UNIVERZITA J. E. PURKYNĚ V ÚSTÍ NAD LABEM

Fakulta životního prostředí

Fytoremediace (Phytoremediation)

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EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

STUVIN - Education, research and innovation of science and technical doctoral programmes on J. E. Purkyně Univerzity in Ústí n.L.,
reg. n. CZ.02.2.69/0.0/0.0/16_018/0002735


Pollution and Bioremediation- An Introduction

Pollution and their consequences

- When harmful substances contaminate the natural environment it is called **Pollution.**
- Intense industrial and agricultural activities worldwide.
- Pollutants such as Heavy metals (Hg and Ni), Petroleum hydrocarbons and pesticides.

Consequences

- Disturbs ecosystem
- Global Warming
- Human health
- Infertile lands

Environmental (Air, Water and Soil/Land) Pollution

Air pollution: Caused by exhausts to the atmosphere from airplanes, vehicles and industrial plants; open burning; gaseous (e.g. methane) releases from open dumps etc. Since our atmosphere is universal, it can be concluded that the earth's air medium is universally contaminated, albeit, to varying degrees.

Water pollution: - Most of our water bodies have been impacted through our water transportation activities; heavy oil spills and leaks to water bodies, direct deposition of wastes to water bodies and contamination through seepages from landfill or dump sites.

Land pollution: - Lands used for industrial activities (gas stations, manufacturing factories, etc) are left contaminated after use. Several releases of chemicals, oil and other substances to the land. Contaminated sites abound everywhere in major locations of the world. Besides the chemicals released on the contaminated lands, several debris and wastes that constitute dangers are left on the sites.

CONTAMINANTS OF CONCERNS

The contaminants of concerns on used sites depend on the previous activities (industrial, commercial or domestic) that took place at the site. The contaminants released to a site that was used as a gas station will be different from those released into a site where mining activities or ore processing took place.

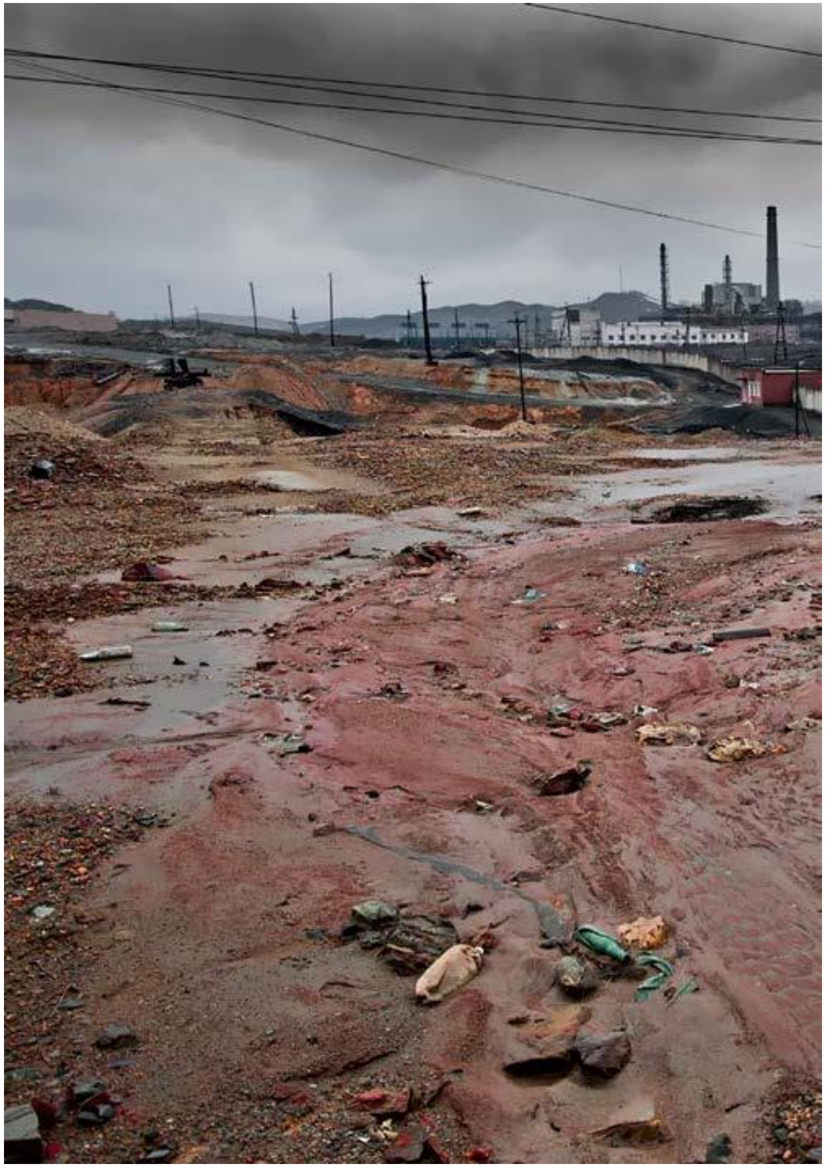
The broad category of contaminants that could be found on most contaminated sites include:

- ❖ Dilapidating Infrastructure - buildings, equipments, and other structures
- ❖ Hazardous chemicals/wastes
- ❖ Surface Debris / non-hazardous wastes
- ❖ Contaminated Soils - metals impacted soils; petroleum hydrocarbon (PHC) impacted soils or polychlorinated biphenyl (PCB) impacted soils
- ❖ Mine openings - adits, vent raises, shafts, exploration ditches/trenches

Soil Pollution

“Soil pollution” refers to the presence in the soil of a chemical or substance out of place and/or present at a **higher than normal concentration that has adverse** effects on any non-targeted organism (FAO and ITPS, 2015).

Soil pollution often cannot be directly assessed or visually perceived, making it a hidden danger.



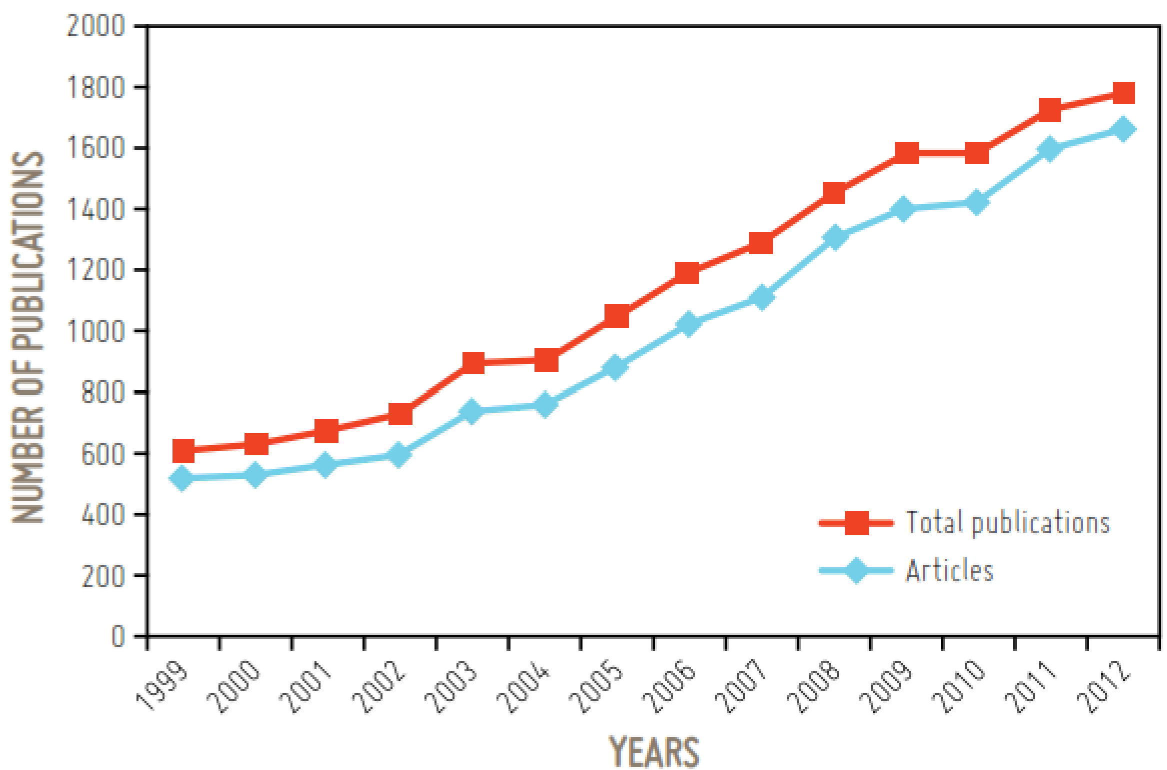
- The main sources of soil pollution are **anthropogenic**, resulting in the accumulation of contaminants in soils that may reach levels of concern.

- Industrial activities including mining, smelting and manufacturing; domestic, livestock and municipal wastes; pesticides, herbicides, fertilizers used in agriculture; petroleum-derived products that are released into or break-down in the environment; fumes generated by transportation — all contribute to the problem.

- So-called "emerging pollutants" are also a growing concern. These include pharmaceuticals, endocrine disruptors, hormones and biological pollutants; "e-waste" from old electronics; and the plastics that are nowadays used in almost every human endeavour.

- The presence of certain pollutants may also produce **nutrient imbalances** and **soil acidification**, two major issues in many parts of the world.

Fortunately, awareness on the importance of soil pollution is increasing around the world, leading to an increase in research conducted on the assessment and remediation of soil pollution.



Number of scientific publications on soil pollution in the period of 1999-2012.
Source: Guo et al., 2014

Soil pollution can result from both intended and unintended activities.

These activities can include the direct deposition of contaminants into the soil as well as complex environmental processes that can lead to indirect soil contamination through water or atmospheric deposition.

Different types of soil pollution

1. POINT-SOURCE POLLUTION

2. DIFFUSE POLLUTION

1. POINT-SOURCE POLLUTION

Soil pollution can be caused by a specific event or a series of events within a particular area in which contaminants are released to the soil, and the source and identity of the pollution is easily identified. This type of pollution is known as point-source pollution.

Anthropogenic activities represent the main sources of point-source pollution.

Examples include former factory sites, inadequate waste and wastewater disposal, uncontrolled landfills, excessive application of agrochemicals, spills of many types, and many others.

Activities such as mining and smelting that are carried out using poor environmental standards are also sources of contamination with heavy metals in many regions of the world.

- Other examples of point-source pollution are aromatic hydrocarbons and toxic metals, which are related to oil products.

The sites range from leakage from tank installations in Greenland, which caused aromatic hydrocarbon and toxic metal levels that exceeded the Danish environmental quality criteria (Fritt-Rasmussen et al., 2012), to accidental leakage from oil refinery storage tanks in Tehran (Bayat et al., 2016).

- Point-source pollution is very common in urban areas.
  - Soils near roads have high levels of heavy metals, polycyclic aromatic hydrocarbons, and other pollutants.
  - Old or illegal landfills, where waste is not disposed of properly or according to its toxicity (e.g. batteries or radioactive waste), as well as disposal of sewage sludge and wastewater, can also be important point-source pollutants.

2. DIFFUSE POLLUTION

- Diffuse pollution is pollution that is spread over very wide areas, accumulates in soil, and does not have a single or easily identified source. Diffuse pollution occurs where emission, transformation and dilution of contaminants in other media have occurred prior to their transfer to soil.

- Diffuse pollution involves the transport of pollutants via air-soil-water systems.

- Complex analyses involving these three compartments is therefore needed in order adequately to assess this type of pollution. For that reason, diffuse pollution is difficult to analyze, and it can be challenging to track and to delimit its spatial extent.

- Many of the contaminants that cause local pollution may be involved in diffuse pollution, since their fate in the environment is not well understood.

Examples of diffuse pollution are numerous and can include sources from

- nuclear power and weapons activities;
- uncontrolled waste disposal and contaminated effluents released in and near catchments; land application of sewage sludge;
- the agricultural use of pesticides and fertilizers which also add heavy metals, persistent organic pollutants, excess nutrients and agrochemicals that are transported downstream by surface runoff;
- flood events;
- atmospheric transport and deposition; and/or soil erosion.

Diffuse pollution has a significant impact on the environment and human health, although its severity and extent are generally unknown.

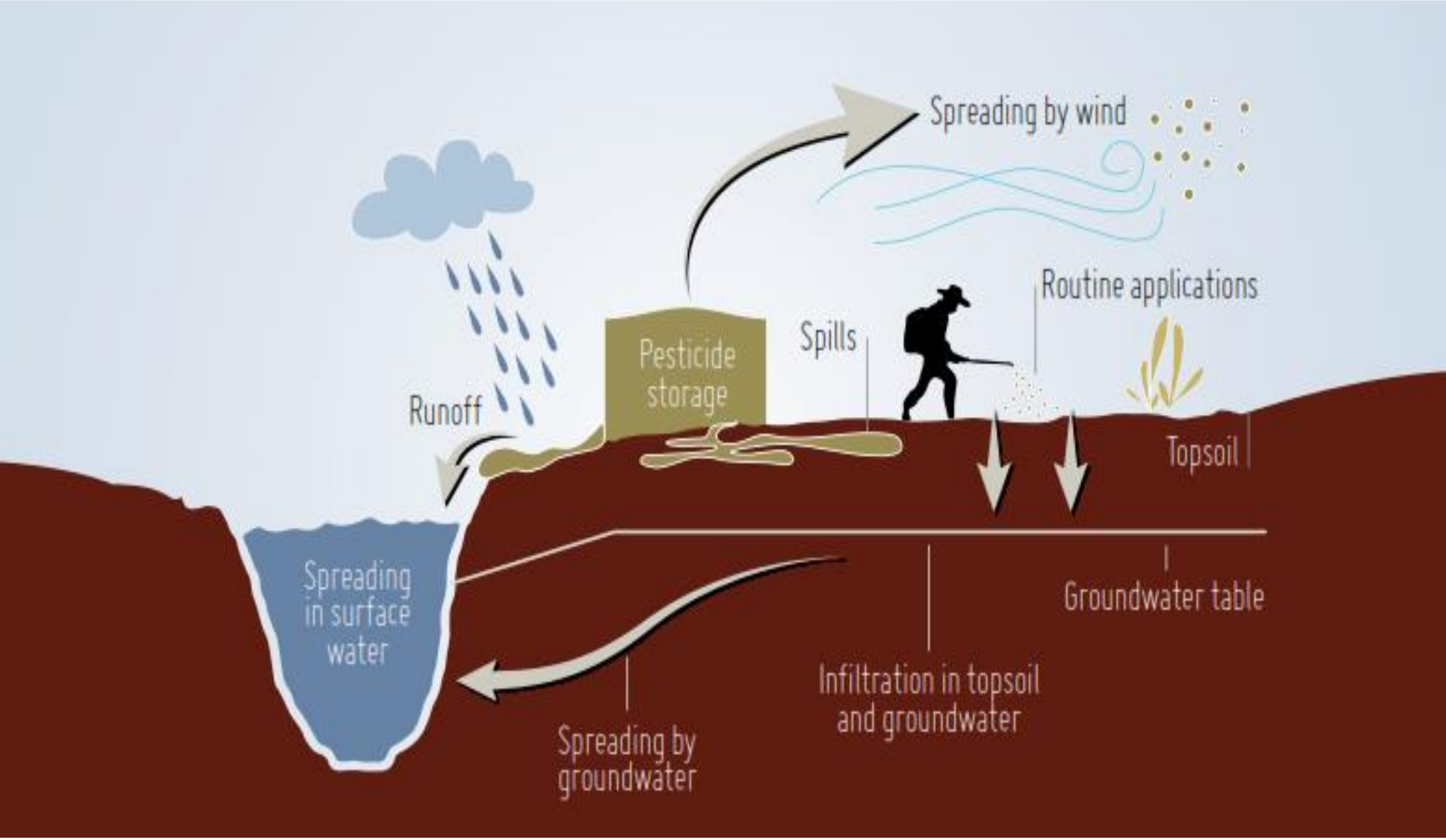
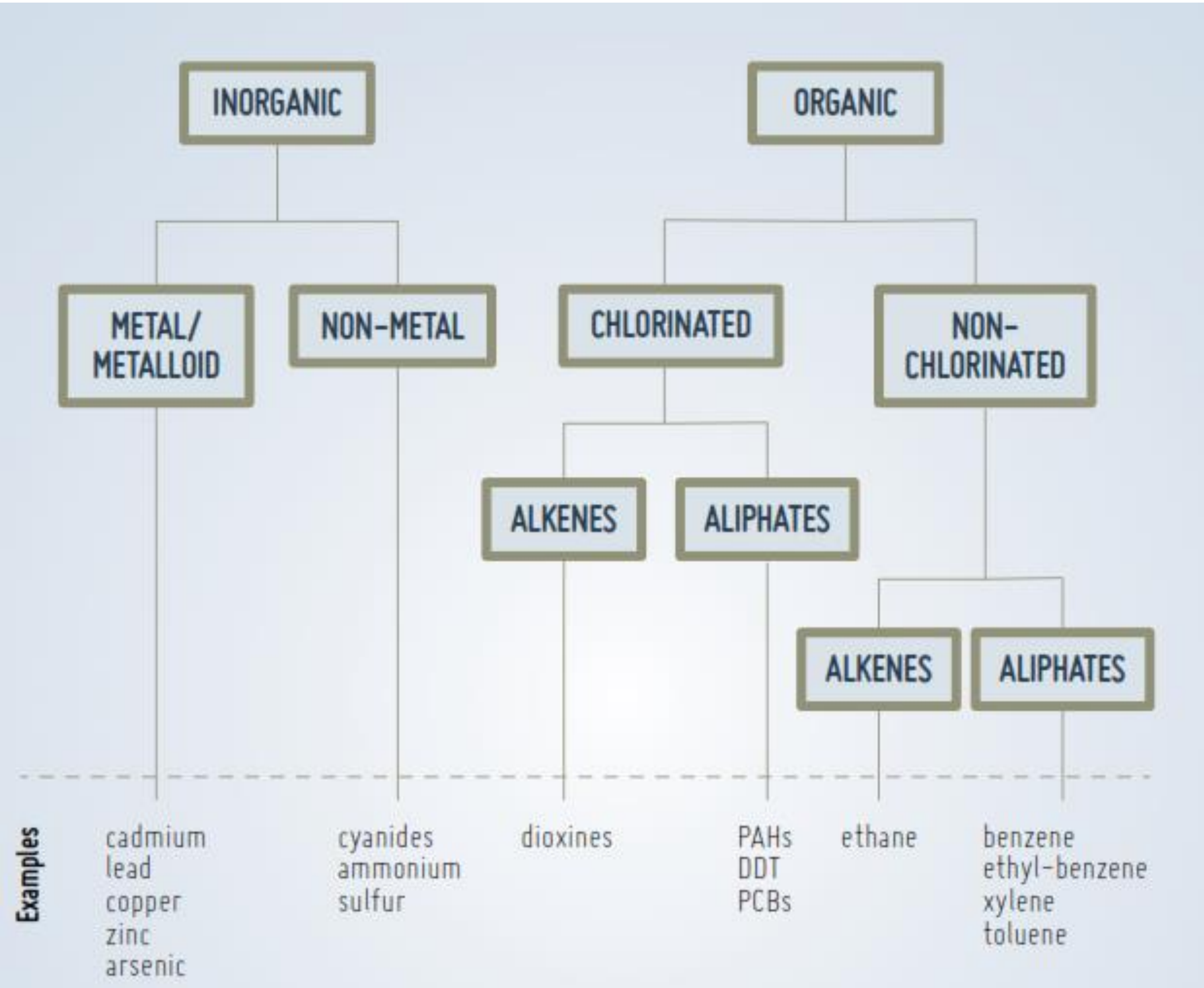


Fig. Transport pathway of pesticides in the environment. Source: FAO, 2000

MAIN POLLUTANTS IN SOIL



SOURCES OF SOIL POLLUTANTS

1. NATURAL, GEOGENIC SOURCES

- Several soil parent materials are natural sources of certain heavy metals and other elements, such as radionuclides, and these can pose a risk to the environment and human health at elevated concentrations.

- Arsenic (As) contamination is one of the major environmental problems around the world. Natural sources of As includevolcanic releases and weathering of As-containing minerals and ores, but also naturally occurring mineralized zones of arsenopyrite (gossans), formed by the weathering of sulphide-bearing rock.

- Natural events such as volcanic eruptions or forest fires can also cause natural pollution when many toxic elements are released into the environment. These toxic elements include dioxin-like compounds and polycyclic aromatic hydrocarbons (PAHs).

- High level of heavy metals have been identified in volcanic soils, mainly mercury (Hg), or with the weathering of the parent material, where high levels of chromium (Cr), copper (Cu), niquel (Ni) and zinc (Zn) have a natural pedo-geochemical origin.

- High levels of Cr and Ni have also been reported in volcanic Indonesian soils, associated with pedo-geochemical origins.

- However, this natural pollution does not normally cause environmental problems due to the regenerative ability and the adaptation capacity of plants.

- The problems arise when the ecosystems are subject to external pressures, which alter their resilience and response ability.

2. ANTHROPOGENIC SOURCES

- Centuries of anthropogenic activities have resulted in a widespread problem of soil pollution around the world.

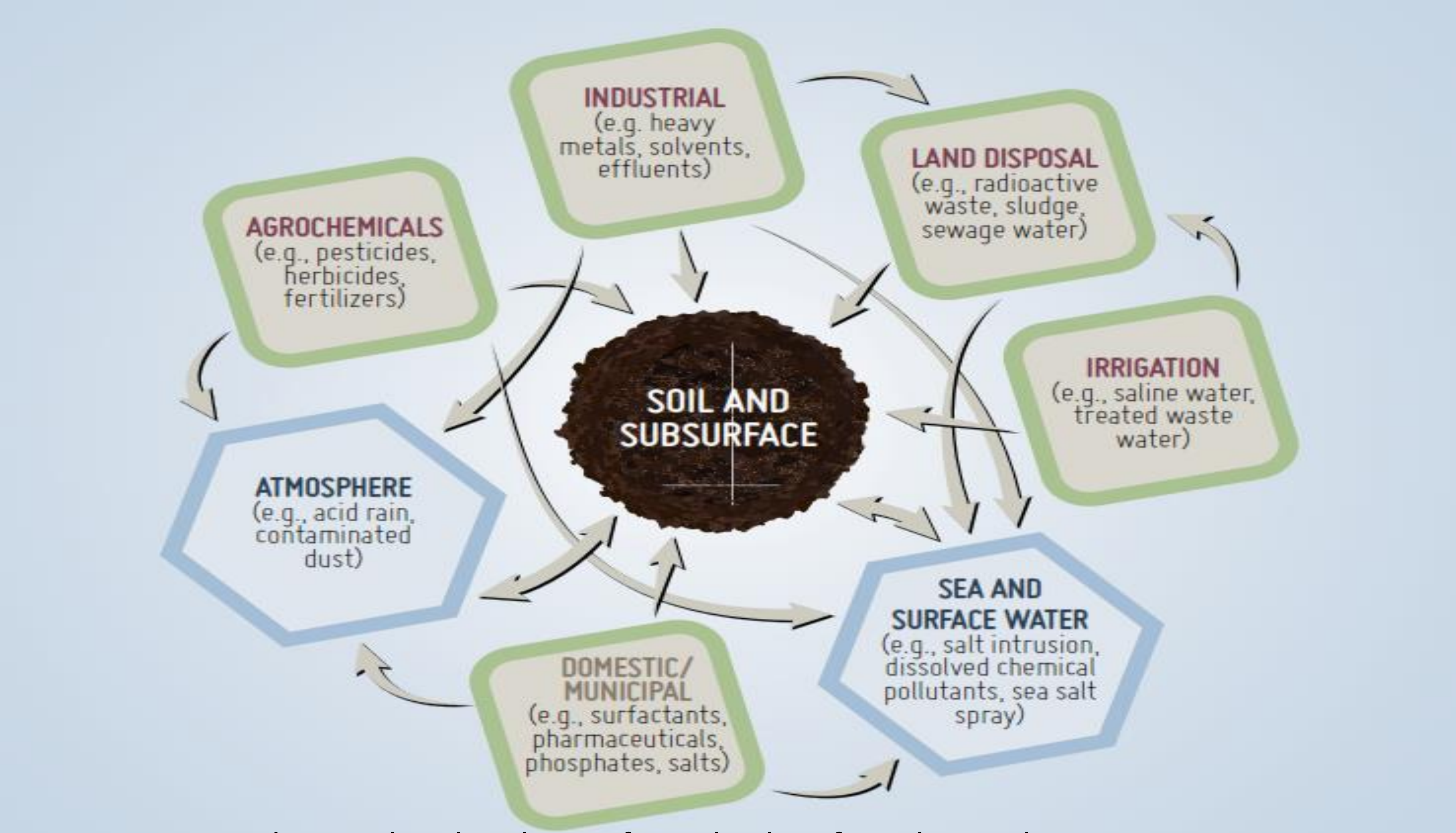


Fig. Potential interrelated pathways for soil-subsurface chemical contamination.

3. MINING

- Mining has had a major impact on soil, water and biota since ancient times. Many documented examples can be found of heavily contaminated soils associated with mining activities around the world.

- Metal smelting to separate minerals has introduced many pollutants into the soil.

- Mining and smelting facilities release huge quantities of heavy metals and other toxic elements to the environment; these persist for long periods, long after the end of these activities.

- Toxic mining wastes are stocked up in tailings, mainly formed by fine particles that can have different concentrations of heavy metals. These polluted particles can be dispersed by wind and water erosion, sometimes reaching agricultural soils.

- The use of phosphate rocks, which are naturally rich in radioactivity, in the production of fertilizers generates a by-product called phosphogypsum, which maintains nearly 80 percent of its original radioactivity due to ^{238}U decay products such as radon, ^{226}Ra, and polonium, ^{210}Po.

- These industries generate a radioactive source of pollution, which constitutes a threat to the surrounding ecosystems and organisms.

- Significant point-source soil pollution occurs from oil and gas extraction due to spills of crude oil and brines. Brines have high salinity levels and can also contain toxic trace elements and naturally occurring radioactive materials.

4. URBAN AND TRANSPORT INFRASTRUCTURES

- The widespread development of infrastructure such as housing, roads and railways has considerably contributed to environmental degradation.

- Their more evident negative effects on soil are soil sealing and land consumption.

- Apart from these known soil threats, another major impact of infrastructure activities is the entry into the soil system of different pollutants.

- Activities linked to transportation in and around urban centers constitute one of the main sources of soil pollution, not only because of the emissions from internal combustion engines that reach soils at **more than a 100 m** distance by atmospheric deposition and petrol spills, but also from the activities and the changes that result from them as a whole.

- A major legacy source of soil pollution associated with transport is lead contamination of soils from leaded gasoline.

- Splashes generated by traffic during rainfall events and runoff, which may be significant if the **drainage system is not well maintained**, may translocate particles rich in **heavy metals from the corrosion of metal vehicle parts**, **tires and pavement abrasion** and other pollutants such as **polycyclic aromatic hydrocarbons, rubber and plastic derived compounds**.

- Soil pollution associated with roads and highways is especially important in urban and peri-urban soils, and can be a major threat when food production occurs in adjacent areas. Foliar deposition and root uptake and transfer to above-ground tissues of bioavailable heavy metals are the main processes observed in roadside soil.

- Grazing in roadside soils is also quite common, and the ingestion of contaminated soil and plants constitutes potential dietary transfer of pollutants affecting animal and human health.

5. WASTE AND SEWAGE GENERATION AND DISPOSAL

- According to a World Bank report, the global production of municipal solid waste was estimated to be 1.3 billion tonnes per year in 2012, varying from 0.45 kg per person and per day in sub-Saharan Africa to 2.2 kg per capita annually in the Organisation for Economic Co-operation and Development (OECD) countries.

- Future predictions are worrying, however, as waste production is expected to rise to 2.2 billion tonnes by 2025.

- Municipal waste disposal in landfills and incineration are the two most common ways to manage waste.

- In both cases, many pollutants, such as heavy metals, polyaromatic hydrocarbons, pharmaceutical compounds, personal care products and their derivative products accumulate in the soil, either directly from landfill leachates that may be polluting soil and under groundwater, or by ash fallout from incinerating plants.

6. MILITARY ACTIVITIES AND WARS

- Until the twentieth century, most conflicts were of local magnitude and had relatively little impact on soils.
- However, modern warfare makes use of non-degradable weapons of destruction and of chemicals that can remain in the affected soils for centuries after the end of the conflict.
- The First and Second World Wars left Europe with a significant heritage of pollution (land mines, remains of ammunitions and leftover chemicals, radioactive and biological toxic agents), not only in the battlefields but also in sites such as shooting areas, barracks and storage of armaments.
- There are approximately **110 million** mines and other unexploded ordnance (UXO) scattered in **64 countries** on all continents, remnants of wars from the early twentieth century up until today.

There is little published evidence on this type of contamination, largely because of restrictions placed by governments of many countries on the publication of material related to warfare.

- In Berlin, for example, more than a thousand hectares presented high levels of contamination.
- Gruinard Island, in western Scotland, is still polluted with anthrax spores that were used as potential biological weapons, despite remediation efforts.
- Mustard gas stored during the Second World War has also contaminated some sites for up to 50 years.

Commonly used military energetic compounds include the explosives

2,4,6-trinitrotoluene (TNT),
hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and
octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX).

Nitroglycerin (NG),
nitroguanidine (NQ),
nitrocellulose (NC),
2,4-dinitrotoluene (DNT), and
various perchlorate formulations

are employed in missile, rocket, and gun propellants.

Apart from above, Heavy metal and oil contamination is also occurs at military sites.

7. AGRICULTURAL AND LIVESTOCK ACTIVITIES

- Trace metals from agrochemicals, such as, Cu, Cd, Pb, As and Hg, are also considered soil pollutants.
- Excess N and heavy metals are not only a source of soil pollution, but also a threat to food security, water quality and human health, when they enter the food chain.

According to their chemical structure, pesticides can be divided into twelve distinct groups, with the main pesticides in each group listed below:

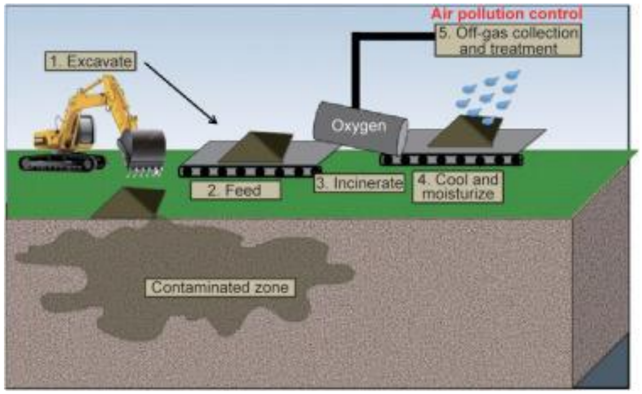
Different Groups	Examples
Organochlorine compounds:	DDT, Methoxychlor, Chlordane, Dicofol. BHC/HCH, Aldrin, Endosulfan, Heptachlor, Methoxychlor, Chlordane,Dicofol
Organophosphorus compounds:	Parathion, Malathion, Monocrotophos, Chlorpyrifos,Quinalphos, Phorate,Diazinon, Fenitrothion, Acephate, Dimethoate, Fenthion;
Carbamates:	Aldicarb, Oxamyl, Carbaryl, Carbofuran, Carbosulfan, Methomyl;
Pyrethroids:	Allethrins, Deltametrin, Resmethrin, Cypermethrin, Permethrin;
Neonicotinoids:	Acetamiprid, Imidacloprid, Nitenpyram, Thiamethoxam;
Organotin compounds:	Triphenyltin acetate, Trivenyltin chloride, Tricyclohexyltin hydroxide, Azocyclotin;
Organomercurial compounds:	Ethyl mercuric chloride, Phenyl mercuric bromide;
Dithiocarbamate fungicides:	Zineb, Maneb, Mancozeb, Ziram;
Benzimidizole compounds:	Benomyl, Carbendazim, Thiophanate methyl;
Chlorphenoxy compounds:	2,4-D, TCDD, DCPA, 2,4,5-T, 2,4-DB, MCPA, MCPP;
Dipyridiliums:	Paraquat, Diquat;
Miscellaneous:	DNOC, Bromoxyl, Simazine, Triazamate.

Methods to reduce pollution

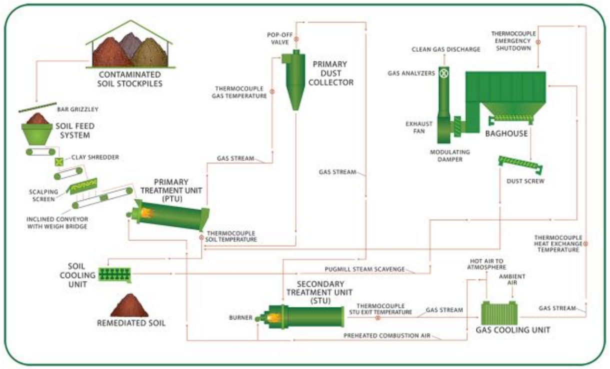
TRADITIONAL WAYS TO REDUCE POLLUTION

- For treating soil contamination(metal), methods such as:

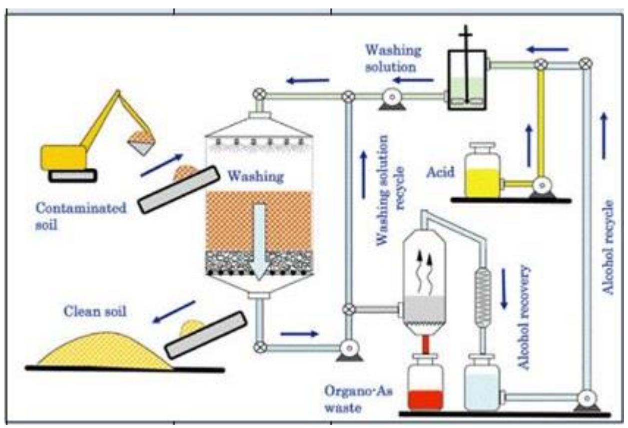
1. Excavation



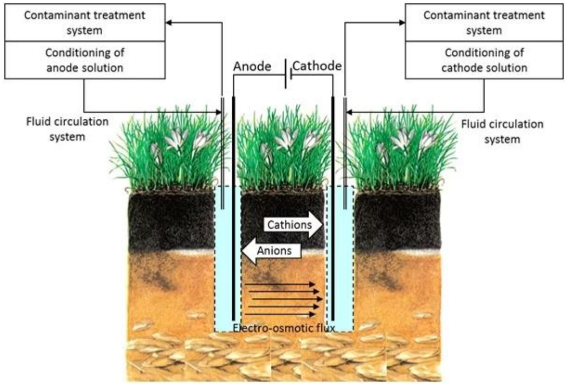
2. Thermal treatment



3. Acid leaching



4. Electro reclamation



All of the above treatments are costly, not environmental friendly and not very effective.

Bioremediation or Environment/Ecofriendly way to reduce pollution

Bioremediation:

Bio (biological) + Remediation (to remediate)

Use of different biological systems to destroy or reduce concentrations of contaminants from polluted sites.
Manages microbes and plants to reduce, eliminate, contain or transform contaminants present in soils, sediments, water or air.

Types of Bioremediation:

1. **Microbial bioremediation** uses microorganisms to break down contaminants by using them as a food source.

2. **Phytoremediation** uses plants to bind, extract, and clean up pollutants such as pesticides, petroleum hydrocarbons, metals, and chlorinated solvents.

Phytoremediation: overview and main terms

Phytoremediation - What is it?

- Definition: Use of green plants and their microorganisms to reduce environmental problems without the need to excavate the contaminant material and dispose of it elsewhere.

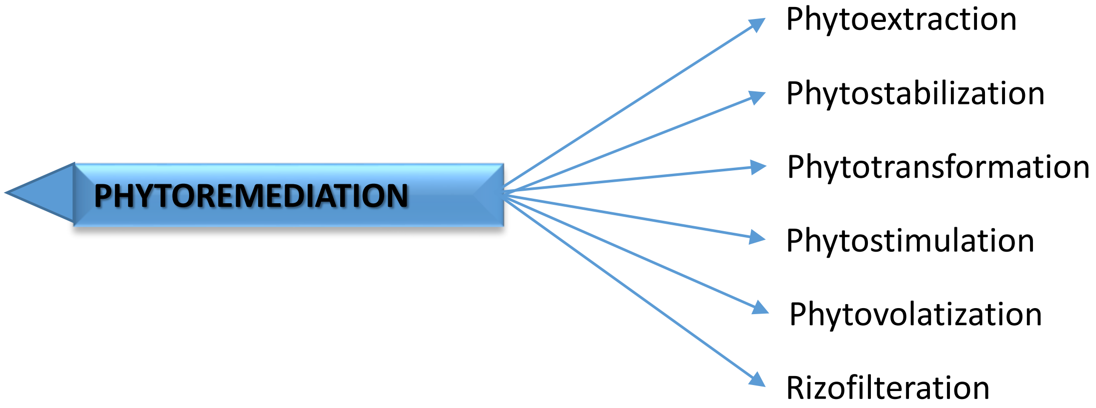
- Natural process - can be an effective remediation method at a variety of sites and on numerous contaminants.

- Selected plant species possess the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants (~350 species).

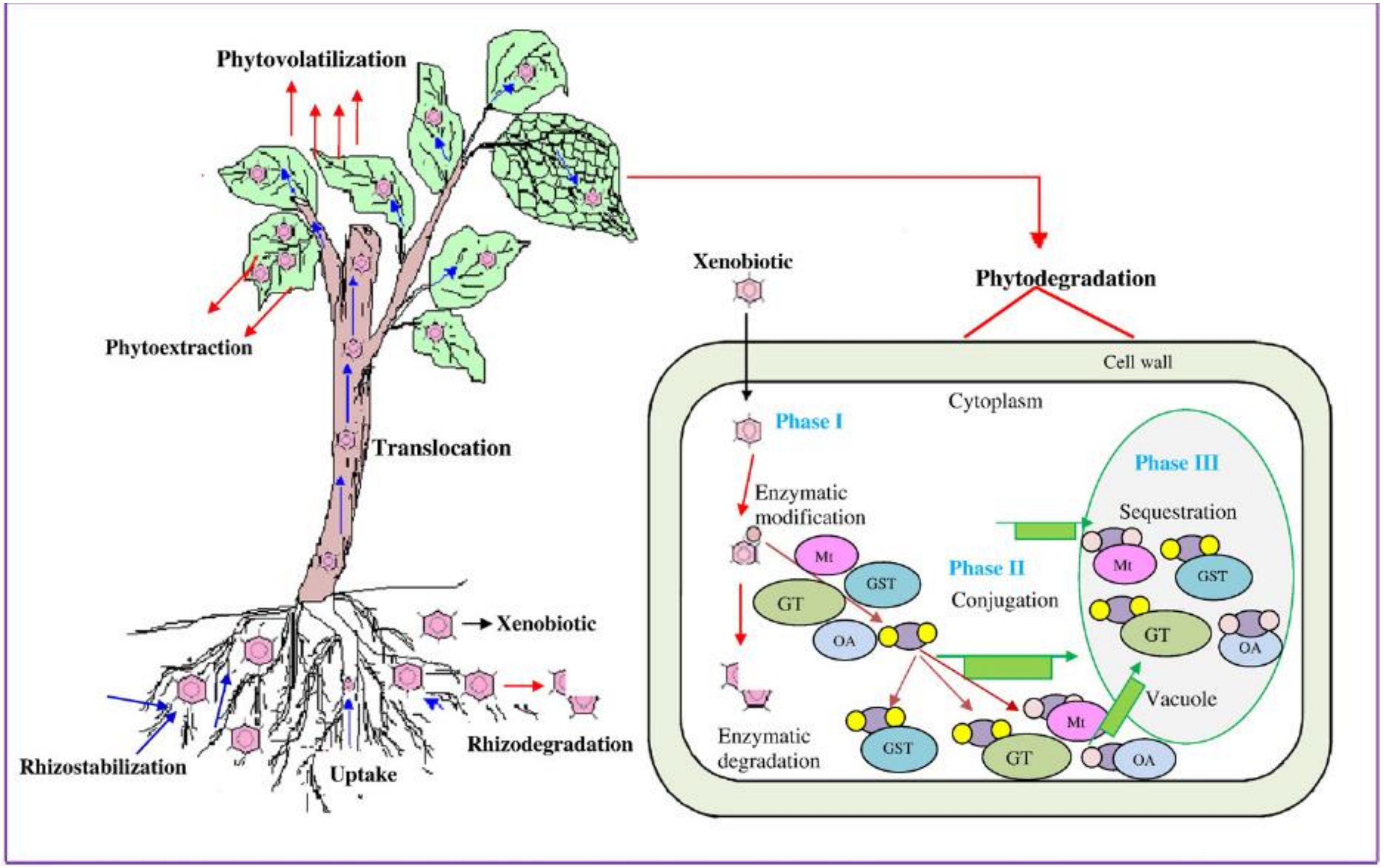
Advantages and Disadvantages of Phytoremediation

Advantages	**Disadvantages**
In situ and *ex situ*	Take several years to remediate a contaminated site
Amenable to a variety of organic and inorganic compounds	Limited to shallow groundwater, soils and sediments
Suited to remediation of large areas of soil	Not as effective for sites with high contaminant concentrations
Costs effective compared to conventional methods	Slower than conventional methods
Easy to implement and maintain & accepted by public	Toxicity and bioavailability of biodegradation products are not known
Fewer spread of contaminant via air and water	Contaminants may be mobilized into the ground water.
Conserves natural resources	Influenced by soil and climate conditions of the site. It does not work in the winter.
Environmentally friendly and aesthetically pleasing to the public	Disposal of contaminants accumulated in plants after harvesting - pollution again!

PHYTOREMEDIATION MECHANISMS



OVERVIEW:



Phytovolatilization
Phytoextraction
Translocation
Xenobiotic
Rhizostabilization
Uptake
Rhizodegradation
Xenobiotic
Phytodegradation
Cell wall
Cytoplasm
Phase I
Enzymatic modification
Mt
GST
GT
OA
Phase II
Conjugation
Phase III
Sequestration
Vacuole
Enzymatic degradation

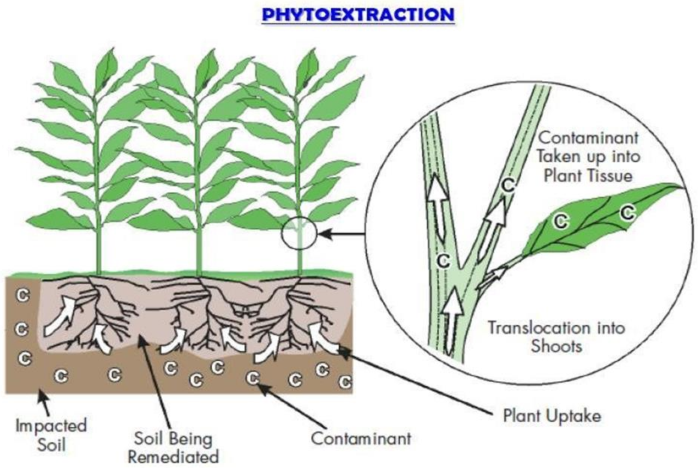
1. PHYTOEXTRACTION:

The uptake of contaminants by plant roots and movement of these contaminants from roots to the above part of plants - by absorbing, concentrating and precipitating the contaminants.

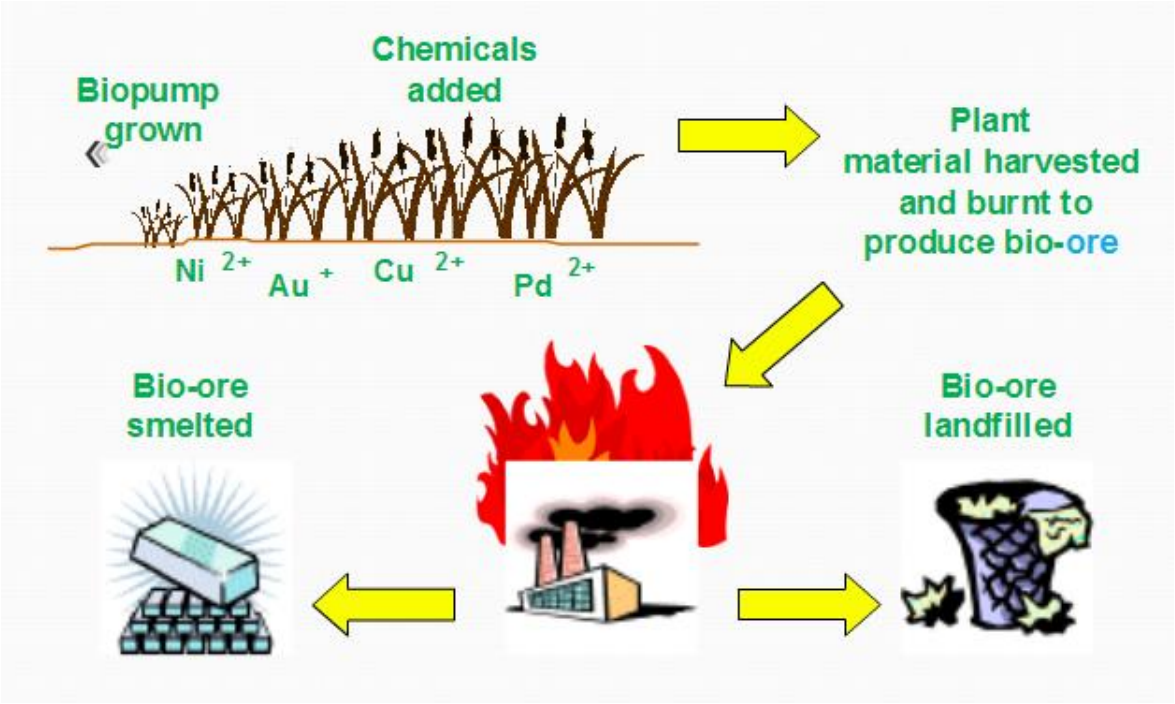
Two ways for phytoextraction:

- **Natural:** where plants naturally takeup contaminants from the soil unassisted

- **Assisted:** use of chelating agents, microbes and plant hormones to mobilize and accelerate contaminant uptake.



THE PHYTOEXTRACTION OPERATION



Advantages:

- Cost is fairly inexpensive compared to conventional methods.
- Contaminant permanently removed from soil.
- Amount of waste material that must be disposed of is decreased up to 95%
- In some cases, contaminant can be recycled.

Limitations:

- Metal bioavailability within the rhizosphere.
- Rate of metal uptake by roots.
- Proportion of metal “fixed” within the roots.
- Cellular tolerance to toxic metals.

2. PHYTOSTABILIZATION

Refers to the immobilization of contaminants in the soil through:

- absorption and accumulation by roots,
- precipitation within the roots.

Eventually, the mobility of the contaminant is reduced, migration to groundwater is prevented and thus bioavailability of metal into food chain is reduced.

Advantages:

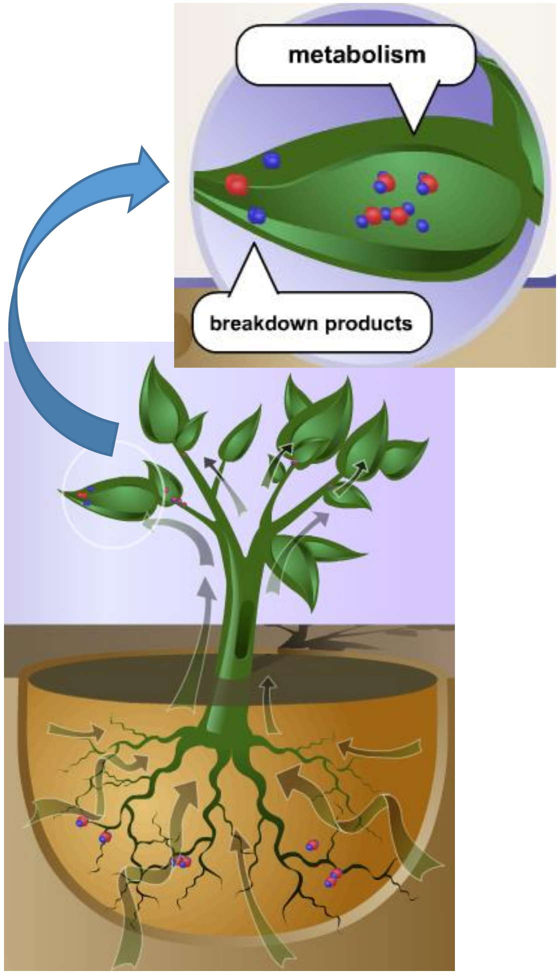
- No disposal of hazardous material / biomass is required
- Very effective when rapid immobilization is needed to preserve ground and surface waters

Disadvantages:

- Contaminant remain in soil
- Application of extensive fertilization / soil amendments
- Mandatory monitoring required

3.Phytotransformation

- Also known as phytodegradation, it is the breakdown of contaminants taken up plants by metabolic processes within the plant.
- Remediate some organic contaminants, such as chlorinated solvents, herbicides, and munitions
- It can address contaminants in soil, sediment, or groundwater.



Advantage:

- Both economically and environmentally friendly

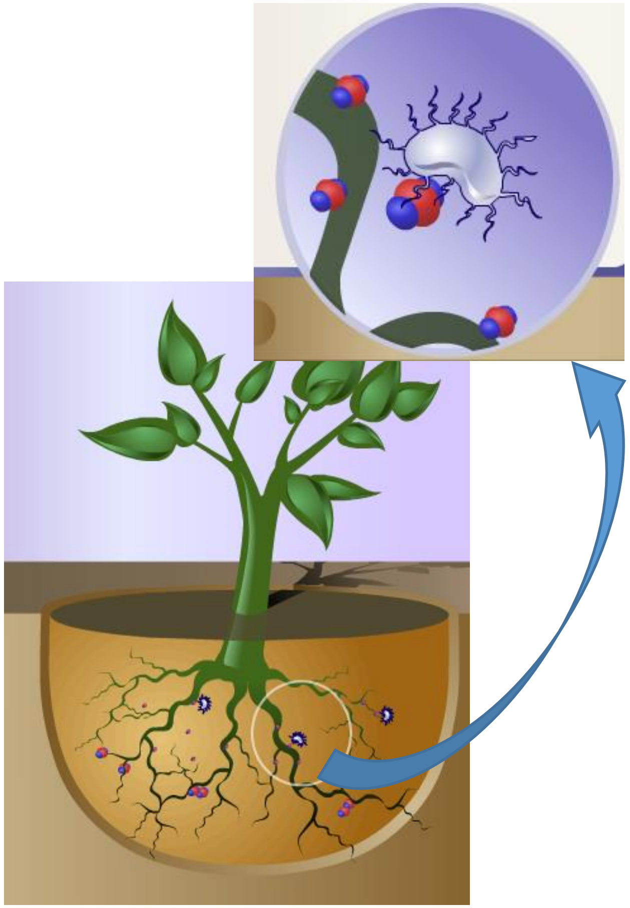
Disadvantages:

- Requires more than one growing season to be efficient
- Soil must be less than 3 ft in depth and groundwater within 10 ft of the surface
- Contaminants may still reenter the food chain through animals or insects that eat plant material

4. Phytostimulation (Rhizodegradation)

Definition: Breakdown of contaminants within the plant root zone, or rhizosphere.

- Carried out by bacteria or other microorganisms flourishing in the rhizosphere.
- Microbes in rhizosphere transform contaminant to non toxic product.
- Works well in the removal of petroleum hydrocarbons.



Advantages:

- *in situ* practice resulting in no disturbance
- No removal of contaminated materials
- Complete mineralisation of the contaminant can occur
- Low installation and maintenance cost

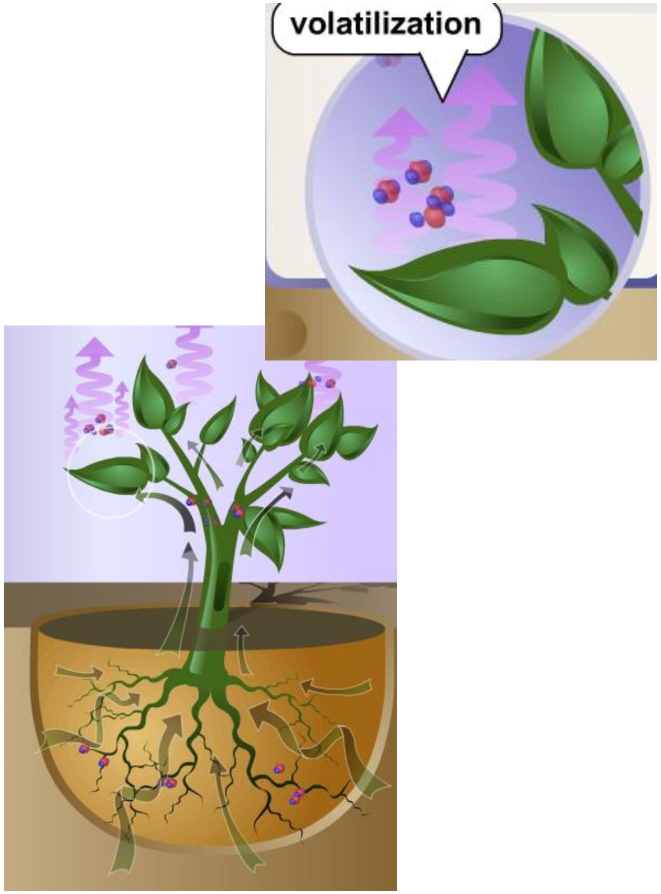
Disadvantages:

- Development of extensive root zone required- takes time
- Root depth limited due to physical structure of soil
- Organic matter from plant may be used as a C source instead of contaminant -> decrease amount of contaminant biodegradation

5. Phytovolatilization

- Involves plants taking up contaminants from soil, transforming them into volatile forms and transpiring them into atmosphere

- Works on organic compounds and heavy metal contaminants, TCE as well.



Advantage:

- The contaminant, mercuric ion, may be transformed into a less toxic substance (i.e., elemental Hg).

Disadvantage:

- The mercury released into the atmosphere is likely to be recycled by precipitation and then re-deposited back into lakes and oceans, repeating the production of methyl-mercury by anaerobic bacteria.

6. Rhizofiltration

- Adsorption or precipitation onto plant roots or absorption of contaminants in the solution surrounding the root zone.
- Used to remediate extracted groundwater, surface water, and waste water with low contaminants.
- Compared to phytoextraction, here the plants are used to address the groundwater rather than soil.

Advantages:

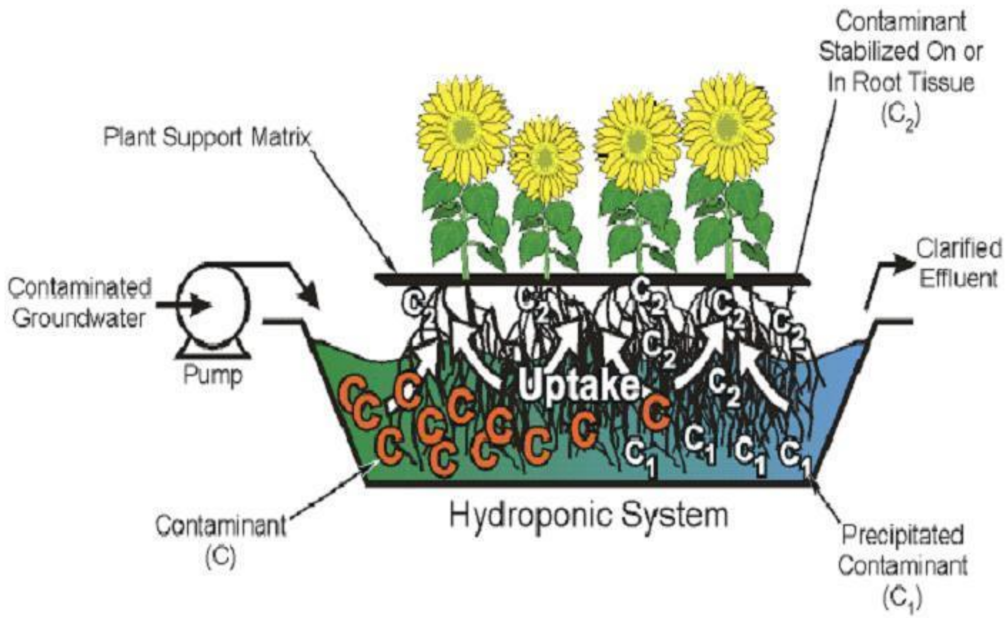
- Ability to use both terrestrial and aquatic plants for either *in situ* and *ex situ applications.*
- *Contaminants do not* have to be translocated into shoots.

Disadvantages:

- Constant need to adjust pH.
- Plants may first need to be grown in greenhouse/ nursery.
- There is periodic harvesting and plant disposal.

Example of Rhizofiltration

- In 1995, Sunflowers were used in pond near Chernobyl.



Conclusion:

- Although much remains to be studied, phytoremediation will clearly play some role in the stabilisation and remediation of many contaminated sites.

- The main factor driving the implementation of phytoremediation projects are low costs with significant improvements in site aesthetics and the potential for ecosystem restoration.

Further reading:

- Kennen K. and Kirkwood N. Phyto: principles and resources for site remediation and landscape design, 2015. Routledge, Taylor &Francis Group, Londin and New York. 346 pages.
- Kulakow P. and Pidlisnyuk V. Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination, 2010, Springer.198 pages.

Plants used in phytoremediation

Common Sunflower

- Sunflowers are native to the Americas. They have been used for dye, medicine, oil, food.
- Common sunflower is from the family Asteraceae, the family of daisies and its botanical name is Helianthus annuus.
- Sunflowers were planted around the Chernobyl region to remove some of the radioactive isotopes released by a nuclear plant meltdown.
- The common sunflower accumulated 2.5-fold more metal (i.e., Zn) in their biomass than was present in the soil.

Indian mustard Brassica Juncea

- Brassica juncea, also known as Chinese mustard, brown mustard, leaf mustard, Indian mustard, Oriental mustard, is a species of mustard plant. The seeds are used to make culinary mustard, particularly Dijon mustard. Also, its flowers can be eaten cooked, or raw.
- Chinese mustard is a high biomass rapidly growing plant with the attribute to accumulate Cd and other metals in shoots.
- It can remove three times more Cd than others, reduce 28% of Pb, up to 48% of Se, and it is effective against Zn, Hg and Cu as well.
- Indian mustard removed radioactive Cs137 from Chernobyl, Ukraine.

Indian grass (Sorghastrum nutans)

- Midwestern U.S. native plant benefits soil and ground water around them.
- Indian grass grows along the roadsides without noticing its power to detoxify common agro-chemical residues such as pesticides and herbicides related to atrazine and metalochlor groups.
- Indian grass is one of the nine members of the graminae family identified by PhytoPet(Bioremediation of Aquatic and Terrestrial Ecosystems), as capable to remediate petroleum hydrocarbons.

Pteris Vittata

- Pteris vittata is indigenous to southern Europe, Asia, Australia, and tropical Africa.
- It is a fern species in the Pteridoideae subfamily of the Pteridaceae.
- The phytoextraction of contaminated soil with As by the plant resulted in a decreased arsenic content in rice grain.

Thlaspi Caerulescens

- It is a flowering plant in the family Brassicaceae
- Is common in Mid-Europe and Scandinavia.
- Plant grows on forest margins, dry hillside meadows, gardens, banks, pastures, lawns, field margins, or bare places.
- Thlaspi caerulescens possesses a high resistance to Zn and accumulates high Zn concentration in its shoots.

Mirabilis Jalapa

- It is a perennial herb found in subtropical and tropical regions.
- Mirabilis jalapa, also referred to as Four o'clock, is native to tropical South America. It got its name as it opens in mid-afternoon and remains open overnight, but closes in the early morning.
- This plant has a long history of cultivation and uses around the world. For instance, it has been recorded around habitation areas and on waste ground.
- Four o'clock is a widely spread species which can be applied to phytoremediation of ≤10, 000 mg/kg petroleum contaminated soil.

Barley

- It is a cereal grain which is part of the family of grasses. In the present day, barley is one of the most widely consumed grains worldwide, however, it was one of the first cultivated grains in history.
- According to a study*, this grain is a suitable choice for phytoremediation of a petroleum-contaminated soil.
- *Farida Irajy Asiabadi , Seyed Ahmad Mirbagheri, Payam Najafi, Faramarz Moatar, Phytoremediation of Petroleum-Contaminated Soils around Isfahan Oil Refinery (Iran) by Sorghum and Barley, Current World Environment, 2014, 9(1)

Willow (Salix species). (White Willow)

- The water loving plants beautify landscapes, however, it’s worth is not confined to its appearance only.
- Their roots have demonstrated viability, accumulating lower levels of heavy metals than Brassicaceae, and they deal with Cd, Ni and Pb, and work even in mixed heavy metals like diesel fuel polluted sites.

Salix Viminalis

- Plant is used in horticulture and its flexible branchlets are used in basketry.
- It is a non-native shrub which is originally from Asia and Europe.
- There is a general increase in Cu accumulation by selected willow organs*.

* Mleczek M., Gasecka M., Drzewiecka K. et al, Copper phytoextraction with willow (Salix viminalis L.) under various Ca/Mg ratios. Part 1. Copper accumulation and plant morphology changes, cta Physiologiae Plantarum, 2013,35, 3251-3259

Paulownia

- It is a genus of 6 to 17 species of flowering plants in the family Paulowniaceae.
- The trees grow fast, putting on around three feet of height every year.
- Paulownia is an effective species for phytoremediation due to high biomass productivity*

*Tzvetlova N., Meladinova K., Ivanova K et al Possibility for using of two Paulownia lines as a tool for remediation of heavy metal contaminated soil.J Environmental Biology, 2015, 1, 145-151.

Apocynum Cannabinum

- Apocynum cannabinum, also known as amy root, dogbane, prairie dogbane, hemp dogbane, rheumatism root, Indian hemp, is a perennial herbaceous plant which grows throughout the United States and in the southern half of Canada.

- It is a phytoremediation plant that is used to sequester lead in its biomass.

Festuca Arundinacea

- It is a species of grass that is native to the Azores, northern Africa (i.e. northern Libya, northern Algeria, Tunisia, and Morocco), western Asia and Pakistan, and all of Europe.
- The plant is used as an ornamental grass in gardens, as well as for phytoremediation. Furthermore, it is an important forage grass throughout Europe.
- Festuca Arundfinacea indicates good usefulness for phytostabilisation of soils that are characterized by a relatively small pollution by Cd.

Poplar tree (Populus deltoides)

- Poplar trees secret lies in the naturally well-designed root system which take up large quantities of water.
- Chlorinated solvents such as trichloroethylene, or the well-known carcinogenic carbon tetrachloride (95% of substance removed) are the organic pollutants that hybrid poplars face better, according to research from National Institute of Environmental Health Sciences.
- PhytoPet -the Canadian database for bioremediation methods, remarks that poplar trees can degrade petroleum hydrocarbons like benzene, toulene and oxylene.

Miscanthusxgiganteus

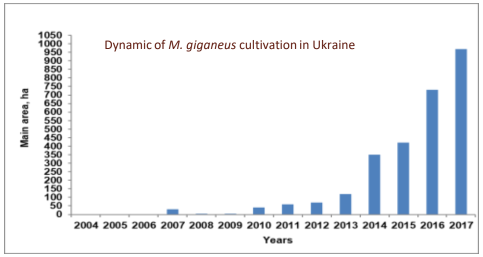
Miscanthus sinensis

Miscanthus sacchariflorus

Miscanthus giganteus

Miscanthusxgiganteus Greef et Deu: biofuel crop and phytoagent

- *M.x giganteus*, a sterile triploid hybrid of:*Miscanthus sinensis*, diploid called Chinese silvergrass,Maidengrass and *Miscanthus sacchariflorus*, tetraploid called Amur silvergrass
- Discovered in Japan in 1935, and for many years was treated as an exotic ornamental plant
- Beginning of 1980s- first plantations were established in Denmark and Germany, than – in other EU countries, including Czech Republic, Poland, Slovakia, Ukraine
- the estimated area of land under miscanthus cultivation in the EU is currently about 20,000 ha, in Ukraine- about 1000 ha
- Annual yield of *Mxgiganteus* across Europe range from 10 to approximately 40 tones/ha, and over 400GJ/ha-1/year*.
- Full establishment of *Mxgiganteus* stand takes from 2 to 5 years, depending of climate conditions, productive life span is estimated between 20 to 30 years*



3rd year of vegetation

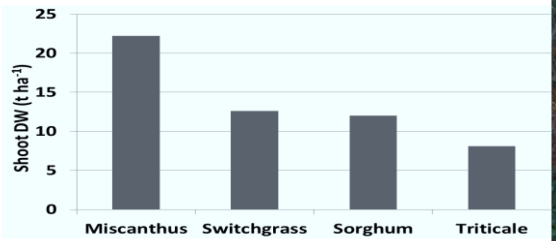
1st year of vegetation

2nd year of vegetation

Kvak V., Stefanovska T., Pidlisnyuk V., Alasmary Z., Kharytonov M., 2018. INMATEH-Agricultural Engineering, 54(1):113-121

Miscanthus x giganteus

- Perennial grass: growth up to 25 years
- Stem height: 3 - 4 m
- High shoot productivity: 15 - 30 t DW ha^{-1}
- Low production inputs
- Soil stabilization
- CO_2 sequestration
- Non invasive species

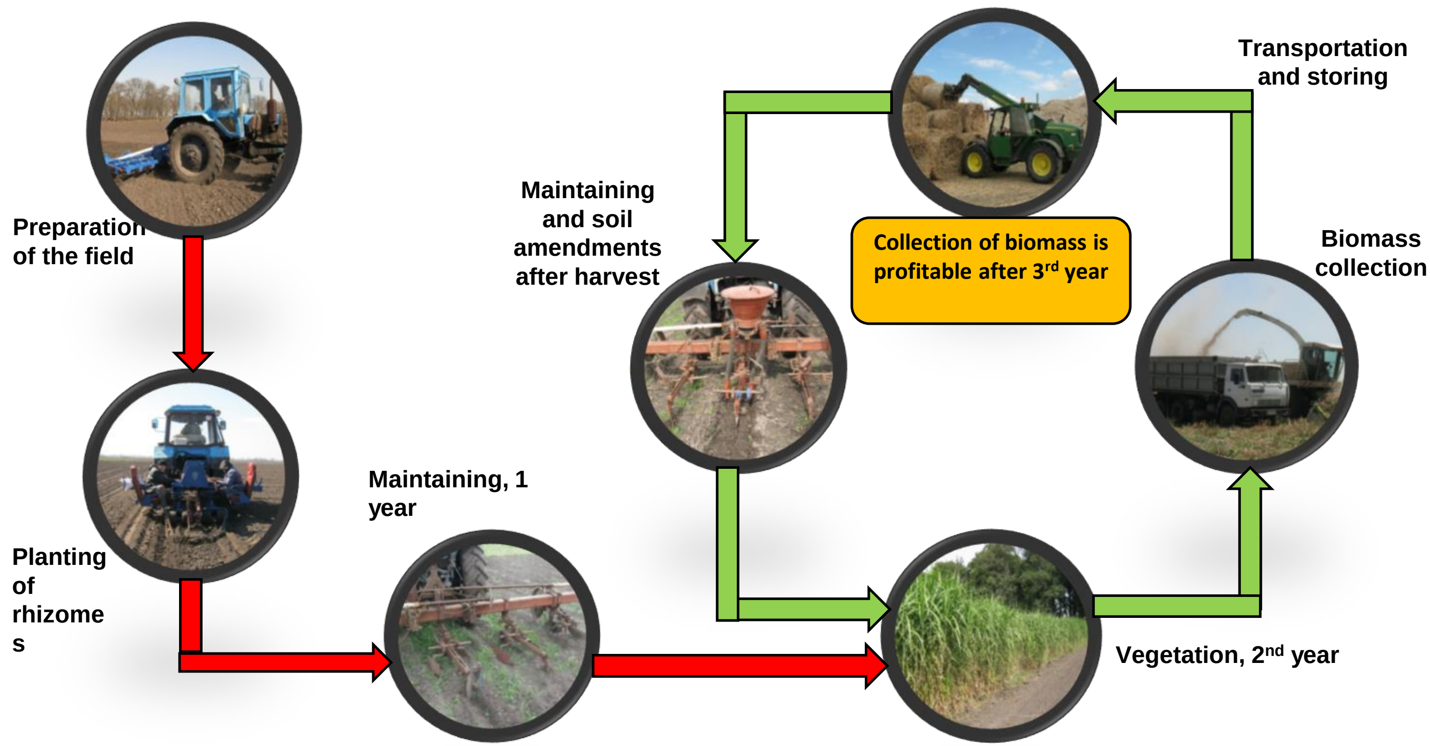


Cadoux *et al*, 2013

Biomass with multiple

heat **biofuel** **mulch** **animal bedding** **building materials** **packaging**

Agricultural technology of growing Mxg



Advantages and disadvantages of *Miscanthus* for phytotechnology with biomass production *

Advantages	Disadvantages
In production	
Perennial, established stands last ~20 years	Takes 2-3 years to fully establish
Effectively suppresses weeds once established	Tall, dense growing perennial grass monoculture with limited wildlife friendly uses
High productivity of biomass compared to other energy crops (20 up to 35 $tons.ha^{-1}.yr^{-1}$)	Bioenergy processing immature technology; expensive pre-processing needed
Uses water efficiently by C-4 photosynthesis; total usage ~ 1 $m.yr^{-1}$	Yields are influenced by water availability; under low-rainfall conditions may be poor
Grows at lower temperatures than other warm season (C-4) grasses; hence longer season	Limited tolerance of low winter temperatures so not suited to severe continental climates
Does not require as much N as sorghum, maize, oil palm, or sugar beets	Off-take of K ~3 x more than coppice willow
Mineral content of biomass relatively low compared to common biomass crops	Mineral nutrient content per unit energy high compared to coal
The winter harvested crop is relatively dry, so drying costs are low	Field drying and mineral leaching results in significant biomass loss as leaf fall

** Pidlisnyuk et al, Critical Review in Plant Science, 2014 ,N1, p.1-19*

Advantages and disadvantages of *Miscanthus* for phytotechnology with biomass production*

In phytoremediation	
Economic return can be obtained from contaminated land with employment and market value of biomass fuels (possibility of developing a more economical approach to remediation of soils with heavy metals such as mine land)	Dedicated energy crops can result in displacement of other crops with significant changes in land use, food crop prices
Easier to clear than trees for the site to be transformed for future use	Sterile hybrid so propagation for initial establishment is labor intensive
In both processes	
Potential for income generation through carbon credits through CO_2 sequestration	Less C storage than forest wood crops over next 50 years
Reduction of soil erosion due to rainfall, or wind. Reduces dust	Can serve as reservoir for insect pests of other species

** Pidlisnyuk et al, Critical Review in Plant Science, 2014, 1, p.1-19*

Phytotechnology with *M.xgiganteus*

- A smelter site in North France showed accumulation ratio <0.1 with tissue Pb ~ 15 mg/kg from soils of 200-500 mg/kg Pb*
- In Rumania a mining area site with > 600 mg/kg Pb showed only 10 mg/kg in leaves. Cd levels likewise were low, about 1 mg/kg from soil with 13. However stem Cd was higher by 2x while stem Pb was lower by 2x relative to leaves. Rhizomes had high levels, about half of soil values

***Nsanganwimana F. et al.2015. Metal accumulation and shoot yield of Miscanthuxgiganteus growing in contaminated agricultural soils: insights into agronomic practices. Agriculture, Ecosystems and Environment, 12, 61-71*

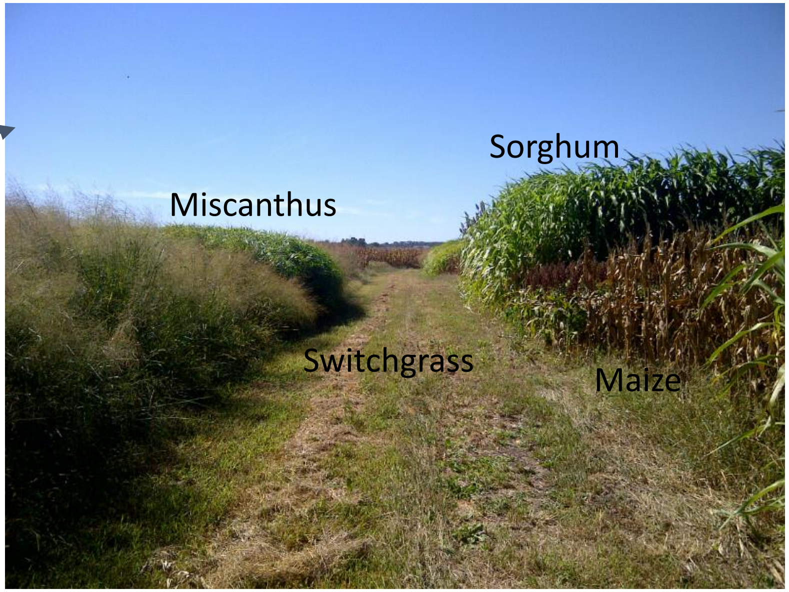
Phytoremediation with Crops Produced for Bioenergy

Thanks to the following co-authors

Lawrence Davis[1A] , Larry E. Erickson [1B], Ganga Hettiarachchi[1C], Kraig Roozeboom[1C]

[1] Depts of: A; Biochemistry, B; Chemical Engineering, C; Agronomy, Kansas State University, Manhattan, KS

Seventh year, study of biomass and bioenergy production with annual and perennial grasses at the Kansas State Agronomy Farm, Manhattan, KS. September 21, 2014



Miscanthus x giganteus, a sterile triploid hybrid of:
Miscanthus sinensis , diploid called Chinese silvergrass, Maidengrass (invasive U.S. south) and
Miscanthus sacchariflorus, tetraploid often called Amur silvergrass, (invasive weed in Minnesota)

Photo credit: Ann Gibson, Vancouver BC

Two views of the same university arboretum plot, different dates/years. Only in late October will the flowers be visible on *M. x giganteus* in Minnesota

Miscanthus x giganteus seems to have hybrid vigor. Its vegetative biomass production is greater than that of either of its progenitor species. This may be because it is much later flowering in northern latitudes and puts its energy into vegetative rather than reproductive material.

Translocation of nutrients to the underground storage organs at end of season, common in perennial grasses of the prairies also give a relatively low ash content. This is a big advantage over annual forage crops designed for maximum reproductive biomass which demands high available soil nutrients. *Miscanthus x giganteus* is not quite so nutrient efficient as tree crops.

Advantages and disadvantages of *Miscanthus* for production and use in phytoremediation*

Advantages	Disadvantages
In production	
Perennial, established stands last ~20 years	Takes 2-3 years to fully establish
Effectively suppresses weeds once established	Tall, dense growing perennial grass monoculture with limited wildlife friendly uses
High productivity of biomass compared to other energy crops (20 up to 35 $tons.ha^{-1}.yr^{-1}$)	Bioenergy processing immature technology; expensive pre-processing needed
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** Pidlisnyuk et al, Critical Review in Plant Science, 2014 ,N1, p.1-19*

Advantages and disadvantages of *Miscanthus* for production and use in phytoremediation*

In use for phytoremediation	
Economic return can be obtained from contaminated land with employment and market value of biomass fuels (possibility of developing a more economical approach to remediation of soils with heavy metals such as mine land)	Dedicated energy crops can result in displacement of other crops with significant changes in land use, food crop prices
Easier to clear than trees for the site to be transformed for future use	Sterile hybrid so propagation for initial establishment is labor intensive
In both processes	
Potential for income generation through carbon credits through CO_2 sequestration	Less C storage than forest wood crops over next 50 years
Reduction of soil erosion due to rainfall, or wind. Reduces dust	Can serve as reservoir for insect pests of other species

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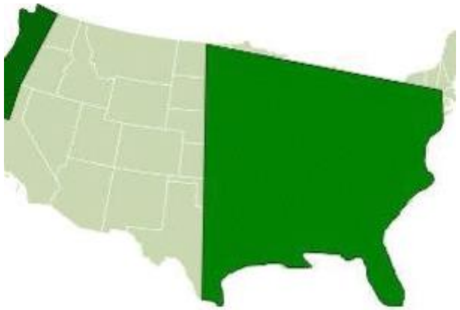


Climatic adaptation in the U.S. is water-limited. Winter minimum temperatures restrict establishment in northerly climates, but if forage remains as insulation, survival is better.

Although a C-4 grass*, Miscanthus x giganteus* maintains functional photosynthesis at lower temperatures than maize and grain sorghum. It requires no exponential growth phase to establish a closed canopy in spring, unlike seed planted annuals.

Germany

Representative European crops

In more humid climates on good soils, *M x g* produces larger plants, greater biomass. Also, genotype selection is being done by breeders

Breeding efforts are extensive in Europe:

<u>Optimisc</u> is a consortium headquartered at University of Hohenheim, Germany
A dozen partner organizations, across Europe, Russia, China, Turkey
Seven trial locations for multiple germplasms (3 dozen from China)
Varietal trial locations from the U.K. to Ukraine, Russia, Turkey, begun years 2012-2013
Testing marginal lands in Germany, begun 2011, Germany
Selection for salinity tolerance, winter hardiness

ted types, improve proces

Blankney,England,
U.K.

Ukraine cooperation with
University of Hohenheim,
location Potasch, Ukraine
German Agrarian Center

Miscanthus productivity, N and water use efficiency

Reported biomass production varies widely, with a significant fraction below-ground. Harvested biomass typically excludes many leaves. N or water use efficiency is usually determined on basis of total above-ground, or harvested biomass. N ~0.25 %

At Manhattan, KS, by direct comparison of replicated plots (n=4), years 2-5
Miscanthus x giganteus, 10-16 Mg/ha (4.5-7 tons/acre) harvestable biomass
Switchgrass (*Panicum virgatum*), 8-14 Mg/ha
Big bluestem (*Andropogon gerardi*), 4-8 Mg/ha

Representative estimates

Iowa > 20 Mg/ha (total aboveground)
Illinois >35 Mg/ha (total after frost)
Mississippi up to 60 Mg/ha (total aboveground), patent claims ~25 Mg/ha
Ireland 12-15 Mg/ha
Germany >20 Mg/ha (*M x g* CV),
Slovakia >24 Mg/ha

Miscanthus tolerance of metals and salt (KSU)

Miscanthus was less tolerant of flue gas desulfurization water than sunflowers in an 18 day test. Hydroponic water use half that of sunflower for same biomass, ~175 mL/g , vs 350 mL/g. Field values would be less efficient (below ground not counted).

Miscanthus grows well in soil with total Pb = 700-1500 mg/kg, poorly with high Zn + Pb chat material even when supplemented with 10 % by wt of composted cattle manure. Total Zn > 4000 mg/kg, total Pb> 2000 mg/kg. Extractable metals expected to be ~ 20 x lower with compost treatment. In earlier field studies Zn toxicity was limiting for other grass crops.

~1000 mg/kg Pb in good soil, from Ft. Riley, KS

Chat, + 10 % compost, 1 % straw

Hydroponic, perlite, Hoagland's soln

~1000 mg/kg Pb in Ft. Riley soil

Miscanthus tolerance of metals and salts

In Slovakia, Sliac soil

Many published lab studies, some with soils having aged contamination show low accumulation ratios for As, Pb, Cd, Zn

Few field studies done on heavily contaminated soils.

Aged contamination with > 500 mg/kg Pb in Poland showed little uptake.

A smelter site in France showed accumulation ratio <0.1 with tissue Pb ~ 15 mg/kg from soils of 200-500 mg/kg Pb.

In Rumania a mining area site with > 600 mg/kg Pb showed only 10 mg/kg in leaves. Cd levels likewise were low, about 1 mg/kg from soil with 13. However stem Cd was higher by 2x while stem Pb was lower by 2x relative to leaves. Rhizomes had high levels, about half of soil values.

Annual yields over three years (g/plot) of aerial part of *Miscanthus giganteus* and *Sida hermaphrodita* (Virginia Mallow) for soil previously contaminated by Zn and Pb **

Plant species	Soil type	pH	2008	2009	2010
Miscanthus giganteus	Loam	5.7 6.3	194 375	1216 1390	1518 2014
	Sand	5.2 6.1	379 546	2067 2087	3084 3454
Sida hermaphrodita	Loam	5.7 6.3	49 130	255 429	854 1199
	Sand	5.2 6.1	248 499	720 1531	1171 2128

Plot size was 1m x 1m. Each plot was filled with loamy or sandy soil, at two different pH levels. More than 20 years previously, the soil in each plot was artificially contaminated by metals. The loam was contaminated with 700 mg.kg^{-1} of soil by Pb and with 1100 mg.kg^{-1} of soil by Zn. The sand was contaminated with 600 mg.kg^{-1} of soil by Pb and 900 mg.kg^{-1} of soil by Zn. In 2008, the year of establishment, two plants were set per plot. Above ground biomass yield was determined for biomass dried several days at 60°C.

***Kocon and Matyka, J.Food Agric.Environ., 2012*

Potential use on degraded lands

Perennial, no tillage once established
Non-invasive sterile triploid plant
Little uptake of heavy metals and metalloids
Low N demand, low ash content in harvest
Is being commercialized, especially under renewables mandates
Phytostabilization can mesh with commercial plans in same region
Aesthetically better than abandoned sites; stabilizes soils
Less costly than contaminant removal most sites

England

Agronomic & economic challenges

Establishment, more like trees than row crops.
High propagule costs mandate wide spacing (typically 1m x1m)
Water is critical for initial establishment, until roots go deep
Minimum 2 year to useful yield, clump radius ~ 1 m/year from planted rhizome
Weed competition problems until canopy closure (1-2 years)

2013 Planting day, Muscatine County, eastern Iowa

Aug 21 stand established

Low density product, must be dried for processing,
Harvest management requires adapted machines,
Product storage and transport both large scale efforts
Distance to market means relatively large transport cost

Vertec Biomass

University of Iowa plan for planting, 2,500 acre (~1000 ha)
Goal 40 % renewables for university energy by 2020.
Funding from university facilities division

University of Iowa miscanthus field

Winter harvest, cutting and baling gives low density product (10-15 lb/cu ft). Processing to pellets increases density ~3-fold, but significant energy cost.

Commercial pilot scale, near Wichita, KS

Policies and markets must be aligned

Ireland

Suitable product outlets needed,
markets may not spontaneously arise
Potential: animal bedding, local heating operations, energy production, biofuel
Has been used for dairy cattle in place of corn stove; slightly lower feed value

Ireland 2013, poor yield, weak market, farmers plowed down their plantings.
These were government subsidized plantings but without sufficient market development.
Independent local markets found by some, including briquettes

Columbia, Missouri attempted co-firing with coal, found poor pellet stability in damp weather, had old power plant not well designed to handle mixed product. (Missouri has renewables mandate)

Commercialization

U.S. efforts primarily driven by government grants, universities
Limited corporate involvement

MFA Oil /Aloterra
Has over 5,000 ha (13,500 acres), target 75,000.
Across sites in Arkansas, SW MO, central MO

Repreve Renewables
Uses **Freedom,** patented, (selected from plant introductions germplasm) ,
Miscanthus x giganteus. Originally wrongly identified as *M. floridulus*
Restricted distribution for contract producers only

Freedom
Giant Miscanthus

Phytotechnologies and Environmental Health

Introduction

- Report “ The Future of Public Health for the 21th Century”, IOM, 2002: public health is in a risk when poor environmental conditions- compromised waster, air, food, housing undermine health
- Remediation technologies are designed to disrupt contamination pathway (environment-receptor) and to reduce exposures:
- a) ground water remediation technology prevents the spread of a contaminant plume and protests drinking water supplies
- b) engineering control put in place to decrease the dispersion of harmful mine tailing dusts act to prevent of harmful mineral particles

Environmental Health

Comprises those aspects of human health, including quality of life, that are determined by physical, chemical, biological, social and psychosocial factors in the environment. It also refers to the theory and practice of assessing, correcting, controlling and preventing those factors in the environment that can potentially affect adversely the health of present and future generations.

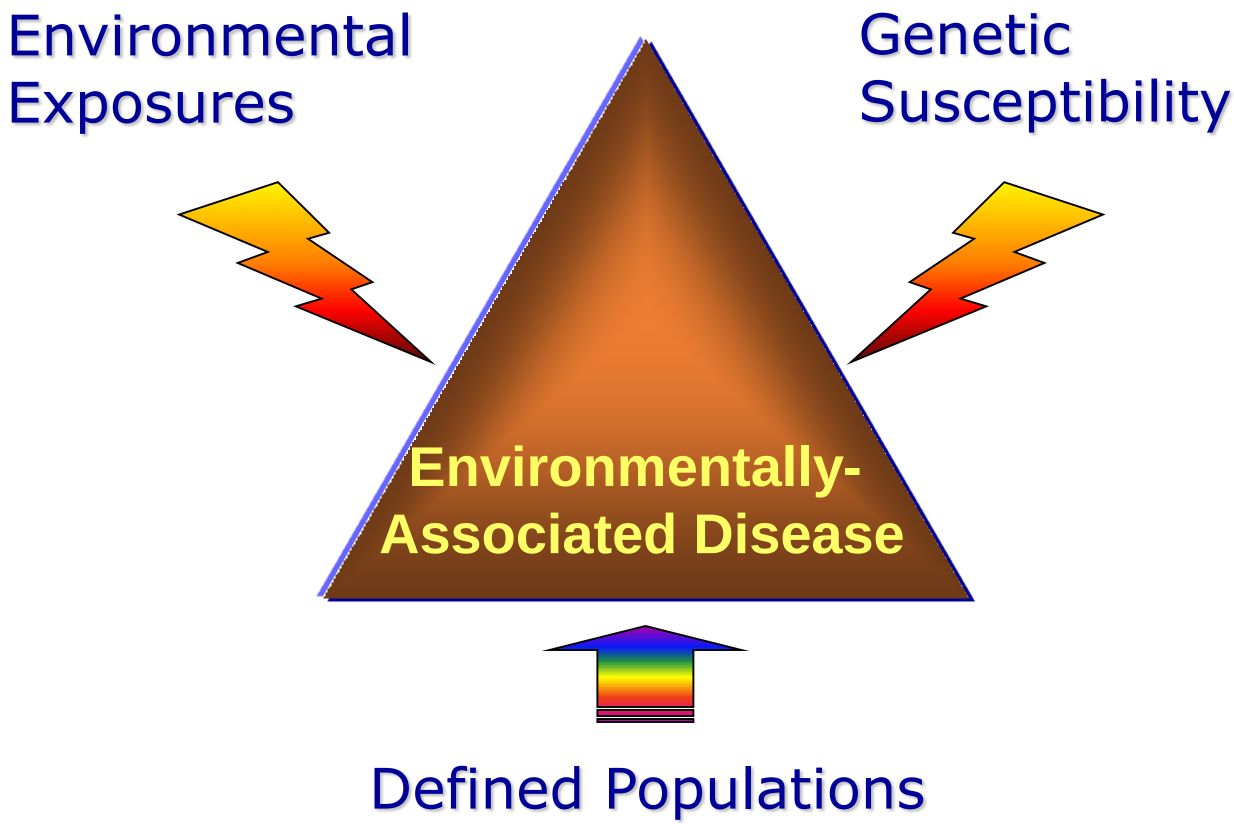
(WHO consultation in Sofia, Bulgaria, 1993)

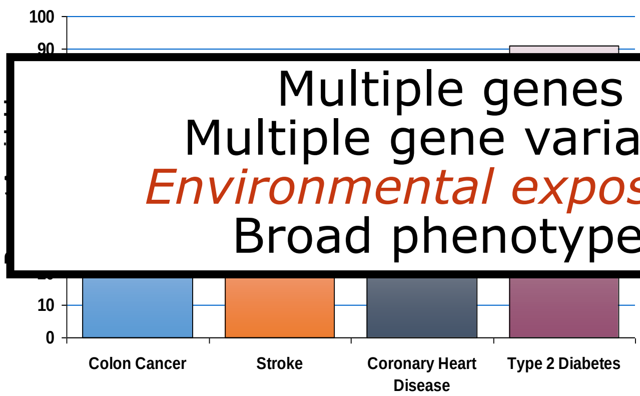
Why Environmental Health?

Inherently complex

- Everyone's 'environment' is different
- Toxins can affect more than one pathway
- Exposure is often at low levels for very long periods and can occur during critical periods of vulnerability
- Most real-life exposures are to a mixture of potential toxins with non-linear interactions

Define the Problem

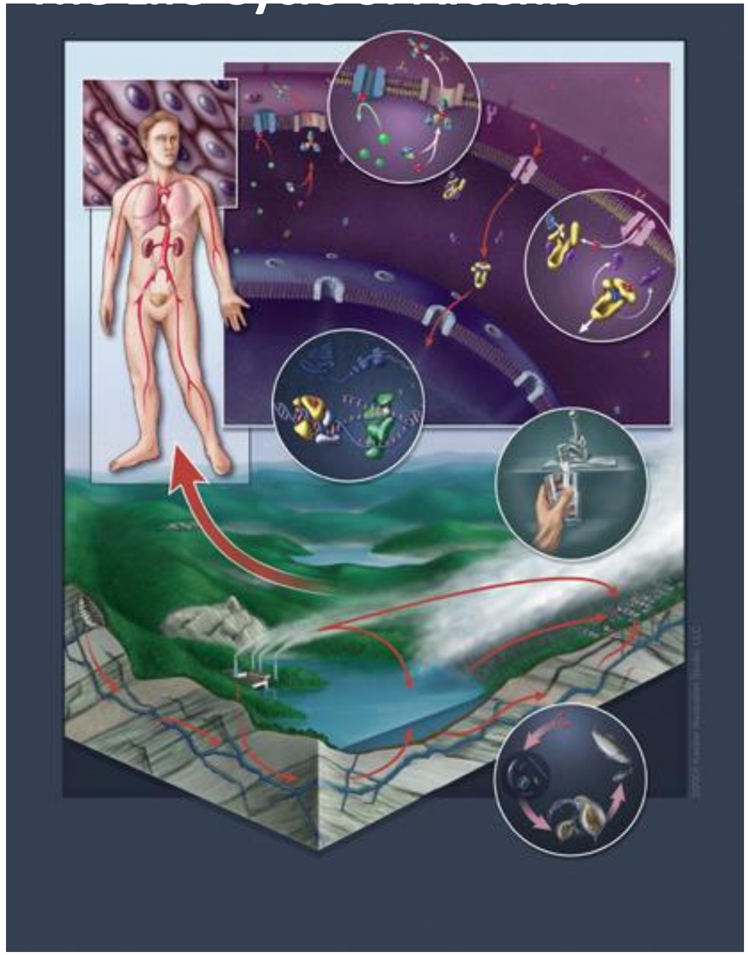




Multiple genes
Multiple gene variants
Environmental exposures
Broad phenotypes

Willett. *Science* 2002; 296:695

- 70-90% of the major diseases in the USA are caused by reversible behaviors and exposures
- Single gene mutations are the major cause of cancers and CVD in < 5% of the cases



Early/Immediate

Skin Lesions:

- Melanosis
- Keratosis

Other Conditions

- Diabetes Mellitus
- Non-pitting edema
- Respiratory lesions
- Cognitive deficits
- Black-foot Disease

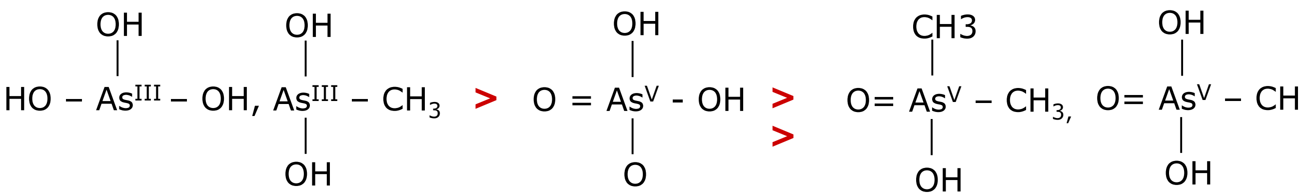
Late

Cancers:

- Skin
- Bladder
- Lung
- Liver

Neuro/Vascular:

- Hypertension
- Stroke
- Neuropathy



Arsenite **Monomethylarsonous Acid MMA^{III}** **Arsenate** **Monomethylarsonic Acid MMA^{V}** **Dimethylarsinic Acid DMA^{V}**

Hughes, M.F. (2002). *Toxicology Letters*, 133:1-16.

Environmental Exposure

↓

Internal Dose

- **Biomarkers of exposure:** arsenic levels in toenail clipping, or urine
- **Metabolism:** metabolite profiles and modulating factors

↓

Biological Response

- **Oxidative Stress:** anti-oxidant response pathway; NFkB pathway; apoptotic pathway
- **Endocrine disruptor:** steroid receptor pathways
- **Proteotoxicity:** regulation of protein malfolding; protein-metal interactions; proteomic profiles; ubiquitination pathways; histone modification
- **Genotoxicity:** mitochondria as a primary target

↓

Early Markers of Disease

- **Biomarkers of effect:** increased expression of COX-2 (observed in bladder cancer); carotid intima-media thickness; metallothionein-3

↓

Disease

Phytotechnologies and community acceptance*

- Phytotechnologies have ancillary positive impacts on the surrounding environment, providing ecosystem services with tangible, quantifiable value for public health and social welfare
- In terms of aesthetics and community acceptance, the importance of the community appeal of phytotechnologies is worth re-emphasizing.
- Public engagement and community acceptance is required and can be key to the long-term success of a clean-up operation.

*Heather F. Henry, Joel G. Burken, Raina M. Maier, Lee A. Newman, Steven Rock, Jerald L. Schnoor, and William A. Suk.Phytotechnologies – Preventing Exposures, Improving Public Health, Int J Phytoremediation,2014,889-899.

Advantages of Phytotechnologies

- **Technical advantages**

- passive and in situ
- inherently controls erosion, runoff, infiltration, and fugitive dust emissions
- applicable to remote locations, potentially without utility access (in some cases requires a supplemental source of irrigation, but this can be solar or wind powered)
- can be used to supplement other remediation approaches or as a polishing step
- can be installed as a preventative measure, possibly as a leak detection system
- can be used to identify and map contamination

Advantages of Phytotechnologies

Pollution Reduction and Resource Conservation

- lower maintenance, resilient, and self-repairing
- considered a green technology and sustainable
- wind- and solar-powered
- improves air quality and sequesters greenhouse gases
- minimal air emissions, water discharge, and secondary waste generation

Phytotechnologies and Clean Water Systems

- Green Liver concept

Green Liver System constructed at the water work facilities of Hefei City at Lake ChaoHu (PR China)

Phytotechnologies and clean air

- House plant that can clean the air in the home: transgenic effect
- Traditional methods – use HEPA filters can remove allergens and dust participles, however, other molecules (small-chloroform or benzene) to be filtered out.
- Common house plant *potros ivy* (Devil's ivy) was genetically modified in a way that plant can absorbs chloroform and benzene. The modified plant can 'eat" these compounds and use them to fuel their own growth.
- The team from UW was inspired by functioning of human liver: a protein cytochrome R450 2E1 which is presented in liver of mammals acts on benzene and thrns it to CO2 and Cl-, which used by plant.

Advantages of Phytotechnologies

- Community benefits/capacity building

- favorable public perception provides a community educational opportunity
- improves aesthetics, reduces noise
- creates habitat (can be a disadvantage—attractive nuisance)
- provides restoration and land reclamation during cleanup and upon completion
- can be cost-competitive
- has the potential for capacity building through involvement of community in maintenance, stewardship, etc.

Linking government, academia, and community partnerships to promote public health

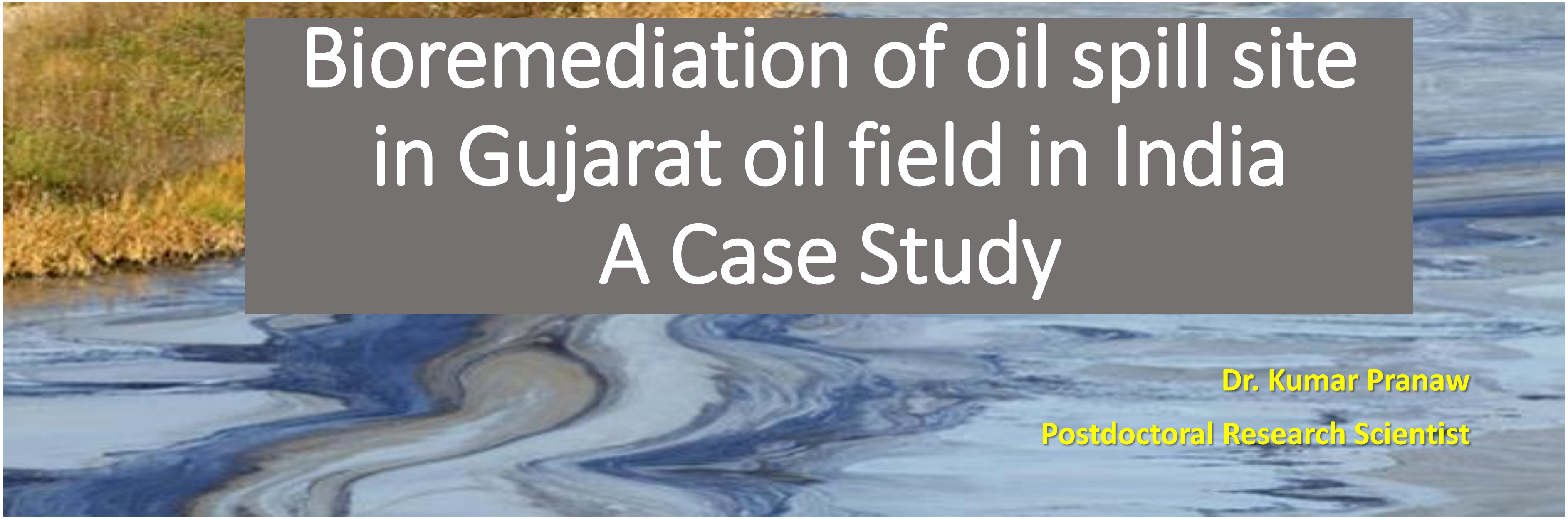
- Connecting phytotechnologists with public health researchers helps to ensure that technology development efforts are focused on the prevention of environmental exposures.
- While public health researchers are well versed in human exposures to toxicants, they are in need of primary prevention solutions that are sustainable.
- The sustainable nature of phytotechnologies (both in terms of economics and energy consumption) further reinforces their potential to reduce exposures within resource-constrained public health agencies worldwide.
- The need for phytotechnology based solutions to reduce exposures will only increase in the future, given the conditions of climate change and the need to conserve water and other ecosystem services. If coordinated with epidemiology studies, phytotechnology field applications could provide important information on how effective these technologies are at reducing disease or exposure.

Further reading:

- Heather F. Henry, Joel G. Burken, Raina M. Maier, Lee A. Newman, Steven Rock, Jerald L. Schnoor, and William A. Suk.Phytotechnologies – Preventing Exposures, Improving Public Health, Int J Phytoremediation, 2014,889-899.
- Maier R. et al, Superfund Research Programmer, Arsenic Uptake in Homegrown Vegetables from Mining-Affected Soils, 2013, University of Arizona



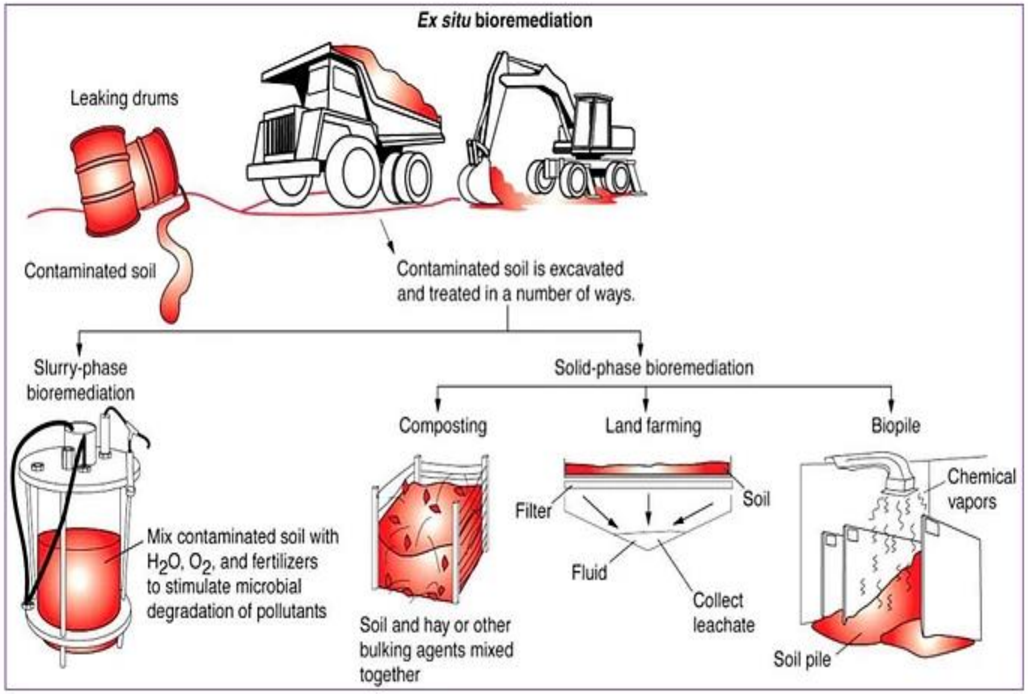




Bioremediation of oil spill site in Gujarat oil field in India A Case Study

Dr. Kumar Pranaw

Postdoctoral Research Scientist



Ex-situ Bioremediation

Based on our definition it seems like we just remove the contaminated material from the environment and then bioremediation happens.

But there are a few options for how to actually carry out the process. When the material is removed from the environment, it can be put into **bioreactors**, large vessels where the contaminated material can be monitored and conditions for bioremediation can be controlled. Biological organisms typically have conditions where they operate best. In bioreactors we can control the mixing rate, temperature, pH, and nutrient levels to suit the organisms breaking down our contaminant.

When bioreactors aren't used, landfarming or biopiles are alternatives.

Landfarming involves spreading contaminated soil into a lined bed (to prevent leaching) and periodically applying nutrients and mixing the soil to boost biological activity.

Biopiling places the contaminated soil into piles that are well aerated and nutrients are added to speed up bioremediation. In all cases, the contaminant levels are monitored to verify that bioremediation is taking place and steps are taken to ensure that contaminated material stays out of contact with the environment.

In June 2008 there was accidental oil spill near city of Gujarat (Western India) due to crude oil trunk line rapture.

Crude oil was spread in large area in farm land. However, land was vacant, thus there was not much loss of crops.

This trunk pipeline was being used for transportation of crude oil from oil producing field to the Gujarat Refinery in Baroda City of India.

- Oil producing company took immediate action and stop pumping of crude oil in affected trunk line.

- Oil Company also immediately barricaded the oil spill site and also prevented the spread of crude oil. The Oil company recovered substantial amount of crude oil accumulated in low lying area at spill site.

- After these primary actions by Oil Company themselves, they approached ONGC TERI Biotech Ltd (OTBL).

- OTBL engaged a team to excavate the entire oil soaked soil. Entire oil soaked soil was excavated by using mechanical excavator and then it was transported to the bioremediation site by using soil dumpers.

- OTBL had carried out the excavation of 14694 m^3 of oil contaminated soil and transported the same to a secured bioremediation pit fitted with High Density Polyethylene (HDPE) liners.
- After dumping of oil soaked soil in secured bioremediation pit, Oilzapper (crude oil degrading bacterial consortium) a patented product was applied for degradation of TPH in oil contaminated soil.
- Oilzapper is a consortium of crude oil degrading bacterial consortium developed by assemble of five species of oil degrading bacteria which could degrade different fraction of TPH.
- After application of Oilzapper (74.5 tonnes) nutrient recipe was also sprayed on oil soaked soil and then tilling of oil soaked soil was done at regular intervals.

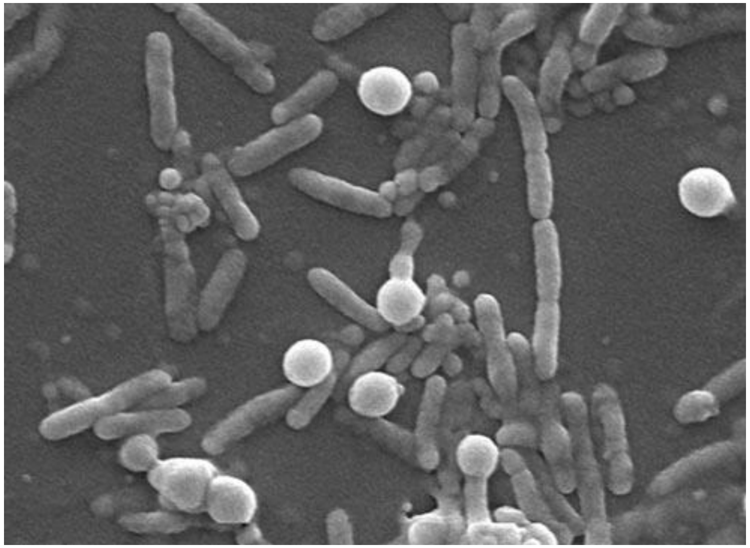
Used HDPE liners

Oilzapper

Application of Oilzapper

Over a period of 4 months soil samples were taken and analysis were carried out.

Oilzapper Intervention



- After seven years of research work, the Microbial Biotechnology laboratory at TERI has developed an efficient bacterial consortium that degrades crude oil and oily sludge very fast.
- This bacterial consortium was developed by mixing four bacterial strains, which could degrade aliphatic, aromatic, asphaltene, and NSO (nitrogen, sulphur, and oxygen compounds) fractions of crude oil and oily sludge.

How does Oilzapper Work?

There are five different bacterial strains that are immobilized and mixed with a carrier material such as powdered corncob. This mixture of five bacteria is called Oilzapper. Oilzapper feeds on hydrocarbon compounds present in crude oil and the hazardous hydrocarbon waste generated by oil refineries, known as Oil Sludge and converts them into harmless CO_2 and water. The Oilzapper is neatly packed into sterile polythene bags and sealed aseptically for safe transport. The shelf life of the product is three months at ambient temperature.

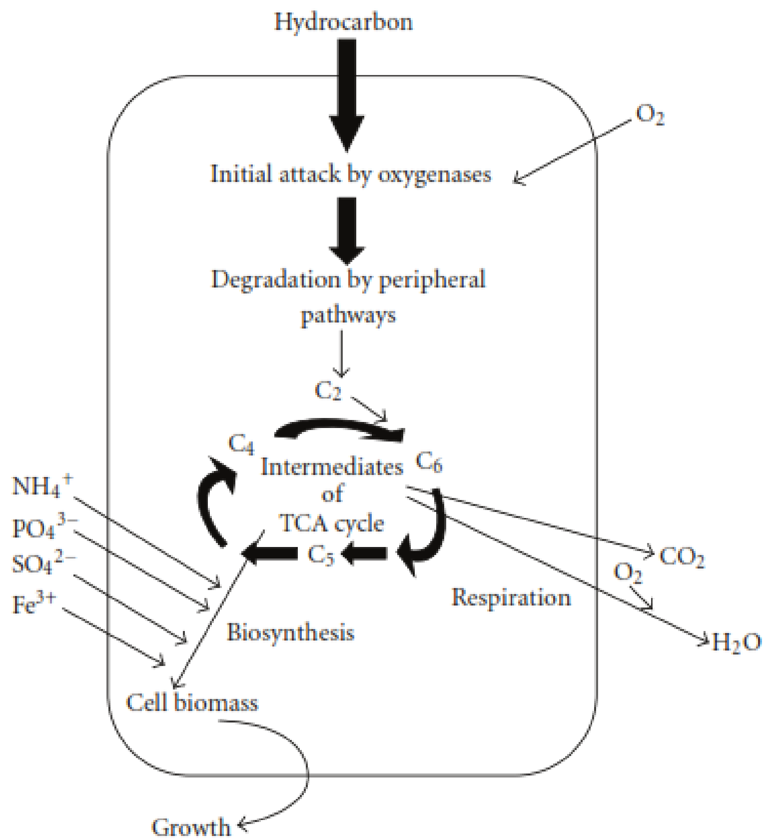


Figure 2: Main principle of aerobic degradation of hydrocarbons by microorganisms.

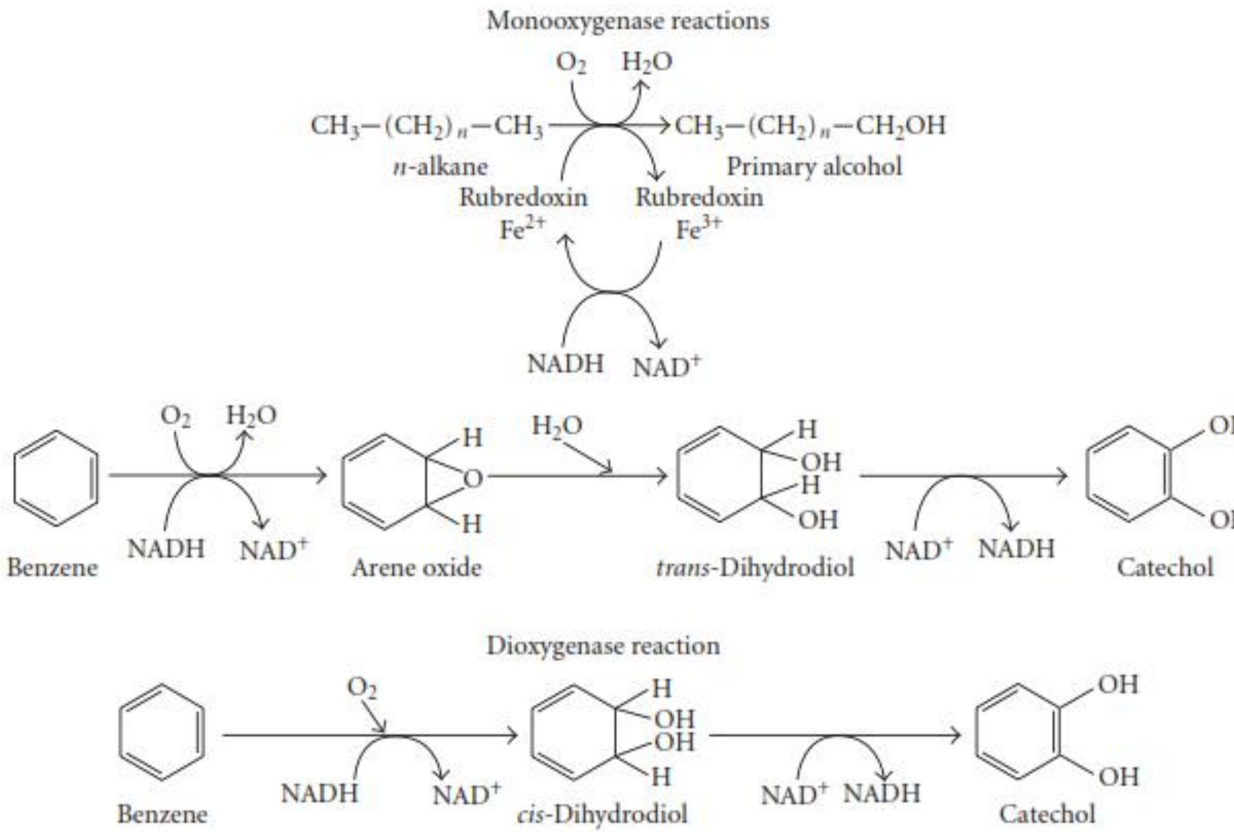


FIGURE 3: Enzymatic reactions involved in the processes of hydrocarbons degradation.

TABLE 1: Enzymes involved in biodegradation of petroleum hydrocarbons.

Enzymes	Substrates	Microorganisms
Soluble Methane Monooxygenases	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus* *Methylosinus* *Methylocystis* *Methylomonas* *Methylocella*
Particulate Methane Monooxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter* *Methylococcus,* *Methylocystis*
AlkB related Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	*Pseudomonas* *Burkholderia* *Rhodococcus,* *Mycobacterium*
Eukaryotic P450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltosa* *Candida tropicalis* *Yarrowia lipolytica*
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter* *Caulobacter* *Mycobacterium*
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*

At zero day (at time of start of bioremediation job) oil content in oil soaked soil was 14.50% and it reduced to 7.31% after two months of initiation of bioremediation.

Oil content in oil soaked soil was further reduced to 3.12% after three month and 1.70% after 3.5 months. After 4 months oil content is same soil was reduced to 0.58% (5800 ppm)

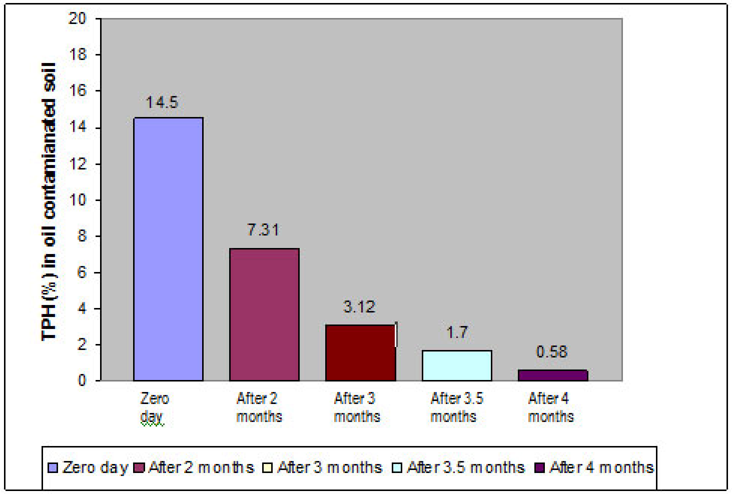


Figure 1: TPH (%) in the oil contaminated soil undertaken for bioremediation at secured bioremediation site situated at North Kadi, Gujarat.

Degradation of different fraction of TPH was also monitored. As shown in Figure 2 that most of alkane fraction were degraded in oil soaked soil in four months.

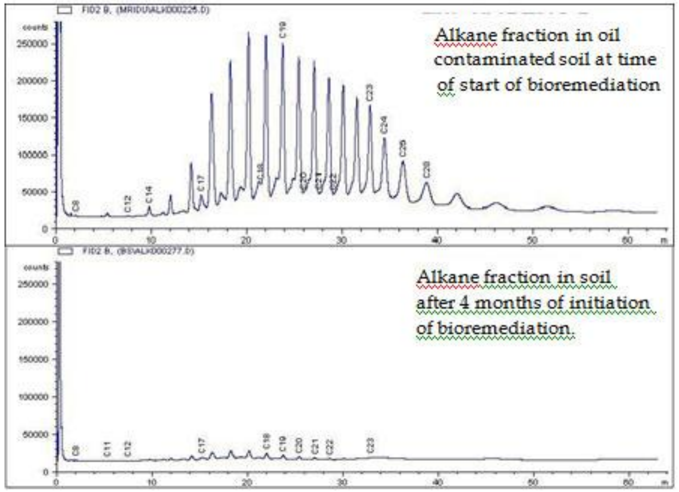


Figure 2: GC fingerprinting indicating the biodegradation of Alkane fraction of TPH in oil contaminated soil

Similarly aromatic fraction was also degraded in soaked soil in four months (Figure 3). After testing of level of oil content in oil soaked soil at bioremediation sites.

Fish toxicity of oil soaked soil was also tested. The fish toxicity test result revealed that in control soil (where only tilling was done) fish could not survive while in bioremediated soil fishes survived.

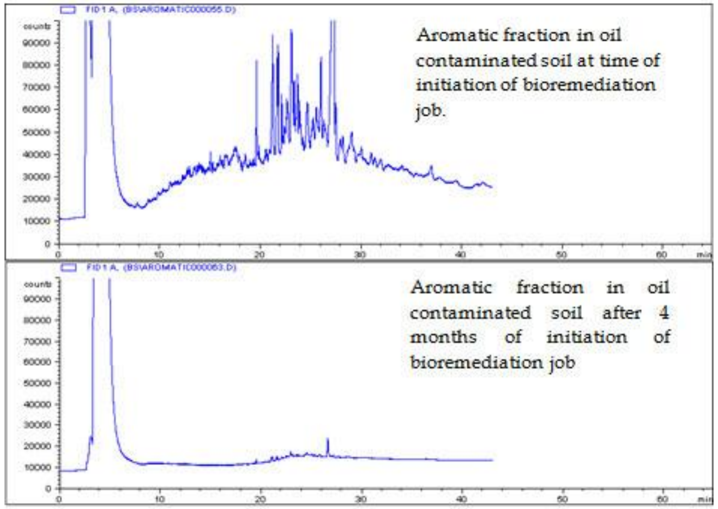


Figure 3: GC fingerprinting indicating the biodegradation of aromatic fraction of TPH in oil contaminated soil

Till date completed project of bioremediation using OILZAPPER

Bioremediation of Oily sludge & Contaminated Soil

- 9180 CUBIC METER Ex *situat* Oil India Ltd., Duliajan Asset, Assam
- 9000 MT *Insitu* MT at Oil India Ltd., Duliajan Asset, Assam
- 7445 MT at Ankleshwar Asset, Gujarat
- 1750 MT at Numaligarh Refinery, Assam
- 3200 cubic meter at Bharat Petroleum Corporation Ltd.
- 1000 MT at Reliance Industries Ltd. NAGOTHANE, Mumbai
- 34421.14 MT at ONGC Mehsana Asset, Gujarat
- 588.60 MT at ONGC Ankleshwar Asset, Gujarat
- 1000 MT at Reliance Industries Ltd. NAGOTHANE
- 300 MT at Reliance Industries Ltd. GADIMONGA
- 500 MT at Numaligarh Refinery, Assam
- 512 MT *Ex situ at* ONGC Mehsana Asset, Gujarat
- 5130 MT at ONGC Ahmedabad Asset, Gujarat
- 4500 MT at Oil India Ltd.Duliajan Asset, Assam
- 68 cubic meter at Hindustan Petroleum Corporation Ltd.
- 160 MT at SB Industries Engineering Pvt. Ltd. BPCL Refinery
- 550 M3 at HPCL-Bahadurgarh
- 4500 MT. at Oil India Ltd, Duliajan, Assam
- 11848 MT at Insitu ONGC Mehsana Asset
- 2500 MT at CTF Ankleshwar in ONGC Ankleshwar Asset, Gujarat
- 500 MT. at ONGC Agartala, Tripura
- 500 Kgs. At TATA Power Company Limited, Mumbai
- 3023 MT at Area-Ankleshwar in ONGC Ankleshwar Asset, Gujarat
- 17323 MT at North Santhal and Jotana ONGC Mehsana Asset
- 1000 MT at Reliance Industries Ltd-Gadimoga East Godavari, Andhra Pradesh
- 36604 MT Cleaning & Restoration at Farmers Land & Other sites in ONGC Ahmedabad Asset
- 8361 MT Line Cleaning & Bioremediation at ONGC Ahmedabad Asset
- 500 MT at Reliance Industries Ltd-Nagothane
- 22984 MT (Sludge Pits) at ONGC Nazira Asset Assam
- 15000 MT at Line Leakage Points in ONGC Mehsana Asset
- 14400 MT at Line Leakage Points in ONGC Mehsana Asset
- 13180 MT at Bechrajee, Lanwa, Balal, Nandasan, Santhal and NK Area of ONGC Mehsana Asset
- 3600 MT at Thol Village at ONGC, Mehsana Asset, Gujarat
- 18311 MT at various sites at Mehsana & Thol at ONGC Mehsana Asset, Gujarat
- 8046 MT at Oil India Ltd, Duliajan, Assam
- 12900 MT at South Santhal area of ONGC, Mehsana Asset, Gujarat
- 500 MT at ONGC, Uran, Maharashtra
- 27614 MT at ONGC, Ahmedabad Asset , Gujarat
- 150 MT at Kesanapalli (W) GGS, ONGC, Rajahmundry Asset, Andhra Pradesh
- 200 MT at Numaligarh Refinery, Assam
- 10680 MT at Sobhasan area of ONGC, Mehsana Asset, Gujarat
- 500 MT at CPF Gandhar, ONGC, Ankleshwar, Asset, Gujarat
- 3838 MT at various sites of ONGC, Assam Asset, Assam
- 500 MT at Reliance Industries Ltd., Gadimoga
- 500 MT at BG Exploration and Production India Ltd., Bhavnagar
- 1741 MT at RDS-GGS-2 in ONGC, Nazira Asset , Assam.

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- Details of the case study: http://www.otbl.co.in/Bioremediation.php

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2	Picture	Mother Earth Vs Capitalism https://lens.google.com/search
4	Picture	Delhi air pollution https://www.shutterstock.com/search/delhi-air-pollution
5	Picture 1	Fishermen clean up oil at an oil spill site near Dalian Port, Liaoning province July 27, 2010. REUTERS/Stringer https://www.businessinsider.com/china-water-pollution-photos-2014
5	Picture 2	Dead fish http://www.mi2g.com/cgi/mi2g/frameset.php?pageid=http%3A//www.mi2g.com/cgi/mi2g/press/030710.php
6	Picture	Land and water Slideshare.net
8	Picture	Soil pollution: a hidden reality, FAO, Room. 2018. https://www.fao.org/3/I9183EN/i9183en.pdf
17	Scheme	FAO and UNEP, Global assessment of soil pollution: Report. Rome. https://doi.org/10.4060/cb4894en
18	Picture 1	Kilauea volcano in Hawaii https: //wordlesstech.com/kilauea-volcano-in-hawaii/
18	Picture 2	Mouint Sinaburg. volcano bbc.org.
18	Picture 3	Fissure 8 erupts in lava hazard zone 1, May 5, 2018. (Photo credit: Bruce Houghton)

20	Picture	Potential interrelated pathways for soil-subsurface chemical contamination. Source: Yaron, Dror and Berkowitz, 2012
21	Picture 1	https://www.mining.com/web/top-producing-powder-river-basin-coal-mines-in-q214/top-producing-powder-river-basin-coal-mines-in-q214-2/
21	Picture 2	https://fountainfoundry.com/
23	Picture 1	https://deca.upc.edu/en/PhD_studies-research/phd/transport-engineering-infrastructures
23	Picture 2	https://www.youtube.com/watch?v=TdP_5gE11_o
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26	Picture 1	http://medievalmanuscriptsunlocked.blogspot.com/2015/
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26	Picture 3	Forbitten Tuna https://www.google.com/
28	Scheme	Soil pollution: a hidden reality.FAO, Room, 2018. https://www.fao.org/3/I9183EN/i9183en.pdf
31	Scheme 1	https://www.researchgate.net/publication/314132914_Thermal_Treatment_of_Hydrocarbon-Impacted_Soils_A_Review_of_Technology_Innovation_for_Sustainable_Remediation/figures?lo=1
31	Scheme 2	https://www.researchgate.net/publication/322369424_Impact_of_Hot_Pressing_Pressure_on_Medium_Density_Fiberboard_MDF_Performance/figures?lo=1

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69	Picture	Source: Pidlisniuk Valentina.
70	Scheme	Miscanthus in Ukraine, 2019. In: Roik, M., Sinchenko, V., Purkin, V., Kvak, V., Gumentik, M., et al. (Eds.), vol. 256. FOP Yamchinskiy Press, ISBN 978-617-7804- 11-5 (in Ukrainian) Kyiv, Ukraine
74	Picture	Source: Davis Lawrence
80	Picture 1	https://upload.wikimedia.org/wikipedia/commons/5/5d/Miscanthus_Bestand.JPG
80	Picture 2	http://www.elektro-wiegand.com/ele1.html
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80	Picture 4	https://www.grace-bbi.eu/crops/
81	Picture 1	https://www.bambushecken.de/chinaschilf-miscanthus-giganteus
81	Picture 2	https://www.bambushecken.de/chinaschilf-miscanthus-giganteus
82	Picture	https://groeneparadijs.nl/media/mf_webp/jpg/media/catalog/product/cache/4749daa9d7edd9f9838910af1a0d84eb/m/i/miscanthus_giganteus_1.webp
83	Picture	Source:Davis Lawrence
84	Picture	Source: Pidlisniuk Valentina
86	Picture	https://www.farmanddairy.com/news/miscanthus-crop-matures-producers-find-uses/154182.html
97	Scheme	Book: Soil pollution: a hidden reality https://www.fao.org/3/I9183EN/i9183en.pdf

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118-120	Schemes	http://www.otbl.co.in/Bioremediation.php