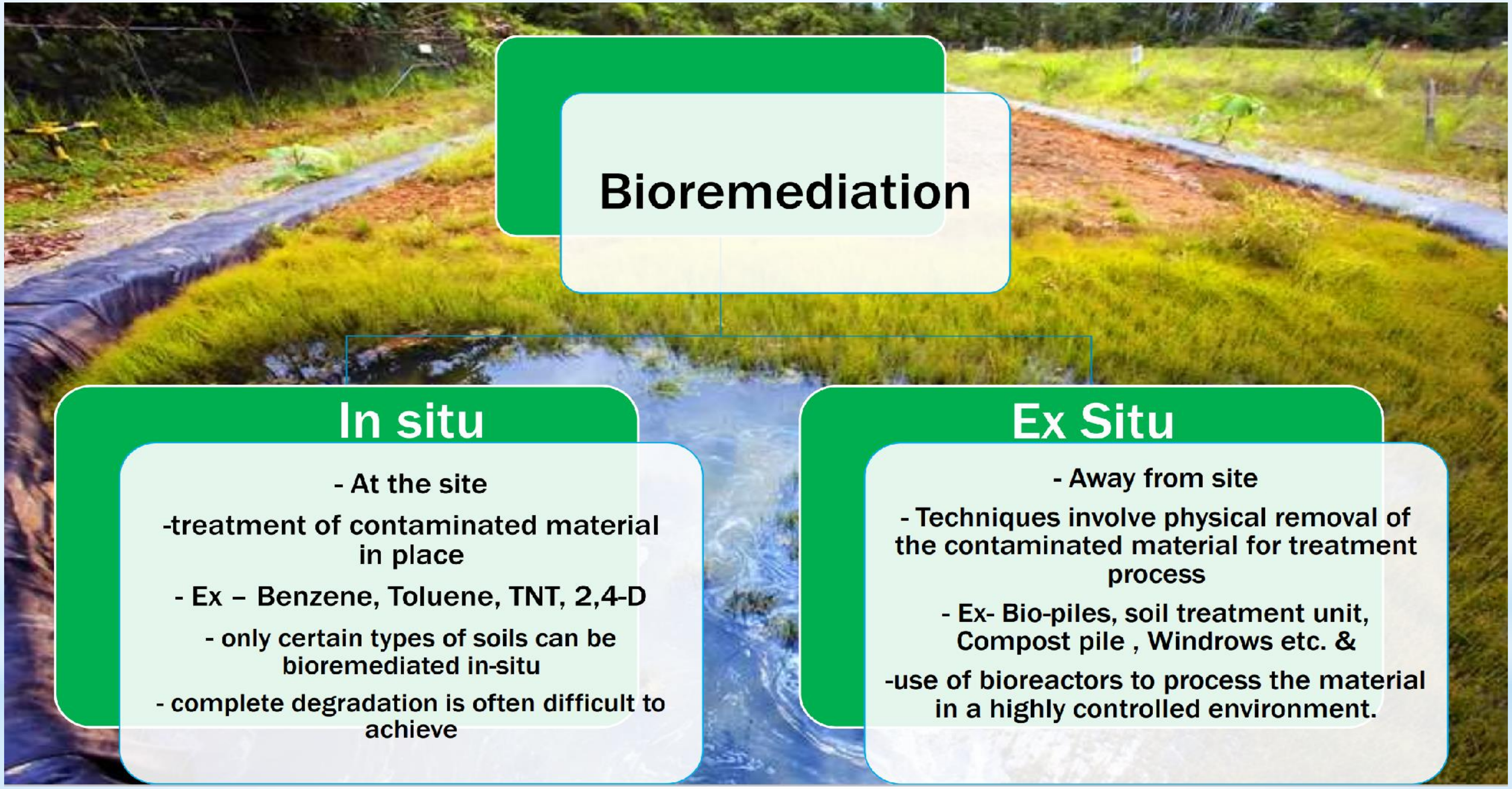
* For large scale remediation in contaminated site.
* High root coverage area causes high accumulation and degradation.
* Both ground water and soil contaminant can be removed.
* Useful by-product generation.
* Prevents soil erosion by large root coverage and direct enzymatic action on contaminant by the microorganism present in root zone.
* Flowering plants keep the polluted site pleasant.
* Landscape eco-tourism.

* Purpose of taking higher genetically modified plants:

Advantages and Disadvantages

- Advantages
  - More efficient than traditional methods of bioremediation.
  - Over all efficiency of G.M. plants will be increases.
  - Easy and enhanced method of cleaning environmental contaminant.
  - Multiple contaminant may be degraded by GMO.
  - The groundwater and soil can be treated at the same time by using in-situ bioremediation by using higher genetically modified plants.
- Disadvantages
  - Risk of getting harmful organism or weed if the experiment goes wrong.
  - May not be economically efficient.
  - Upsetting the ecosystem.
  - Ethical issues.
  - Disrupting crop yield (may be)

* The basis of removal and transportation of wastes for treatment, basically there are two methods.
    * In situ bioremediation
    * Ex situ bioremediation

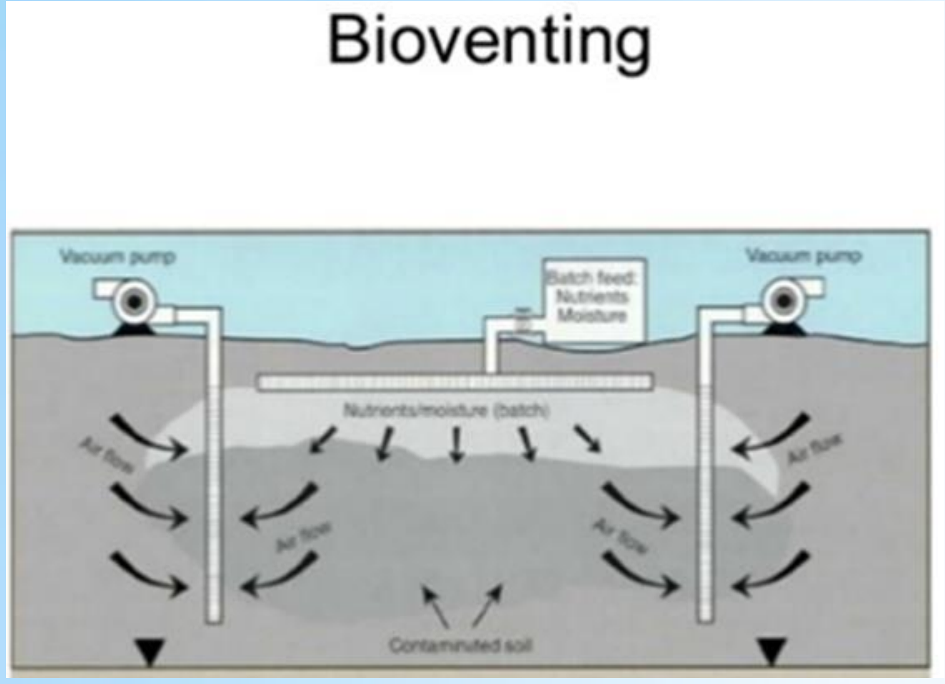


*Environmental clean-up process

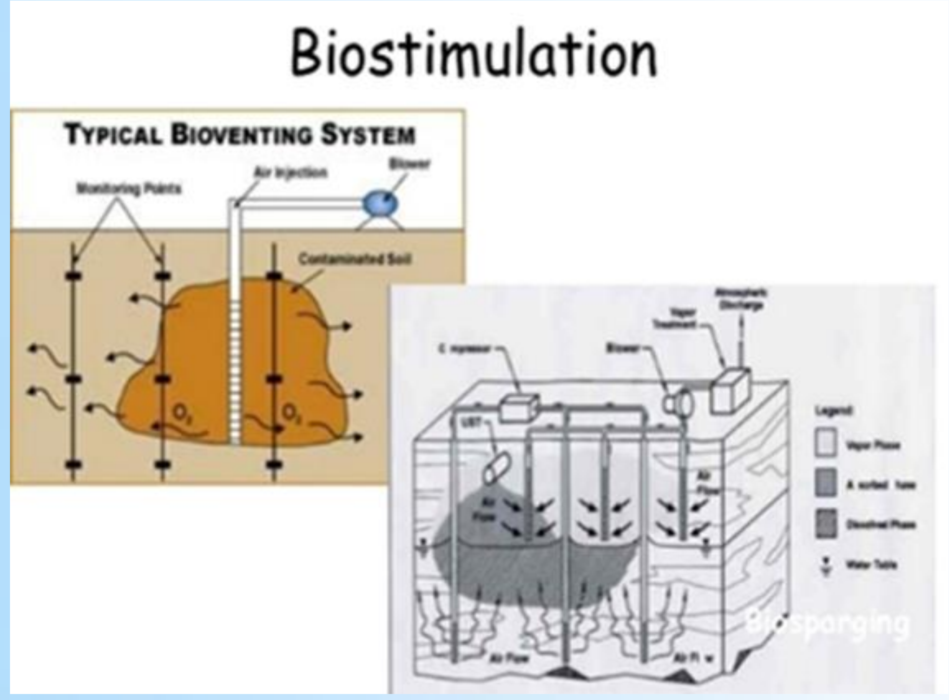
* It involves direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water).
  * It has been successfully applied for clan-up oil spillages, beaches etc.
  * There are 2 types of insitu bioremediation
    - Intrinsic bioremediation
    - Engineered bioremediation

In-situ Bioremediation

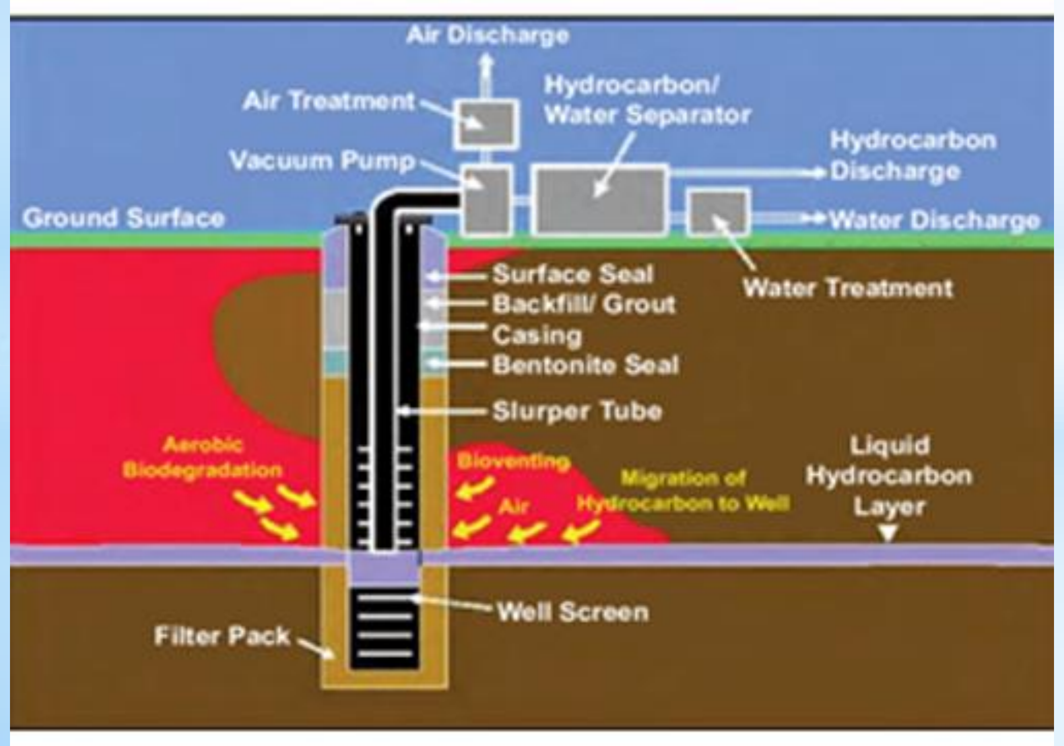
* Types of In-situ Bioremediation (1)



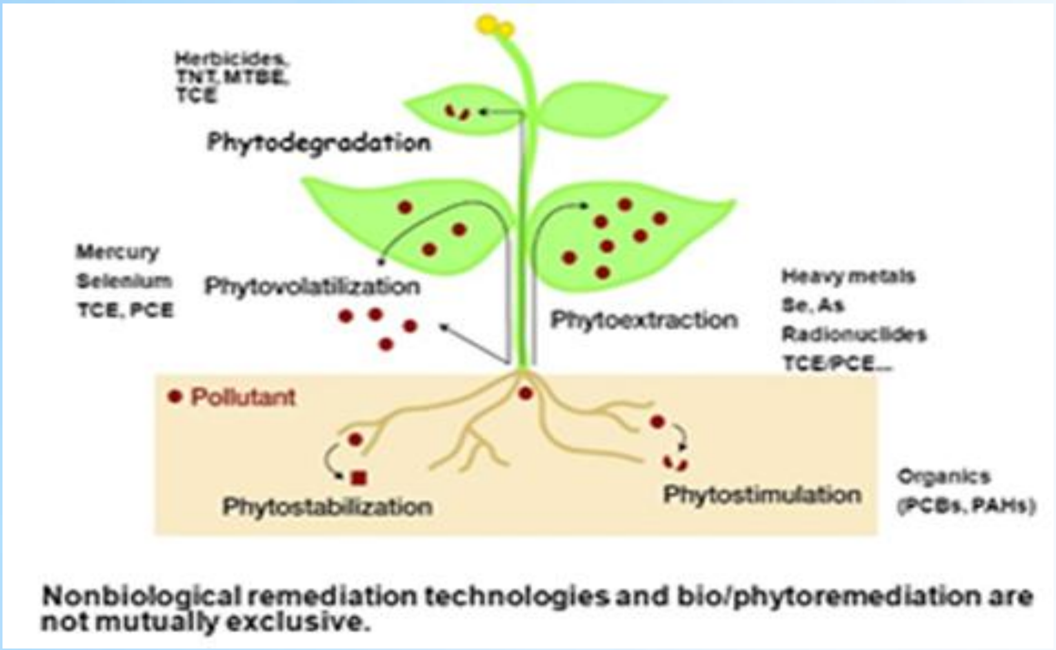
Oxygen venting takes place through soil to stimulate the growth of micro-organisms present in the soil.



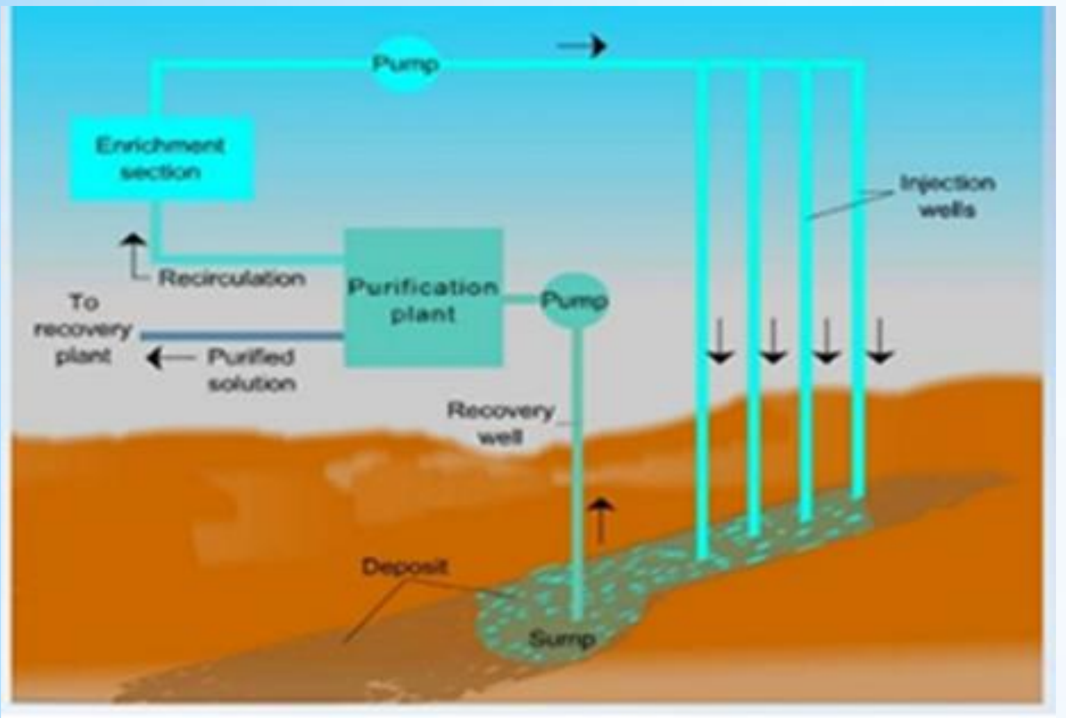
Here specific nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses) are injected at the site to stimulate indigenous microbial activities



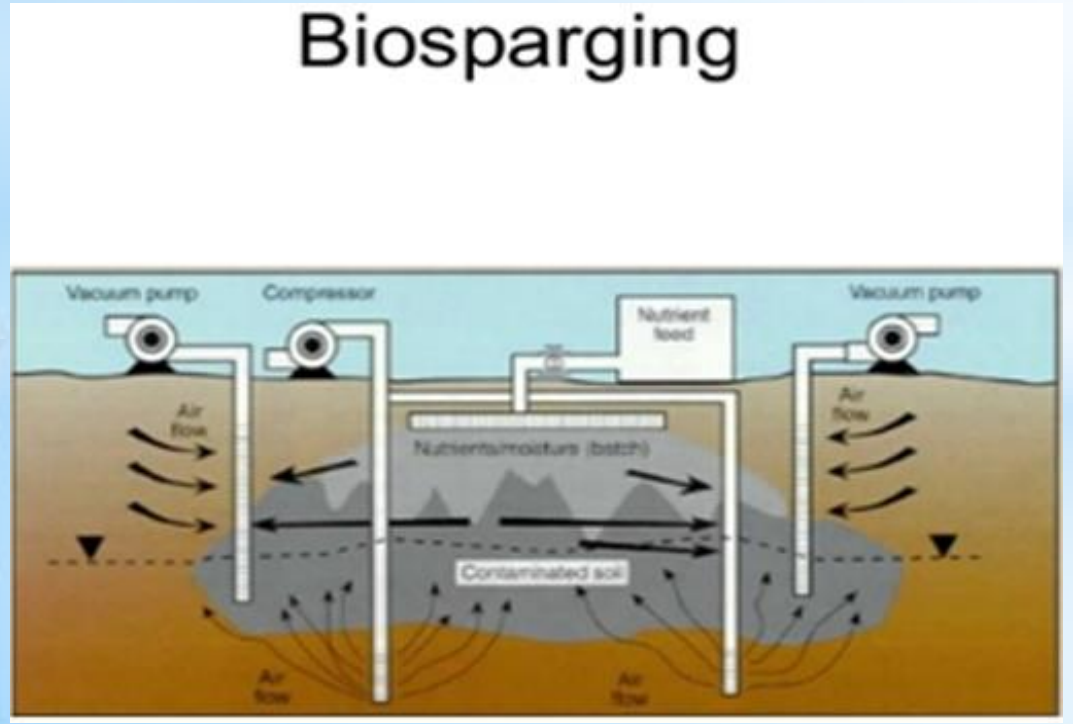
Vacuum-enhanced dewatering technology is used to remediate hydrocarbon contaminated sites, mainly used in petroleum hydrocarbon contaminated soils. It is also applicable at sites with a deep ground water table (>30ft.)



Using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants.



Process to extract metals from their ores using microorganisms which feed on nutrients in the minerals, causing the metal to separate from its ore.



Uses indigenous microorganisms to biodegrade organic constituents in the saturated zone. Oxygen and nutrients are injected into the saturated zone to increase the activity of the indigenous microorganisms.

* Types of In-situ Bioremediation (2)

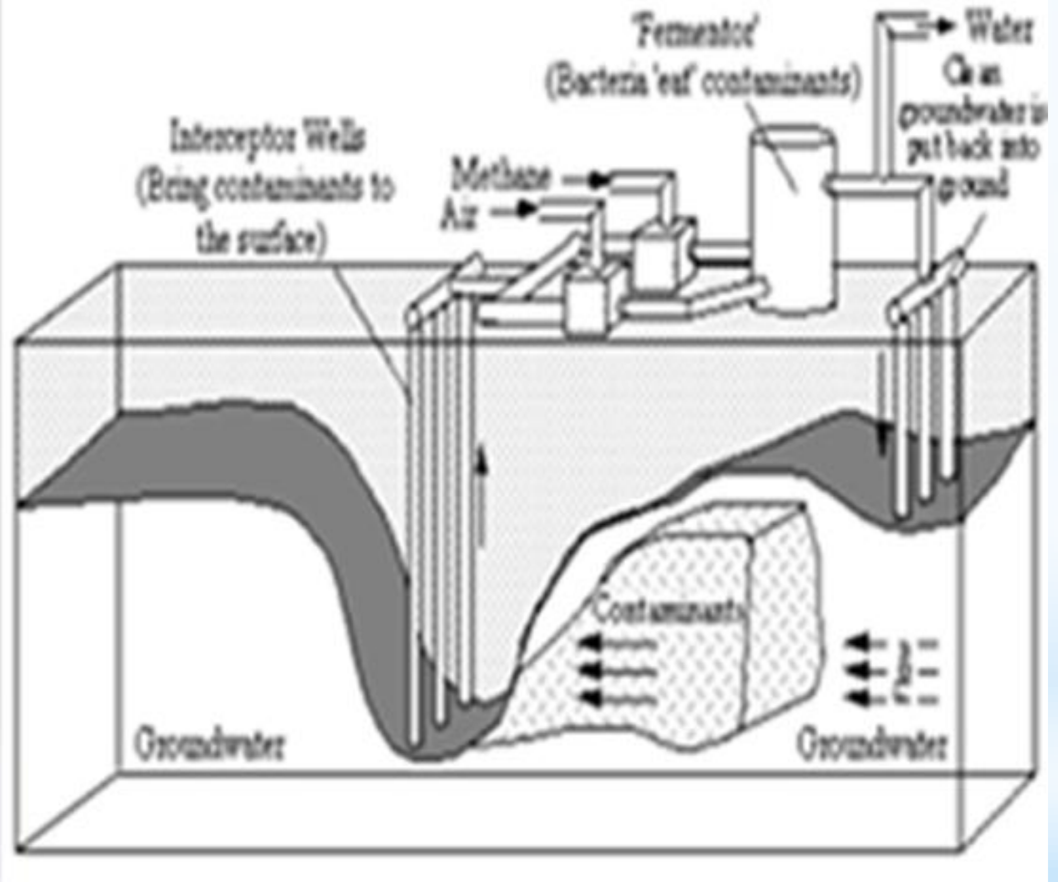
* The waste or toxic materials can be ***collected*** from the polluted sites and the bioremediation with the requisite microorganisms can be carried out at ***designed*** places.

* METABOLIC EFFECT OF MO’S ON XENOBIOTICS:
  - ¤ Detoxification ¤ Activation
  - ¤ Degradation ¤ Conjugation
* TYPES OF REACTIONS IN BIOREMEDIATION:
  - » Aerobic bioremediation
  - » Anaerobic bioremediation
  - » Sequential bioremediation

Ex-situ Bioremediation

* Types of Ex-situ Bioremediation (1)

Treatment is performed in the upper soil zone or in bio treatment cells. The soil is turned over regularly allowing air to mix with the excavated soil so that microorganisms present in the soil can efficiently break down the contaminants in soil



It is a vessel like container where biological degradation of contaminants is controlled. Its function depends on contaminated soil or sludge or water, oxygen transfer and mixing.

Here piles of soil are placed over top of a vacuum pump which pulls air through the pile of soil to allow oxygen to get thought the soil through aeration process to the micoorganisms. Microbial activity results in the breakdown of the petroleum constituents in the soil.

Composting is an aerobic method of decomposing organic solid wastes thereby used to recycle organic materials. In this process organic material is decomposed into a humus-like material, known as compost, which is a good fertilizer for plants.

The most efficient composting occurs with an optimal carbon: nitrogen ratio of about 25:1

* Types of Ex-situ Bioremediation (2)

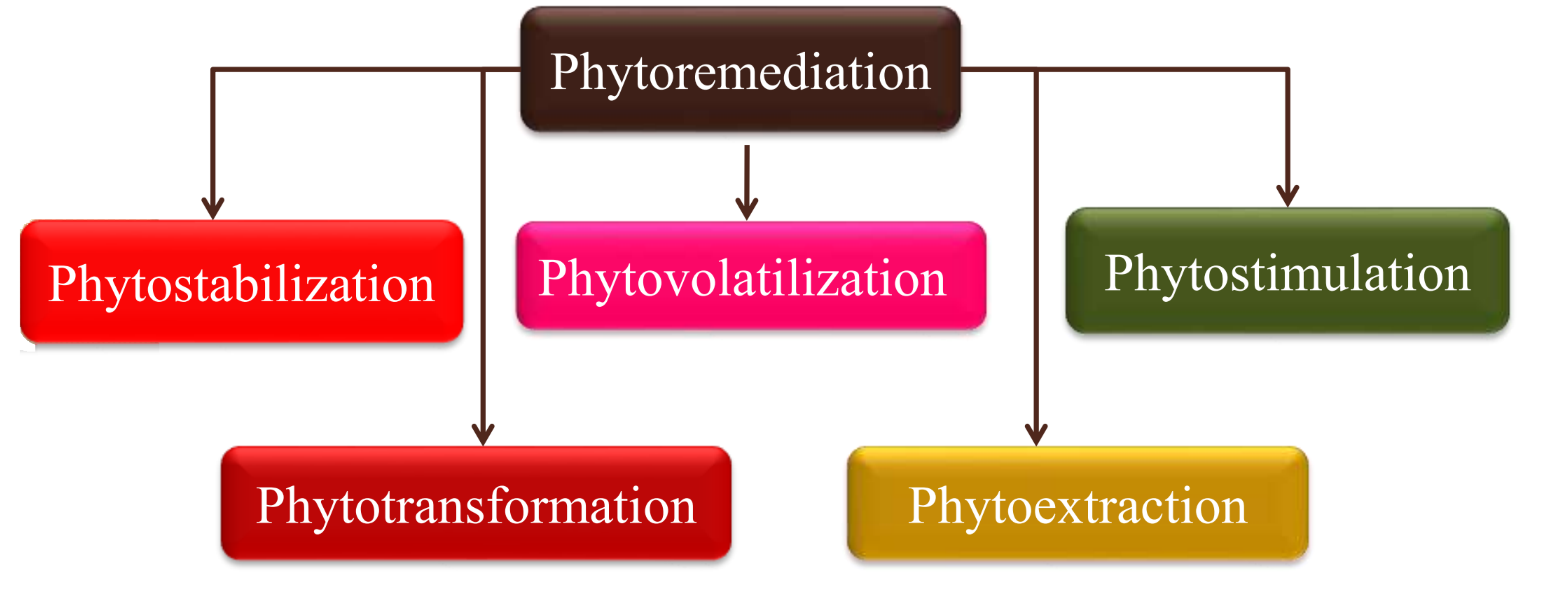
1. Biostimulation	2. Bioaugmentation	3. Intrinsic Bioremediation
• The method in which bacteria are motivated to start the process of bioremediation.	• Microorganisms that can clean up a particular contaminant are added to the contaminated soil and water.	• Process takes place in soil and water because these two places are always full of contaminants and toxins.
• In this method, first the experts release nutrients and other important substances in the soil where there is need or removing the contaminants.	• Bioaugmentation is more commonly and successfully used on contaminants removed from the original site, such as municipal waste water treatment facilities.	• This process is also called as natural attenuation.
• These are in the form of gas or liquid. It increases the growth of microbes in that area.		• Also means use of the microorganisms to remove the harmful substances from soil and water.
• As a result bacteria and other microorganisms remove the contaminants quickly and efficiently.		• Especially those sites are treated with this method, which are underground, for example underground petroleum tanks.

*Types of bioremediation :

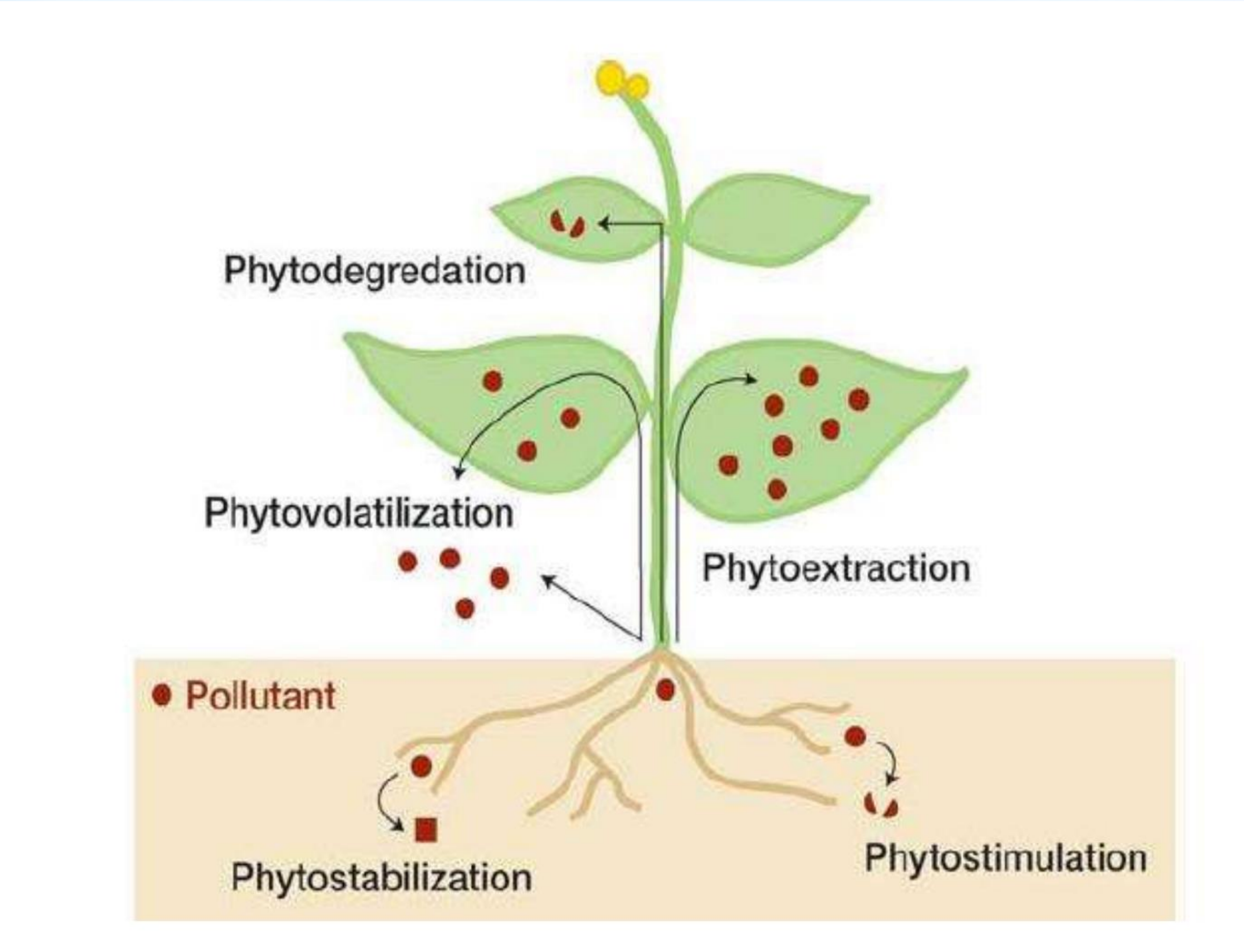
Accumulators and Excluders

* ***Plants*** colonizing metalliferous soils have evolved physiological mechanisms which enable them to ***tolerate*** metal toxicity.
* These mechanisms do not generally suppress metal uptake but result in internal detoxification.
* ***Two basic strategies*** of plant response are suggested, accumulators and excluders.
* ***Accumulators***:
  * Where ***metals are concentrated*** in plant parts, ***above-ground*** plant parts from ***low or high soil levels***.
* ***Excluders***:
  * Where ***metal*** concentrations in the ***shoot*** are ***maintained constant*** and low over a wide range of soil concentration ***upto a critical soil value*** above which the mechanism ***breaks down*** and unrestricted transport results.
* ***Indicators***:
  * They are seen as a further mode of response where ***proportional relationships*** exist between metal levels in the soil, uptake and accumulation in plant parts.
* The physiological properties of accumulator and excluder species are considered in relation to metal ***tolerance mechanisms***.

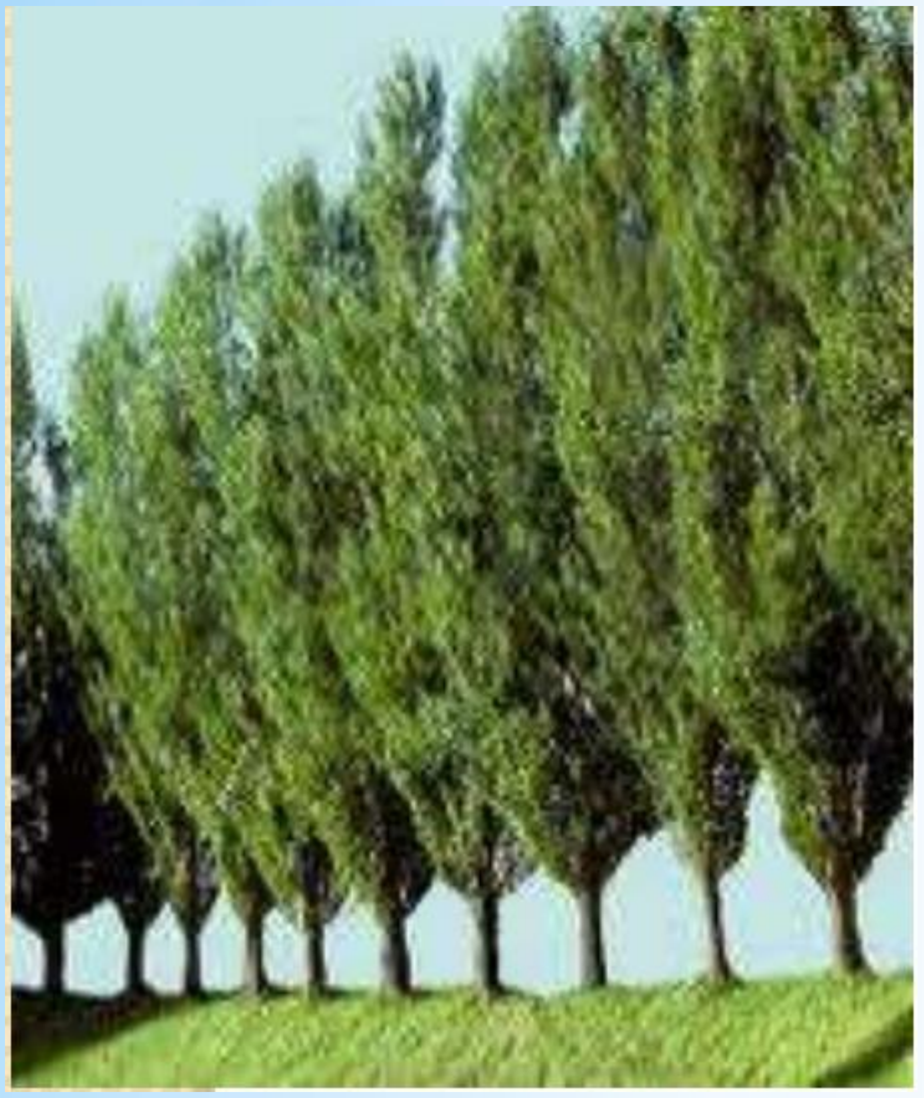
Phytoremediation is use of plants for accumulation, removal or conversion of pollutants



*Phytoremediation



Approximately 400 plant species have been classified as hyperaccumulators of heavy metals, such as grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, water hyacinth, etc.

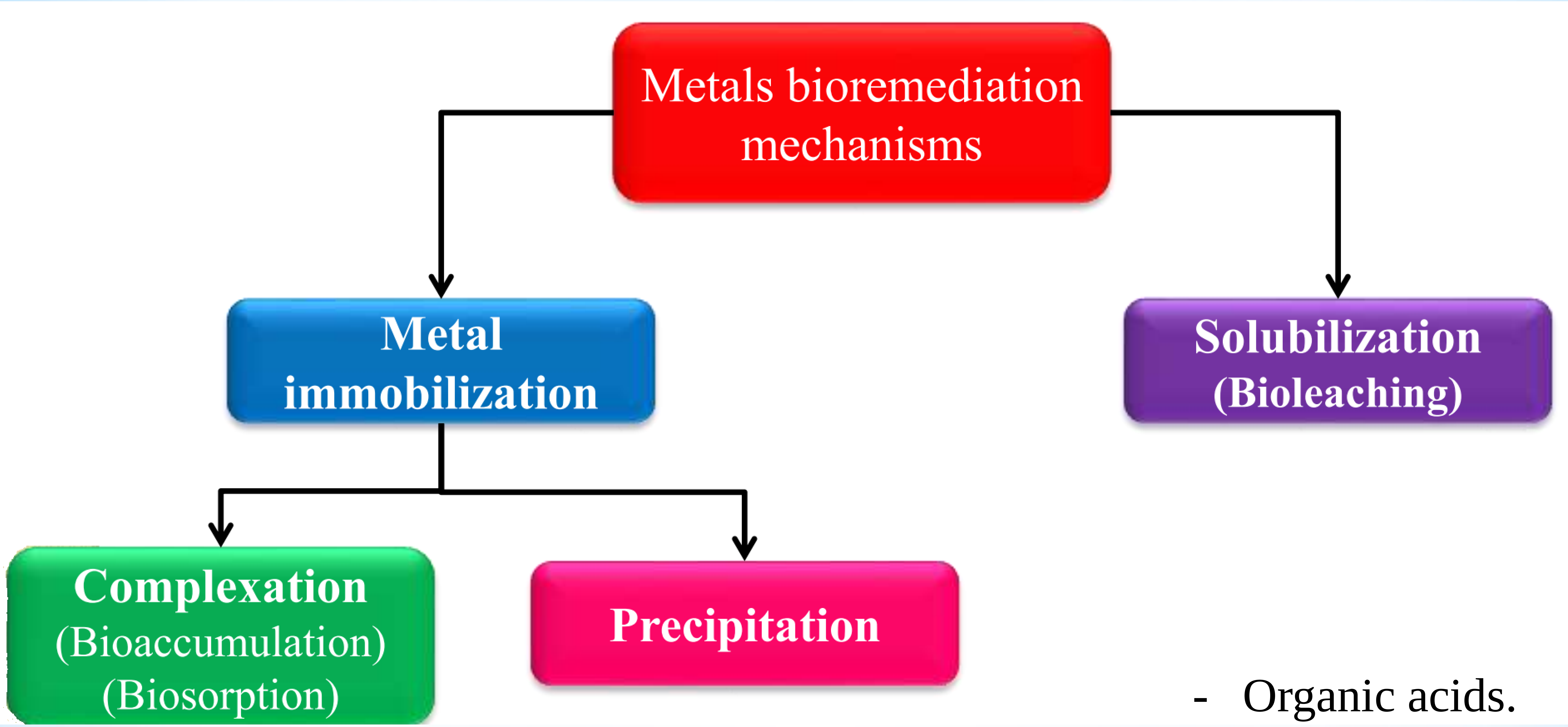




- The root exudates of these plants play an important role in phytoremediation as it activate the surrounded microorganisms.
- Genetic engineering are used as in case of BT protein or insect pheromones producing plants to reduce the use of pesticides.

* Effective and low cost , environmental friendly.
* Soil clean up of heavy metals and organic compounds.
* Pollutants are absorbed in roots, thus plants removed could be disposed or burned.
* Sunflower plants were used to remove cesium and strontium from ponds at the Chernobyl nuclear power plant.
* Transgenic plants with exogenous metallothionein (a metal binding protein) used to remove metals .

Phyto-Remediation

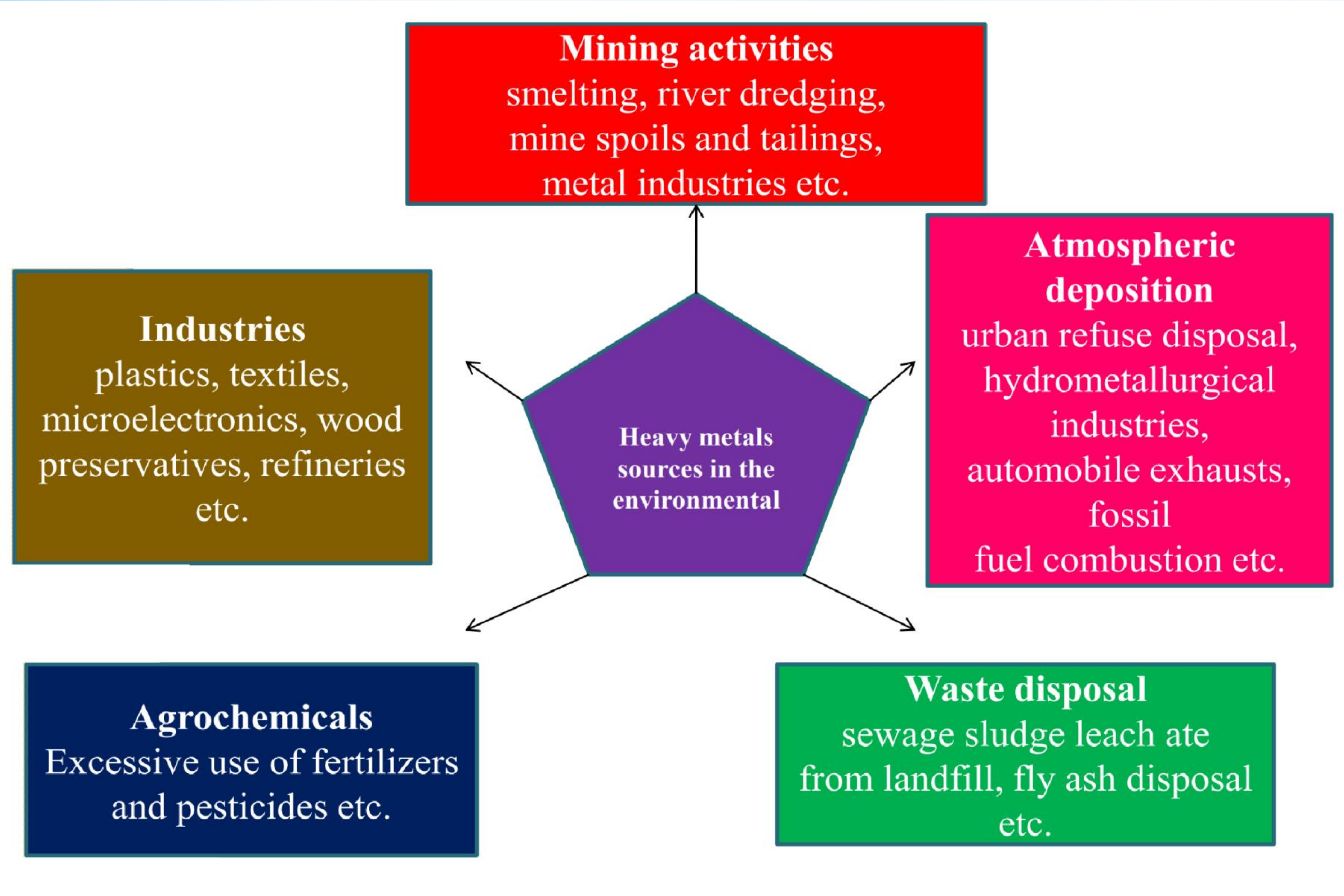


- Organic acids.
- Siderophores.
- Root exudates.

- Exopolysaccharide
- Lipoproteins

- H_2S producing bacteria
- Siderophores.
- Metal reduction.

* Bioremidiation from metal contaminated environment



* Sources of heavy metals in the environment

* The ***biosurfactants*** are chemical compounds characterized by ***hydrophobic*** and ***hydrophilic*** (non-polar and polar) regions in one molecule (amphipathic molecules).

* Biosurfactants from bacteria, cyanobacteria, fungi and yeast are classified into:

    1. Glycolipids.
    2. Lipopeptides.
    3. Phospholipids.
    4. Glycoproteins.
    5. Polymeric biosurfactants.

*Biosurfactants

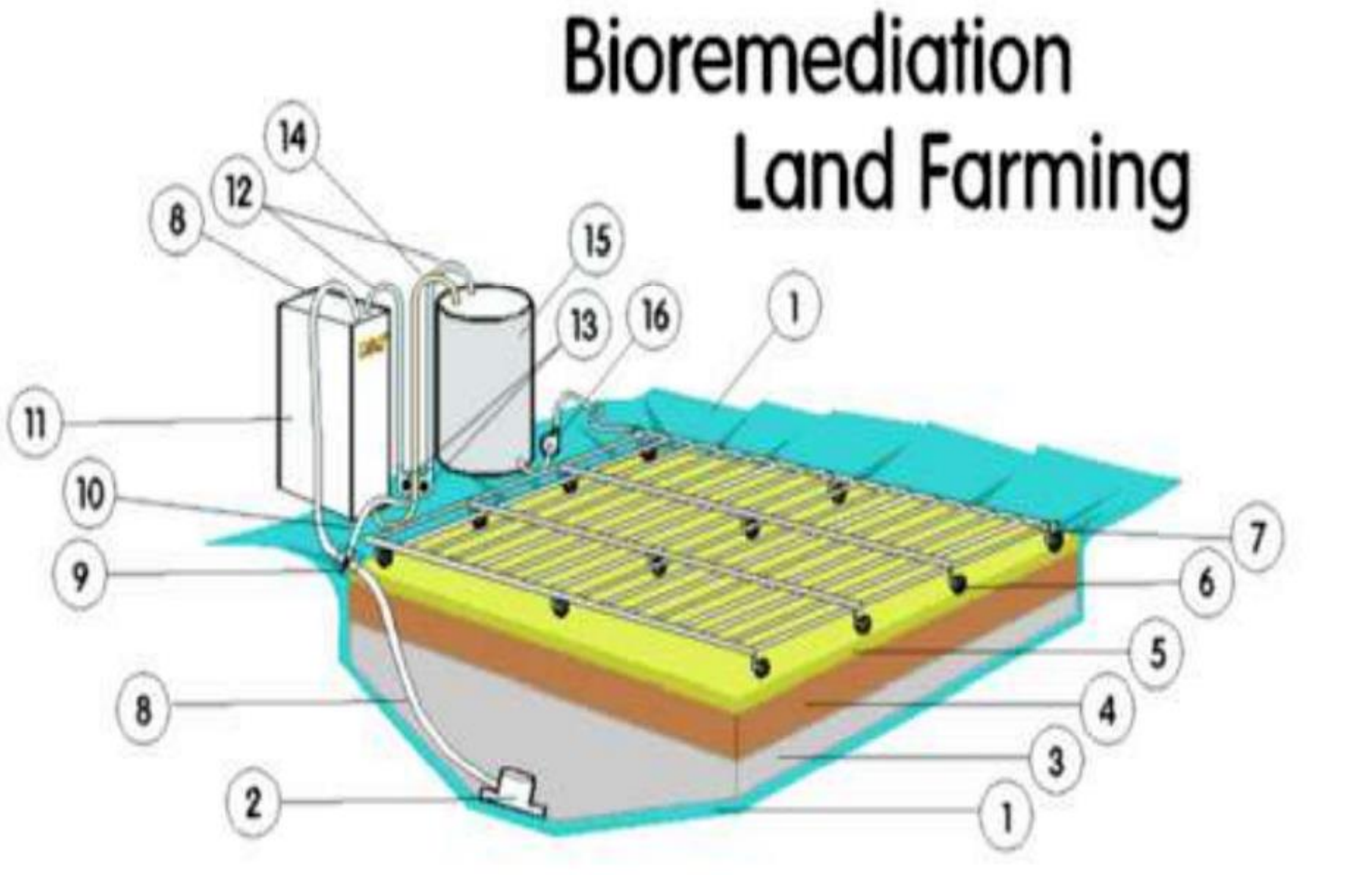
* Biosurfactants Producing Gem

* A genetically engineered *Pseudomonas aeruginosa*.
  * This new strain can produce a glycolipid emulsifier.
  * It can ***reduce*** the ***surface tension*** of an oil water interface.
  * The reduced interfacial tension ***promotes*** biodegradation of oils.

* A variety of pollutants are discharged in the environment from a large no of industries & mills.

1. Bioremediation of Dyes
2. Bioremediation in the paper and pulp industry

* Bioremediation of Industrial Wastes



SLURRY-PHASE BIOREACTOR

LANDFARMING-BIOREMEDIATION

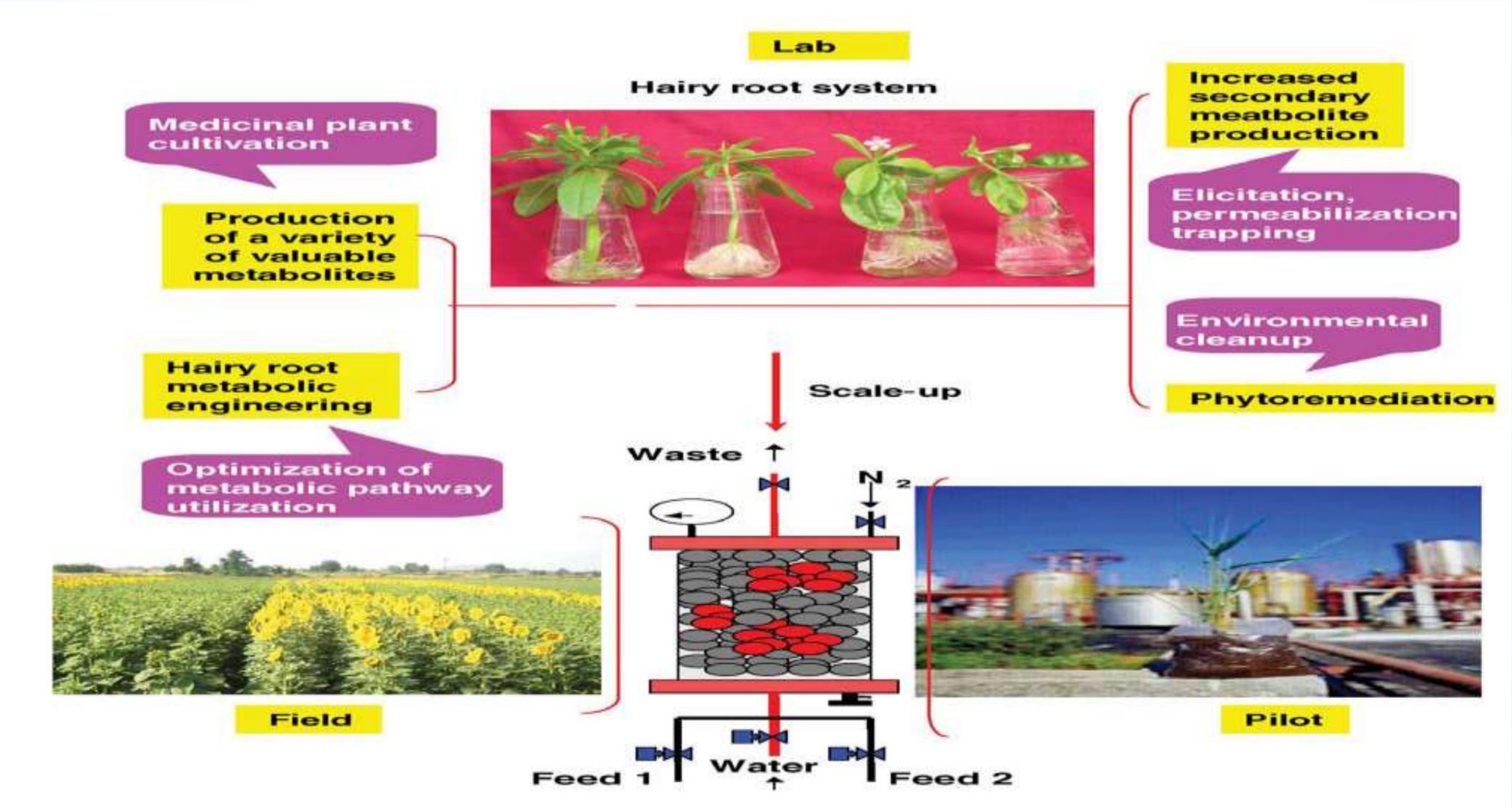
* Bioremediation of Contaminated Soils & Waste Lands

Summary of bioremediation strategies.

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxic concentrations of contaminants

* These activities are supported by ***a set of legislative and regulatory promotional measures*** such as Policy Statement on Abatement of Pollution, 1992; and the National Environment Policy, 2006.
* The major actions on Abatement of Pollution and Environmental Cleanup are as follows:
  * Clean Technology (CT),
  * Control of Pollution (CP),
  * Environment Education (EE),
  * Environmental Impact Assessment (IA),
  * Environmental Information (EI),
  * Environmental Information System (ENVIS),
  * Environment Research (RE),
  * Forest Protection (FPR),
  * Hazardous Substances Management (HSM) ,
  * Climate Change(CC),
  * Clean Development Mechanism (CDM).

Prevention And Control of Pollution



* Bioremediation From Lab-pilot –Field Scale

* Bioremediation is a natural process and is therefore perceived by the public.
* Bioremediation is useful for the complete destruction of a wide variety of contaminants.
* Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
* Bioremediation can often be carried out on site, often without causing a major disruption of normal activities.
* Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

ADVANTAGES OF BIOREMEDIATION

* Bioremediation is limited to those compounds that are biodegradable.
* Not all compounds are susceptible to rapid and complete degradation.
* There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
* Biological processes are often highly specific: microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
* It is difficult to extrapolate (deduce) from bench and pilot-scale studies to fullscale field operations.
* Bioremediation often takes longer than other treatment options.

Disadvantages Of Bioremediation