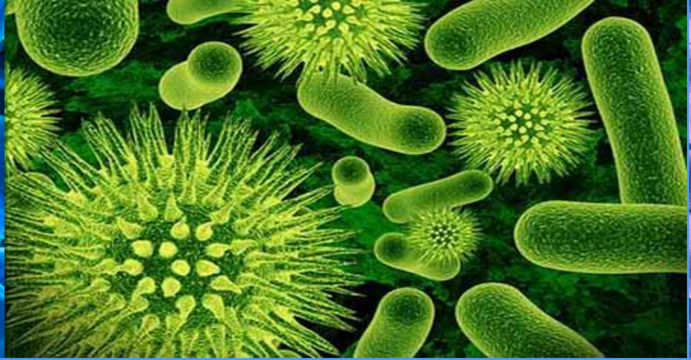
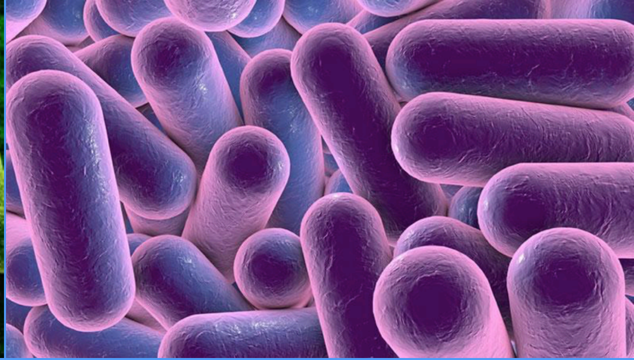
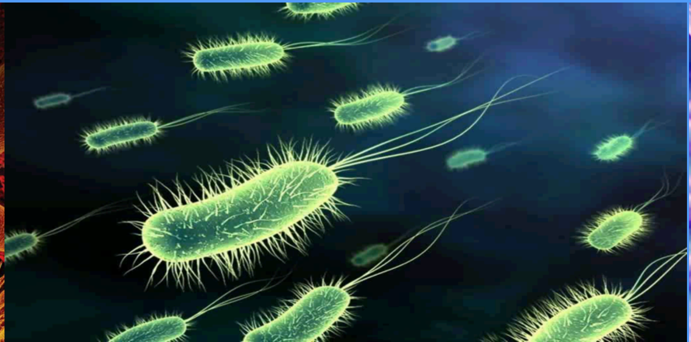




Biological Methods

Jerry Walsh, Jenna Bishop, Quinn Albertson, Shane Galloway, Carlos Rivera, Tom Devenney, Jeff Green


Physical Mechanisms

In Situ Methods

Permeable Reactive Barriers

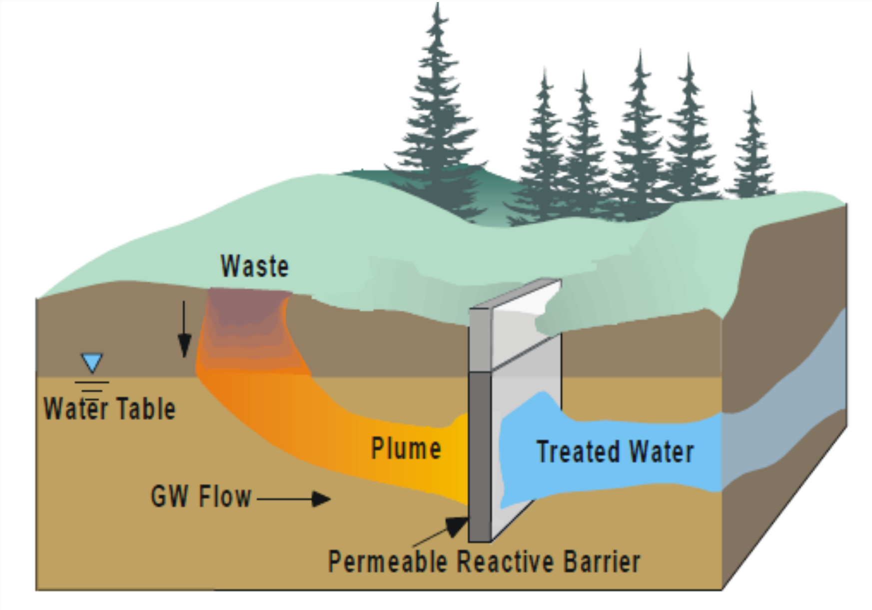
Biostimulation

Bioaugmentation

Phytoremediation

Permeable Reactive Barriers (Biobarriers)

- Semi-permeable reactive media in flow path
- Chemically or biologically-mediated reactions transform contaminants into non-toxic or immobile products
- Hydrocarbons need to be oxidized
- Chlorinated solvents and nitrate need to be reduced
- Microorganisms help mediate redox reactions to gain energy and materials for synthesis



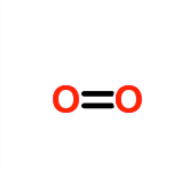
Reactive Media

Reactive Medium	Removal Mechanism	Contaminants Removed
Organic Material	Microbial sulfate reduction and precipitation	Acid mine drainage
Organic Material	Microbial nitrate reduction	Nitrate
Oxygen/Nitrate-Releasing Compounds	Microbial degradation	BTEX
Resting-State Microorganisms	Microbial cometabolism	Chlorinated aliphatics

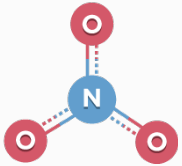
Oxidative Biodegradation

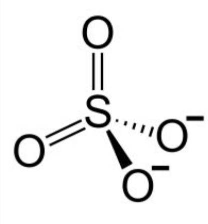
- To respire electrons removed from contaminants, need electron acceptors
- Aerobic methods → Molecular Oxygen
- Anaerobic methods → Nutrients (nitrate, sulfates, CO_2, ferric iron, metal oxides, etc)
- Aerobic methods are generally preferred to provide more energy to the microbes
- Biochemical oxygen demands (BOD's) often exceed available oxygen, so adding oxygen to the system can stimulate oxidative biodegradation

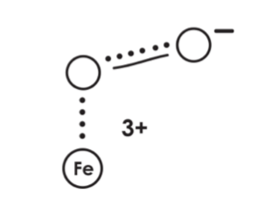
Aerobic:

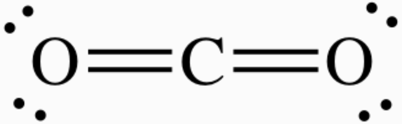


Anaerobic:



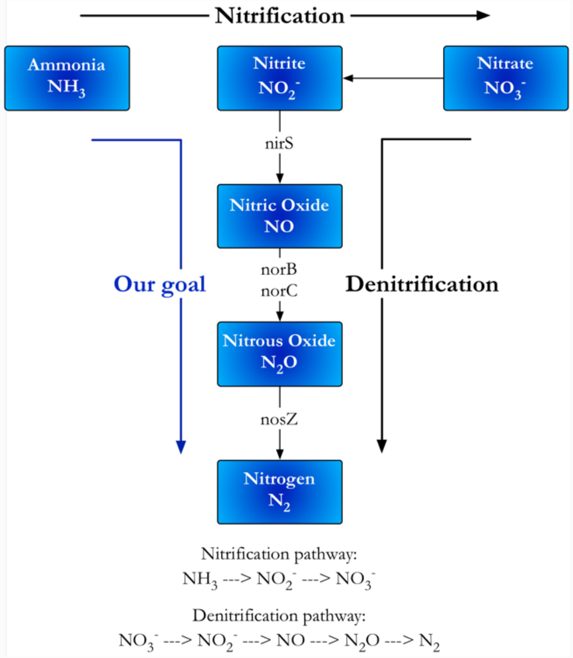






Reductive Biodegradation

- Electron donors are required, which anaerobic environments more abundantly produce
- Organic matter is a well-suited electron donor for this process
- This method works well for TCE, hexavalent chromium, sulfate, and nitrate contamination
- Heterotrophic denitrification to remove nitrate

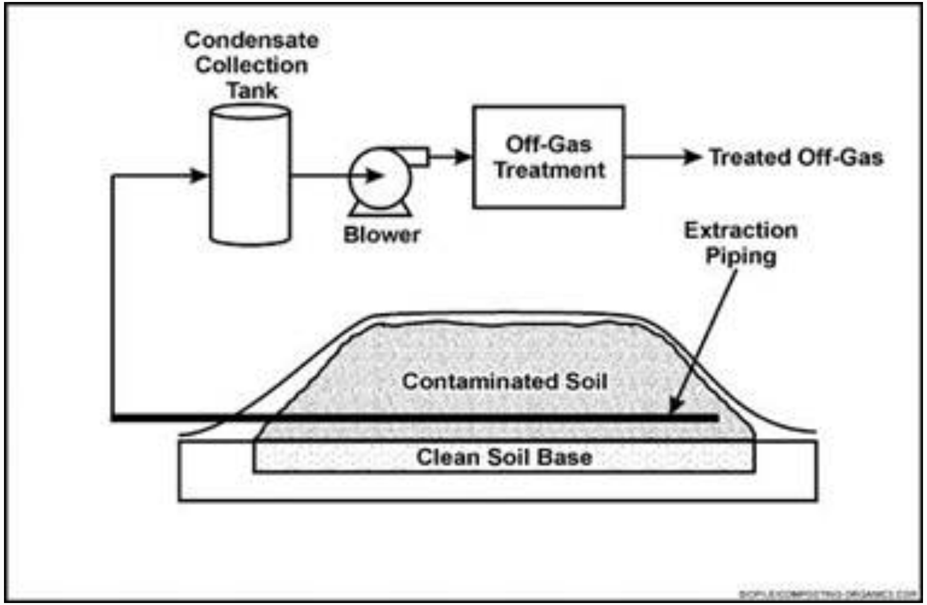


Biostimulation

- Modification of environment to stimulate existing bacteria capable of bioremediation.
- Only works if correct bacteria are present.
- Often done through use of phosphorous, nitrogen, carbon, and oxygen.
- Chemicals usually added through injection wells.
- EPA approved method.

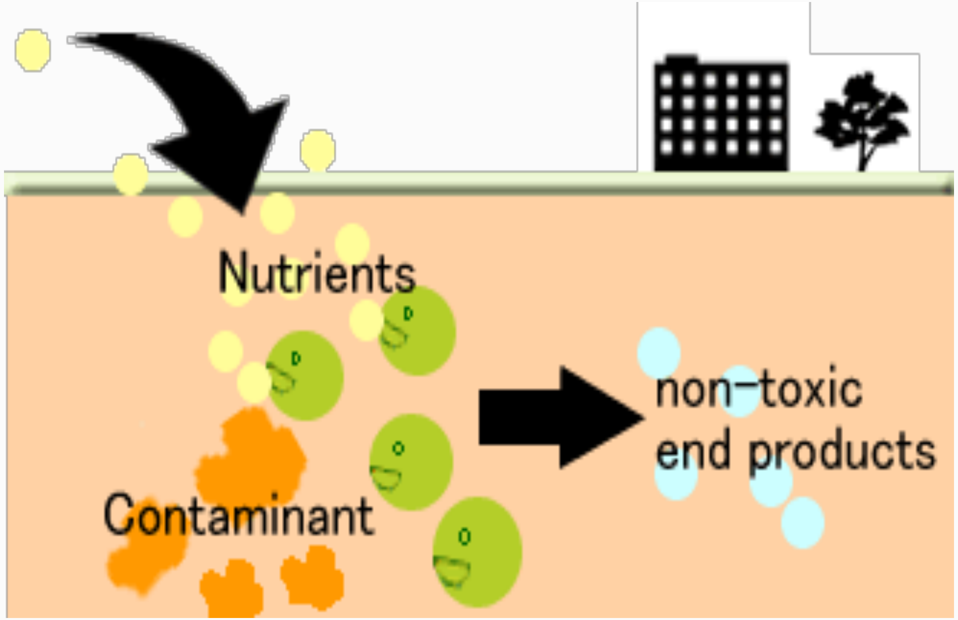
Advantages

- Low Costs
- Can be tailored to the specific site conditions.
- Approach can be passive, semi-passive, or active.
- Involves the use of already present microorganisms.
- Can be enhanced by bioaugmentation.



Disadvantages

- Method relies on easy dispersal of additives.
- Local geology can make it tough for microorganisms to receive necessary additives.
- Fractures in surface create pathways for the additives, preventing even dispersal.
- Weather can also disrupt spread of chemicals.

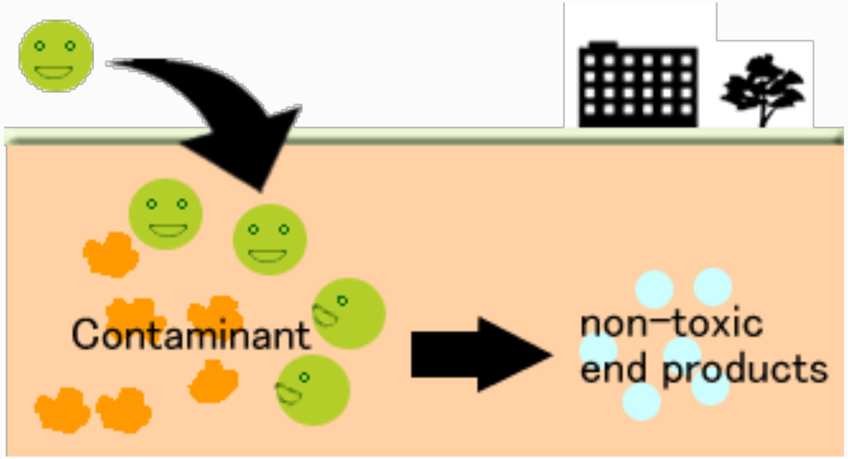


Bioaugmentation (Seeding)

- Addition of specific microbes to promote microbial activity

- Initial steps:
    - Feasibility studies
    - Tailoring a competent microbial formula

- Most common options:
    - Addition of a pre-adapted consortium
    - Addition of a pre-adapted pure strain
    - Introduction of a genetically-engineered bacteria
    - Addition of biodegradation-relevant genes packaged into a vector

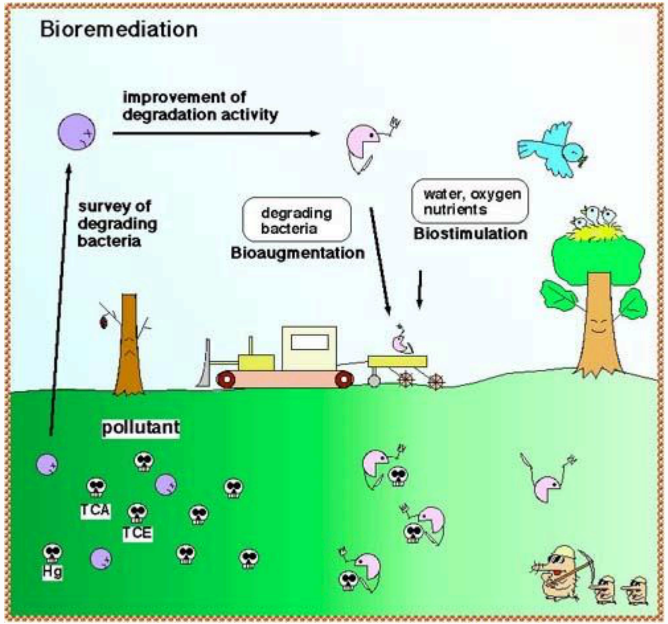


Bioaugmentation (continued)

- Sites contaminated with high metal concentrations and organic pollutants
    - Microbial consortium to mitigate both
        - Adds biodiversity to the site
- Strained field applications if no encapsulation or immobilization
    - Agar, agarose, alginate, gelatin, gellan gum, kappa-carrageenan, acrylate copolymers, polyurethane, polyvinyl alcohol

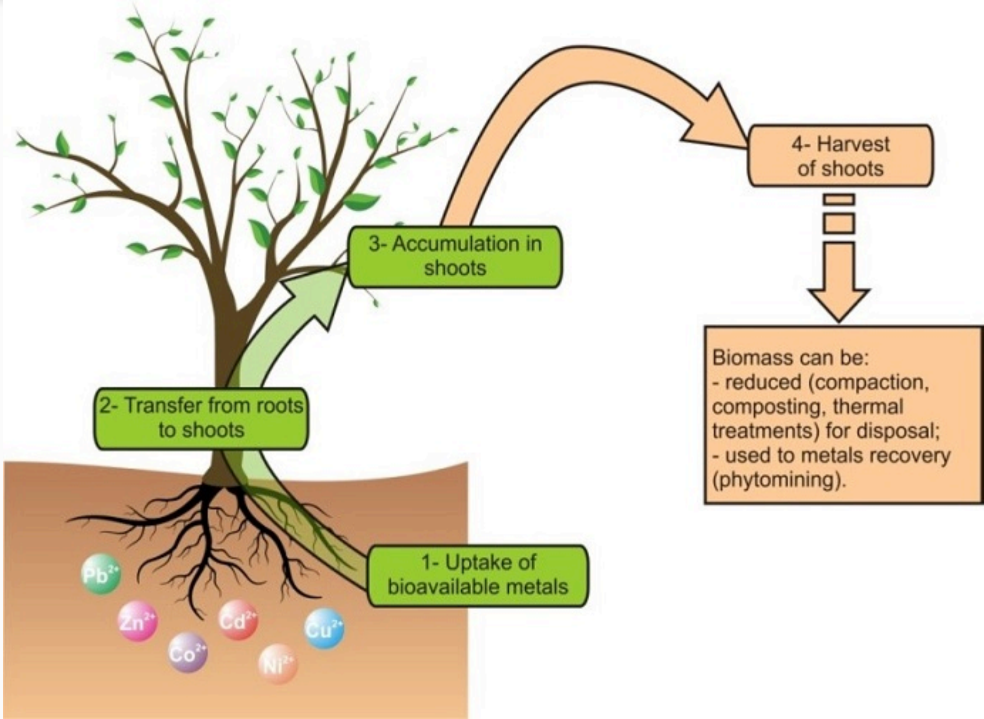
Biostimulation and Bioaugmentation

- These two methods are often used hand-in-hand
  - Provides the correct microbes to remediate
  - Provides these microbes with nutrients and environment needed to remediate
- Often a response to DNAPL's at the bottom of aquifers
- Example: enhanced reductive dechlorination



Phytoremediation

- Use of living green plants for removal or containment of hazardous chemicals in soil, air or water.
- Can be used in soil or static water.
- Uses plants abilities to metabolize various molecules in their tissues.
- Often used for the restoration of abandoned metal mines.



Hyperaccumulators

- Plants capable of growing in soils with very high metal concentrations.
- Absorb the metal through their roots, and concentrated in their tissues.
- Release back into environment in less toxic state.
- Due to differential gene expression.
- High concentrations of metals in their leaves deter herbivores.

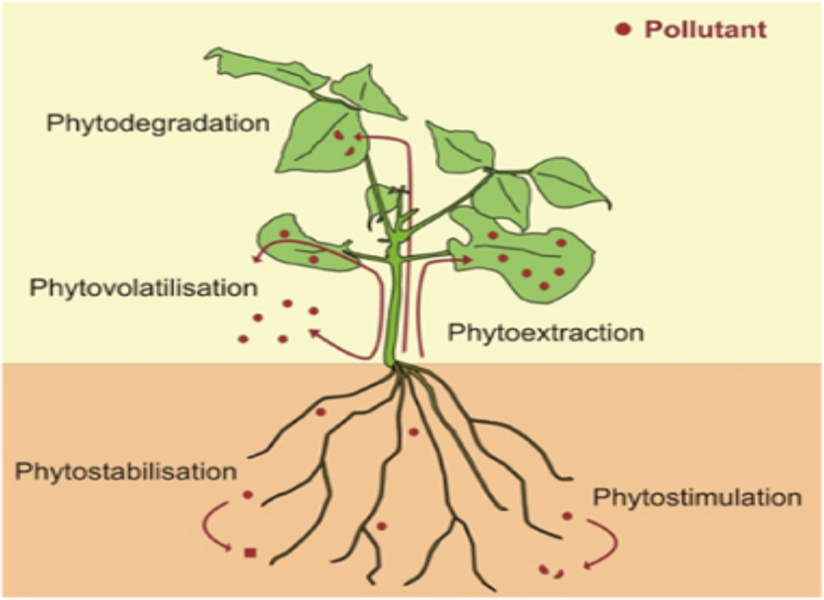


Figure 1 – Schematic representation of possible fates of pollutants during the phytoremediation processes. Figure adapted from (Pilon-Smits, 2005).

Advantages

- Lower costs than traditional methods.
- Easy to monitor and track plants
- Allows for possible recovery of valuable metals for reuse.
- Method is natural, minimizing disruptions to surrounding environment and ecosystems.

Disadvantages

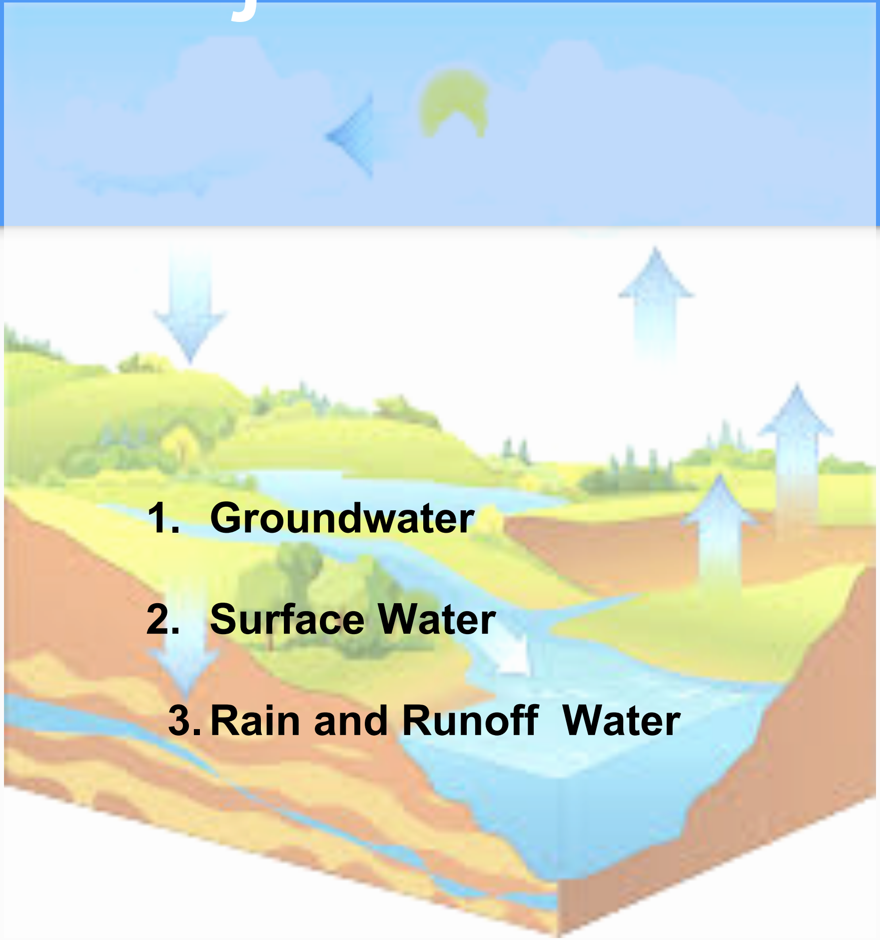
- Range is limited to surface area and depth of the roots.
- Long term commitment.
- Can not completely prevent leaching of chemicals into groundwater.
- If level of toxicity is too high, plants may not survive.
- Bioaccumulation in plants must be removed before food chain is interrupted.

Influencing Factors

Influencing Factors

	HIGH Production (Increased levels of remediation)	LOW Production (decreased levels of remediation)
Cation Exchange Capacity (CEC)(A.K.A Buffering Capacity)	CEC of soil < 0.25 (mEQ/kg)	CEC of soil < 100.00 (mEQ/kg)
Type of Aquifer	Confined (Limestone or Sandstone)	Unconfined
Type of soil	Silty Clay Loam	Well Sorted Sandy Gravel
pH Conditions	6 - 8	1 - 6
Permeability of soil (k)	10^{-7} - 10^{-11} (cm^2)	10^{-3} - 10^{-7} (cm^2)
Hydraulic Conductivity (K)	10^{-4} - 10^{-6} (cm/s)	5 - 10^{-1} (cm/s)

Major Sources & Sub-Sources



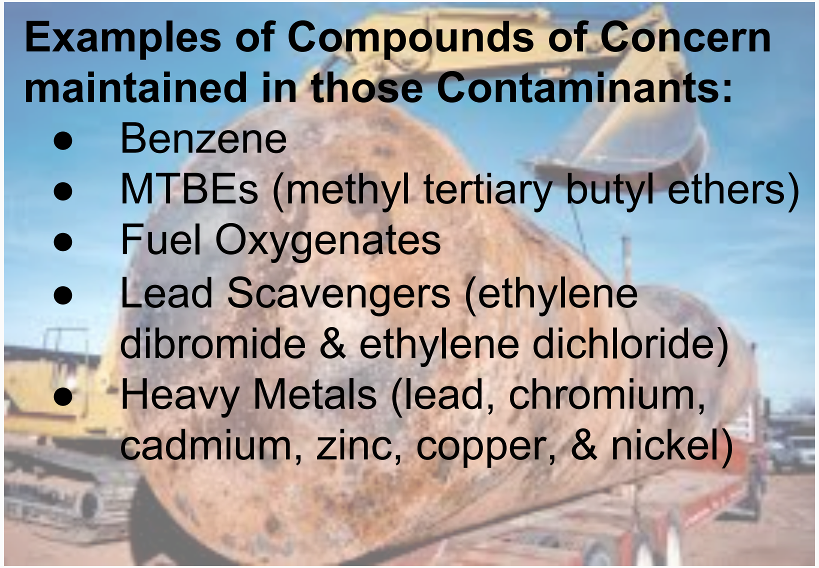
1. Groundwater
2. Surface Water
3. Rain and Runoff Water

I. Storage Tanks
II. Septic Systems
III. Uncontrolled Hazardous Waste
IV. Landfills
V. Chemical and Road Salt
VI. Atmospheric Contaminants

(Underground) Storage Tanks

General Potential Contaminants Residing in USTs:

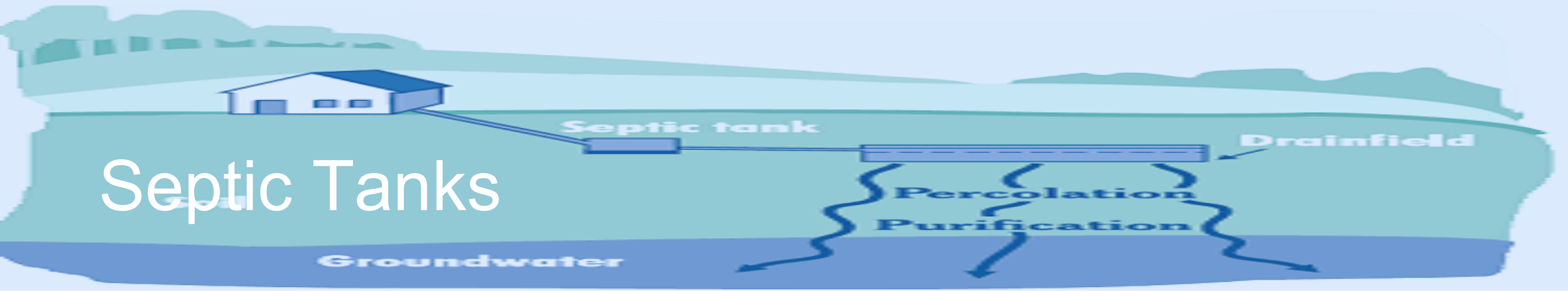
- Oils
- Chemicals
- Gasoline
- Petroleum
- Hazardous Substances



Examples of Compounds of Concern maintained in those Contaminants:

- Benzene
- MTBEs (methyl tertiary butyl ethers)
- Fuel Oxygenates
- Lead Scavengers (ethylene dibromide & ethylene dichloride)
- Heavy Metals (lead, chromium, cadmium, zinc, copper, & nickel)

Septic Tanks



General Potential Contaminants:

- Human & Animal Fecal Matter
- Human & Animal Urine
- Other Human & Animal Extrections
- Insect & Microbial Extrections
- Vectors (disease transmitting insects)
- Miscellaneous Humus (dead org. matter)
- Detergents
- Oils
- Household and Industrial Septic System Cleaners

Examples of Compounds & Microbial Life that reside in the contaminant:

- Bacteria (Tetanus, E. coli, L. monocytogenes, S. pneumoniae)
- Viruses (Hepatitis A,
- Fungi (Meningitis)
- Parasites (Cryptosporidium, Giardia, Meningitis)
- Prions
- Industrial Solvents (Trichloroethane or Methylene chloride)

Uncontrolled Hazardous Waste

Major Potential Contaminants:

- Corrosive Acids & Bases
- Toxins & Highly
- Concentrated Toxins
- Compressed Gases
- Cryogens
- Pyrophorics
- Water reactives
- Explosives

Examples of Compounds Maintained in the Contaminants:

- Alkali Metals
- Nitrogen Dioxide
- Nitric Acid
- Sulfuric Acid
- Hydrogen Sulfide
- Organic Mercury Compounds
- Iodine
- Ammonium Nitrate
- Alcohols (methanol, ethanol)
- Chromic Acid
- Antifreeze
- Ozone

Landfills

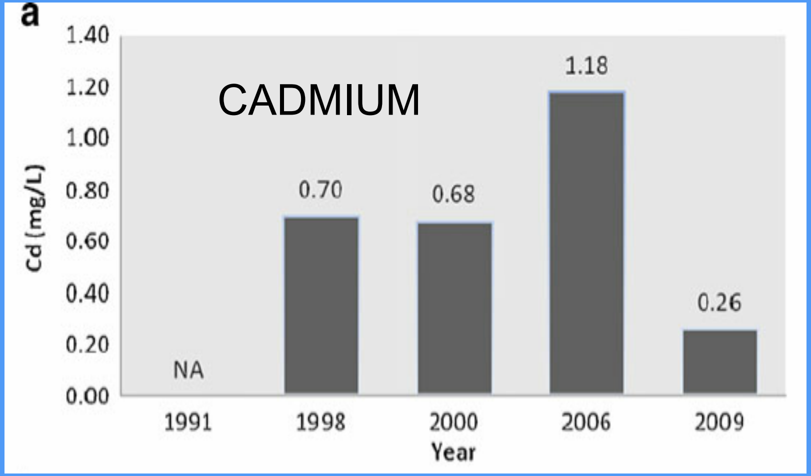
General Potential Contaminants:

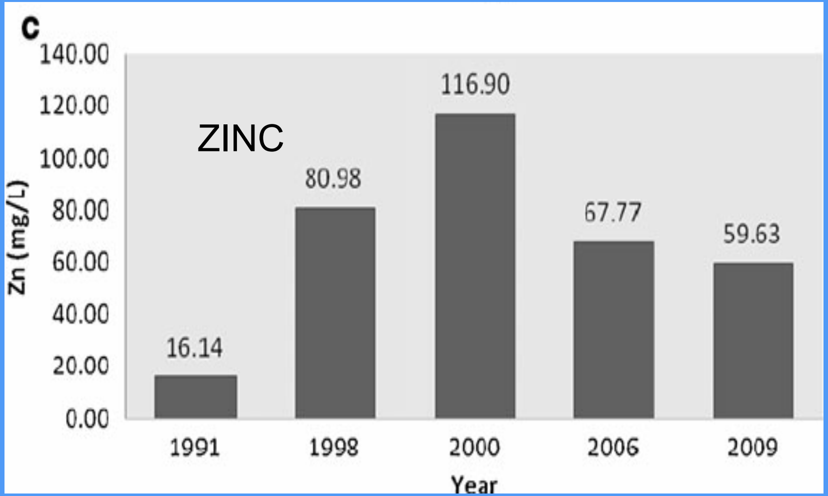
- Leachates
- Methane Gas
- Similar contaminants pertaining to USTs, Septic Tanks, and Uncontrolled Hazardous Waste.

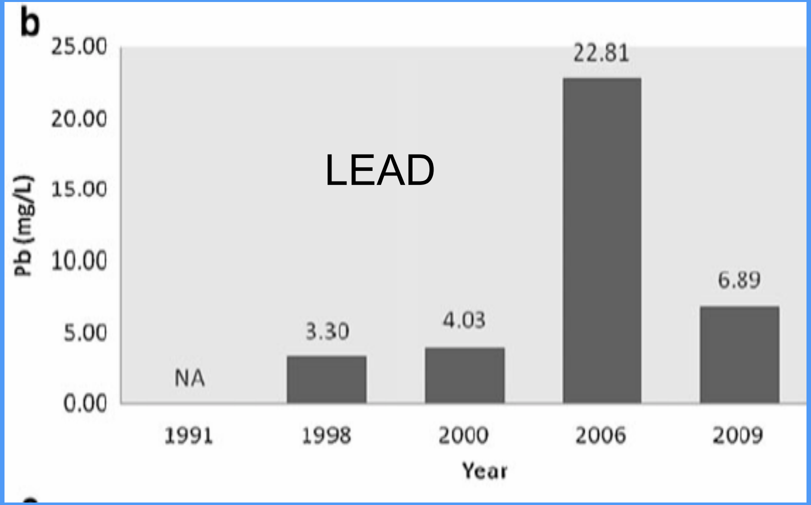
Chemicals and Road Salts

General Potential Contaminants:

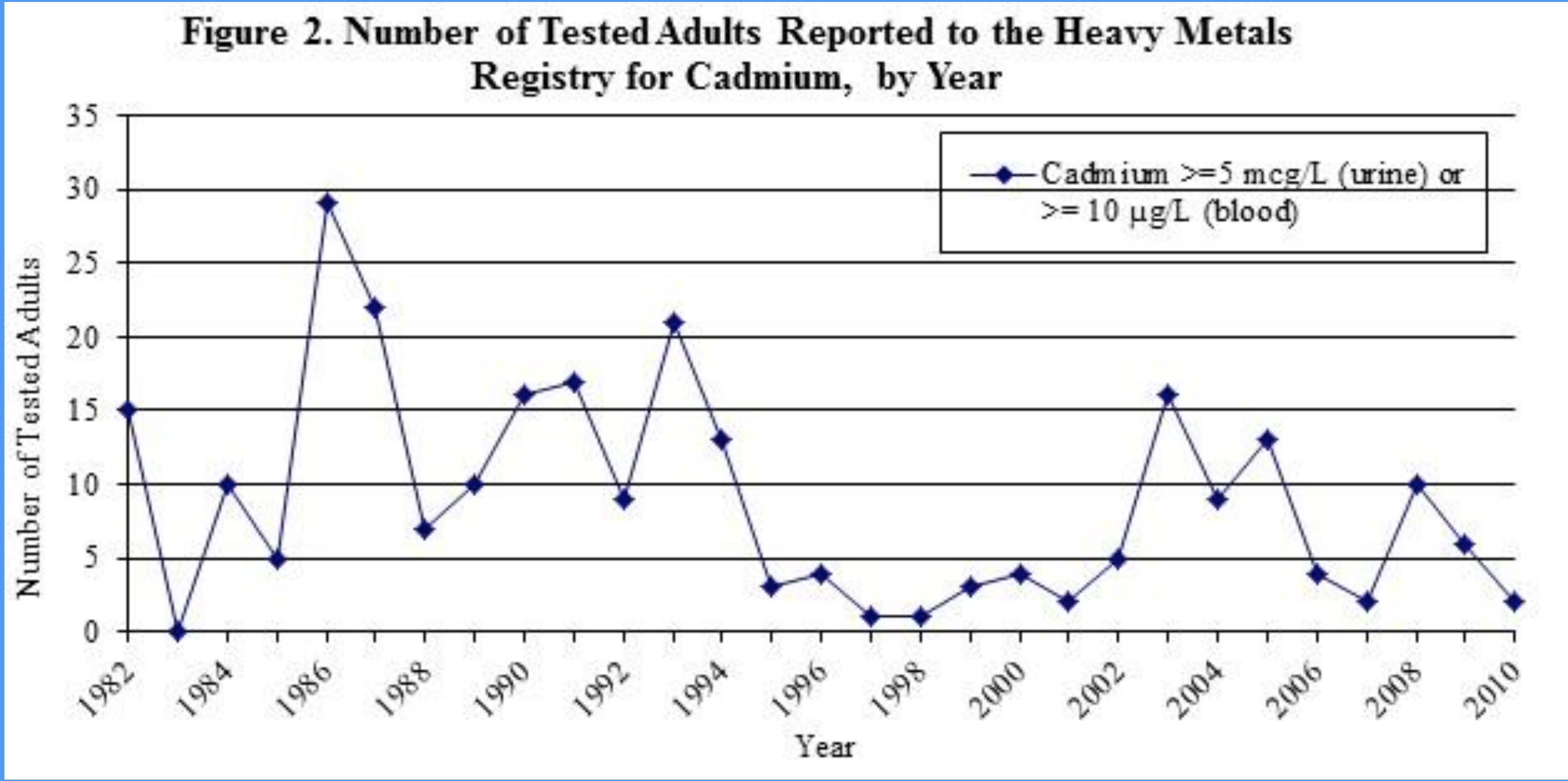
Heavy Metal Contamination vs. Time







Heavy Metal Contamination vs. Time



Field implementation

Sampling/Testing to determine treatment type

- Direct detection by PCR
- Microcosm testing - allows sample to grow on its own in a lab
  - ID types of organisms present
  - Helps determine the rate at which the bacteria digest
  - Shows extent of current digestion and amounts of nutrients, oxygen

Biostimulation

- Bioventing - increases oxygen concentration in the vadose zone
  - Air, oxygenated water, hydrogen peroxide, or nitrate
  - Can be injection or withdrawal
  - Passive barometric flow can be induced or mechanical blower
  - Monitoring wells for movement of the plume

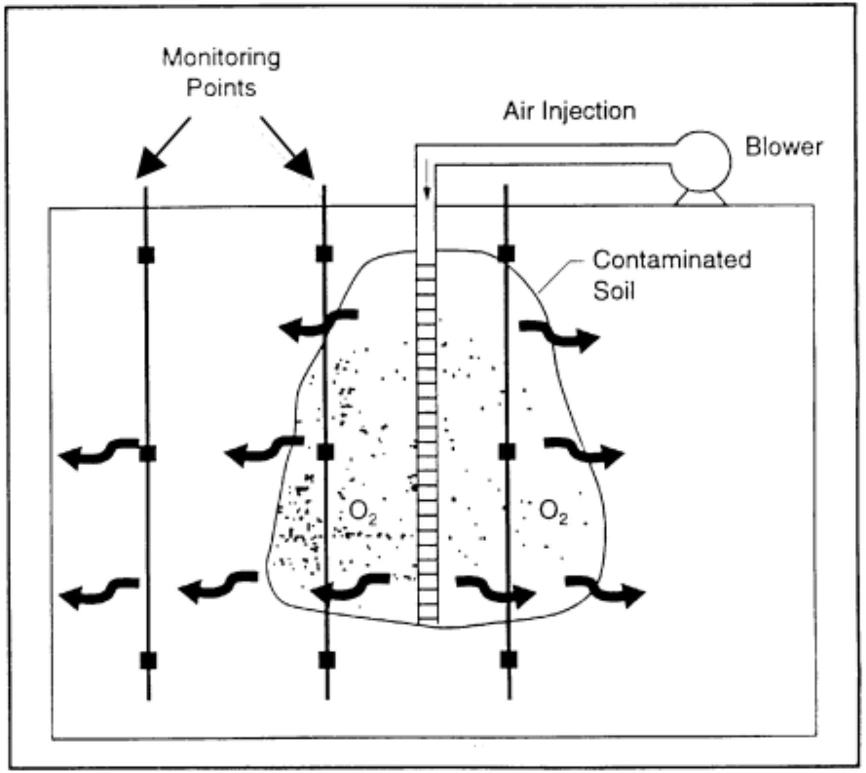


Figure 3-2. Typical bioventing system (AFCEE, 1994).

http://www.publications.usace.army.mil/Portals/76/Publications/EngineerManuals/EM_1110-1-4001.pdf?ver=2013-09-04-072852-030

Bioaugmentation

Introduces new microorganisms to the environment for biodegradation

- Dehalococcoides are the most common - remove halogens from contaminants
    - Usually a mixture of multiple types of bacteria
    - Ex. - Bachman Road culture and Pinellas culture

Also done by well injection, or if soils are shallow, spray irrigation

Usually a dose of electron donors is added first, then the culture

- Can be continuous or batch

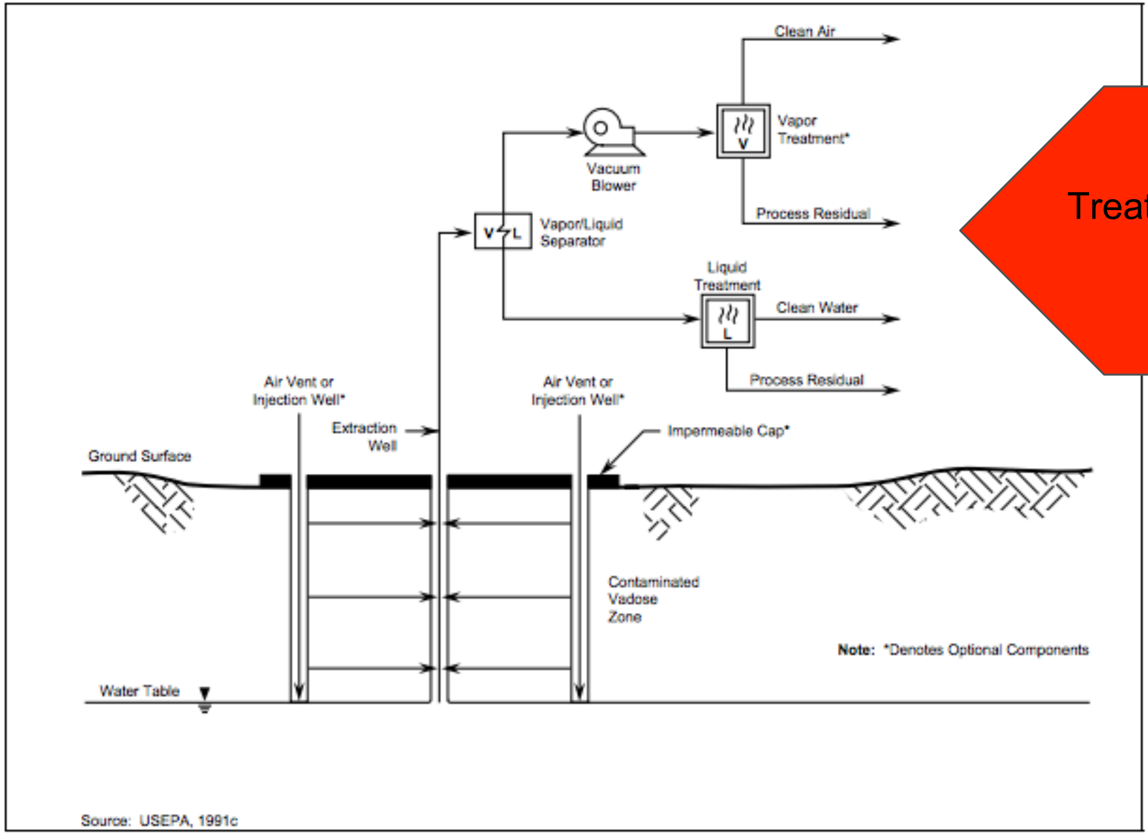


Figure 3-1. Generic soil vapor extraction system.

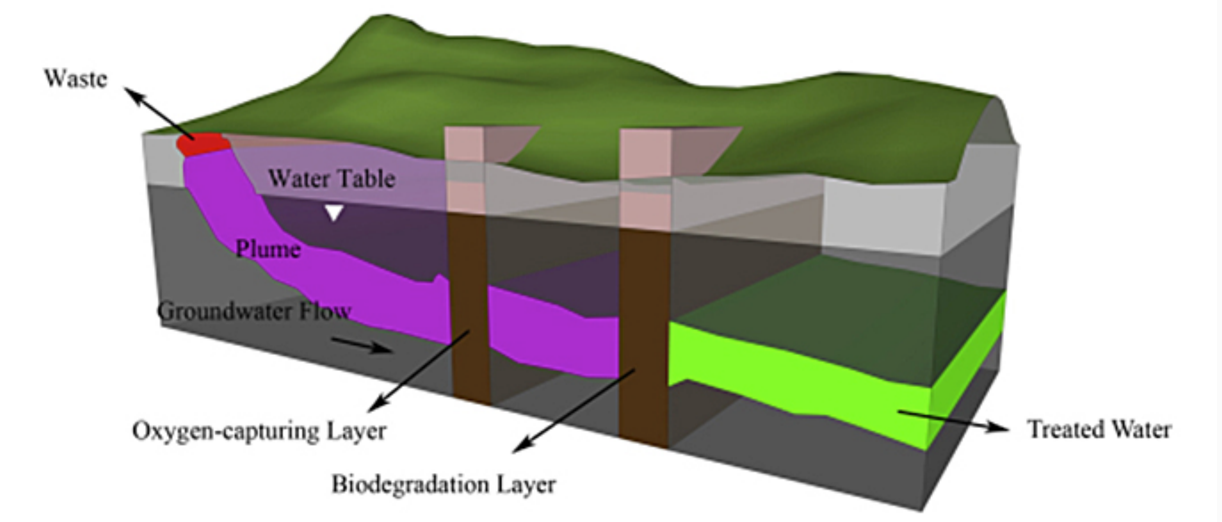
Treatment of removed gas

http://www.publications.usace.army.mil/Portals/76/Publications/EngineerManuals/EM_1110-1-4001.pdf?ver=2013-09-04-072852-030

Biobarriers

Another way to apply biostimulants or bacterial cultures

- Minimizes movement of contaminant
- Installed by digging a large trench
- Usually no more than 50 feet



http://vertexenvironmental.ca/wp-content/uploads/2015/10/PRB-biobarrier-nitrate-treatment_Web.jpg

Installment of biobarriers

Sheet and pile (traditional)

- Uses an excavator
- Can achieve depths up to 200 feet

Installment cont’d

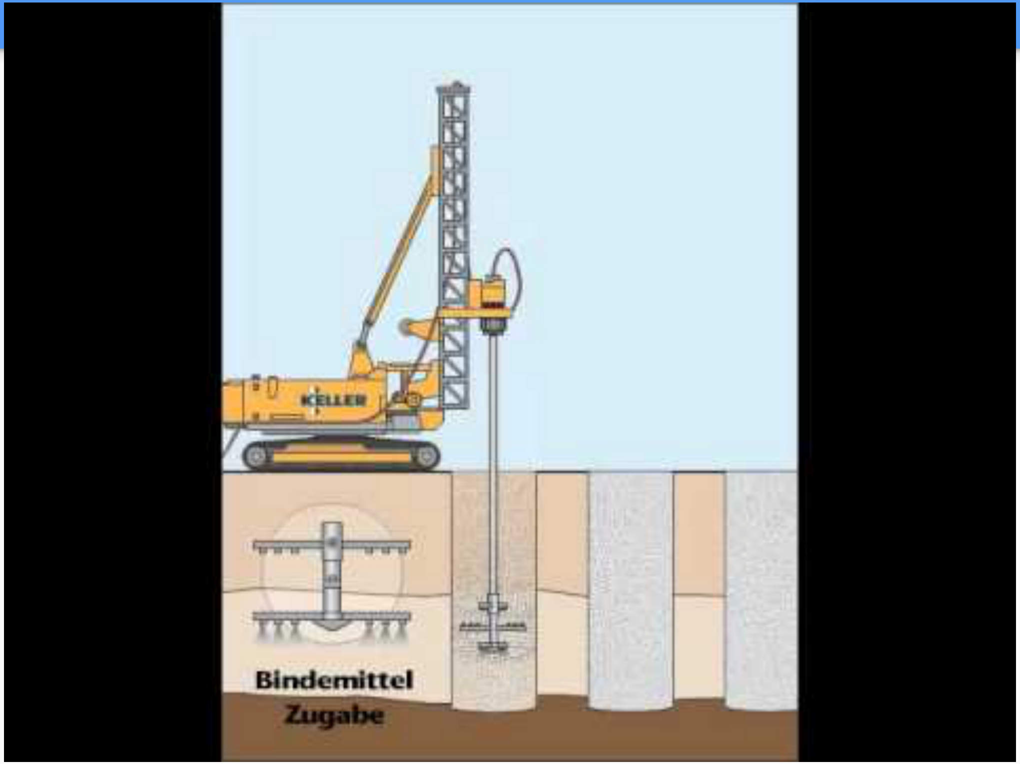
Continuous/one-pass trenching

- 12 to 36 - inch wide hole
- 20 - 50 feet deep
- Continuous means refill happens simultaneously

Installment cont'd

Deep soil mixing

- Virtually any depth



Survival of the cultures

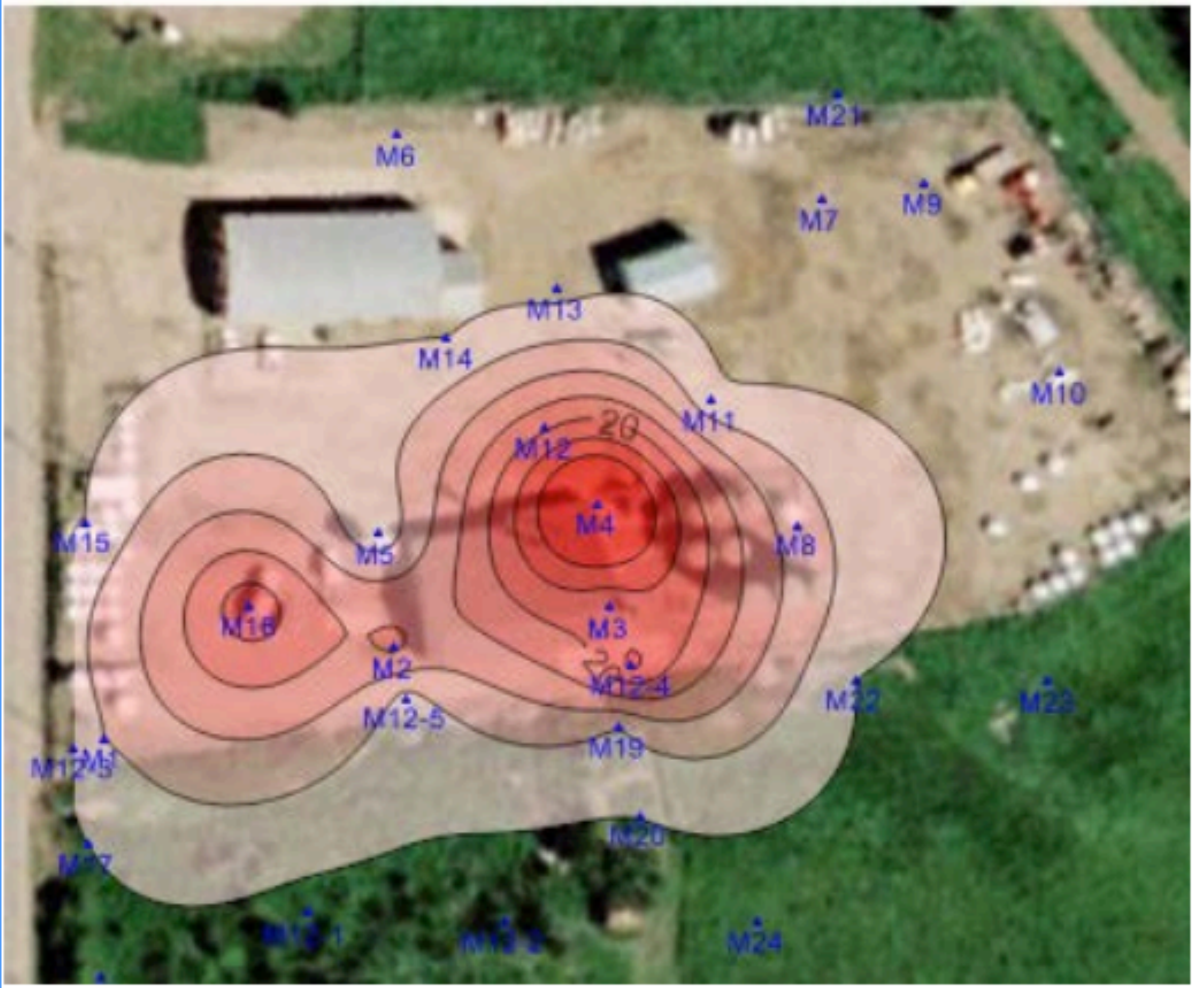
- Once installed, these require minimal maintenance
- Non-native bacteria are often out-competed by endogenous cultures
    - Can also be eaten by protists, especially if there are multiple injections
- Bacteria that use a different source of food than native species can fare better

Demonstration level

Permeable Reactive Barrier for Denitrification

- System put in by Pinter & Associates
- Site in Northern Alberta at an Active Fertilizer distribution facility
- Nitrate plume traveling south

Plume


M21
M6
M7
M9
M13
M14
M10
M11
20
M12
M4
M15
M5
M8
M16
M3
M2
M12-4
M22
M23
M12-5
M19
M20
M24

Denitrification

- Need organic carbon
- Produces alkalinity
- Must have anaerobic conditions

PRB Design

- Pine wood chips used as the carbon source, last for 25+ years
- PRB hyrdraulic conductivity higher than soils
- Water table level is at an average of 0.8 meters below ground level
- Placed the PRB south of the facility
- Helped the nearby landowner by:
    - Improving drainage of area
    - Reclaimed about an acre of land

Construction

JUNE 2012

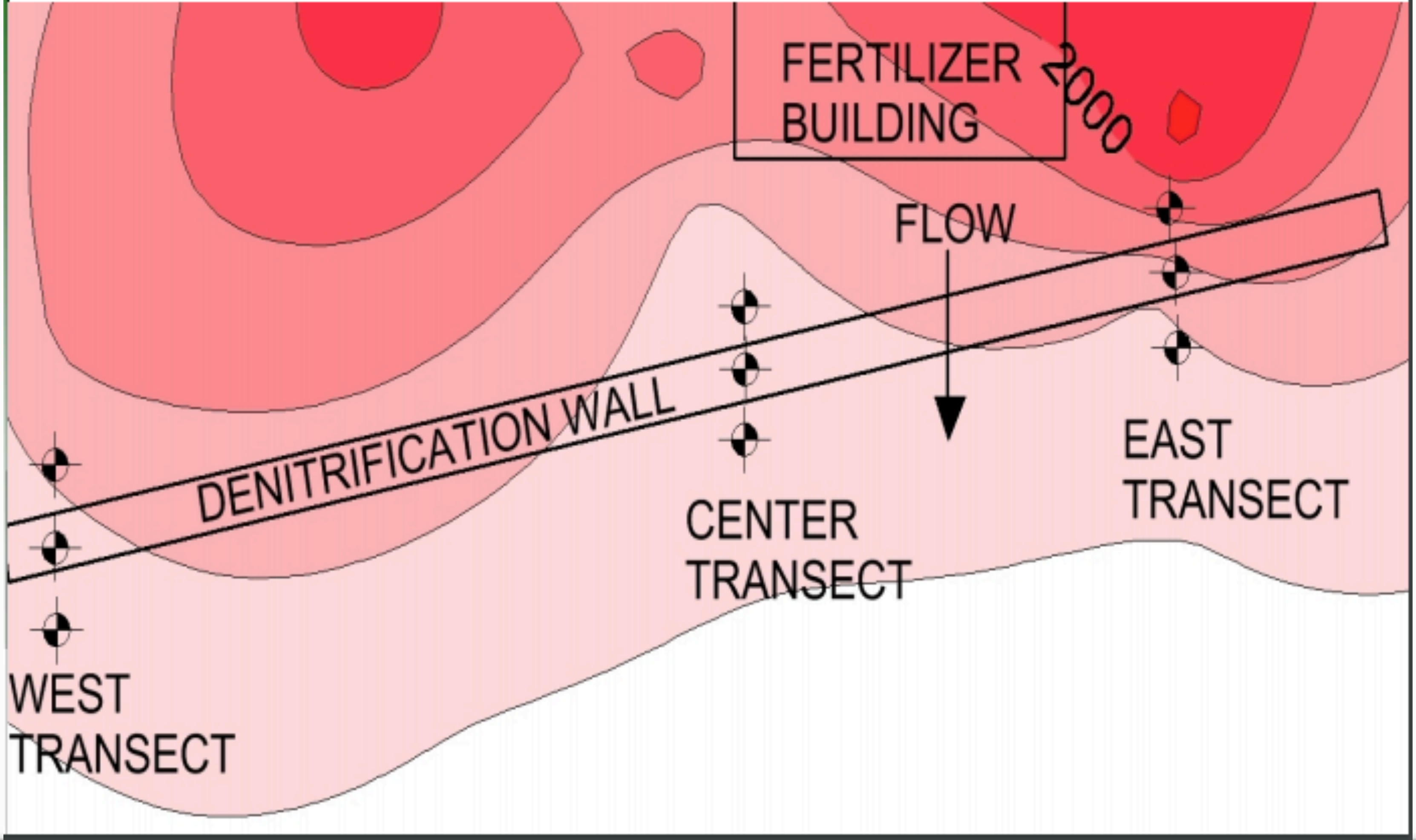
Took about a month to complete

Post-Construction

May 2013

Post-Construction

August 2013


FERTILIZER
BUILDING
2000
FLOW
DENITRIFICATION WALL
CENTER
TRANSECT
EAST
TRANSECT
WEST
TRANSECT

Results

Results

- Average nitrate reduction of 95%
- Nitrate concentrations have not shown reduction yet downstream due to the retention time of the PRB
- Sulphate reduction of 85% in some areas

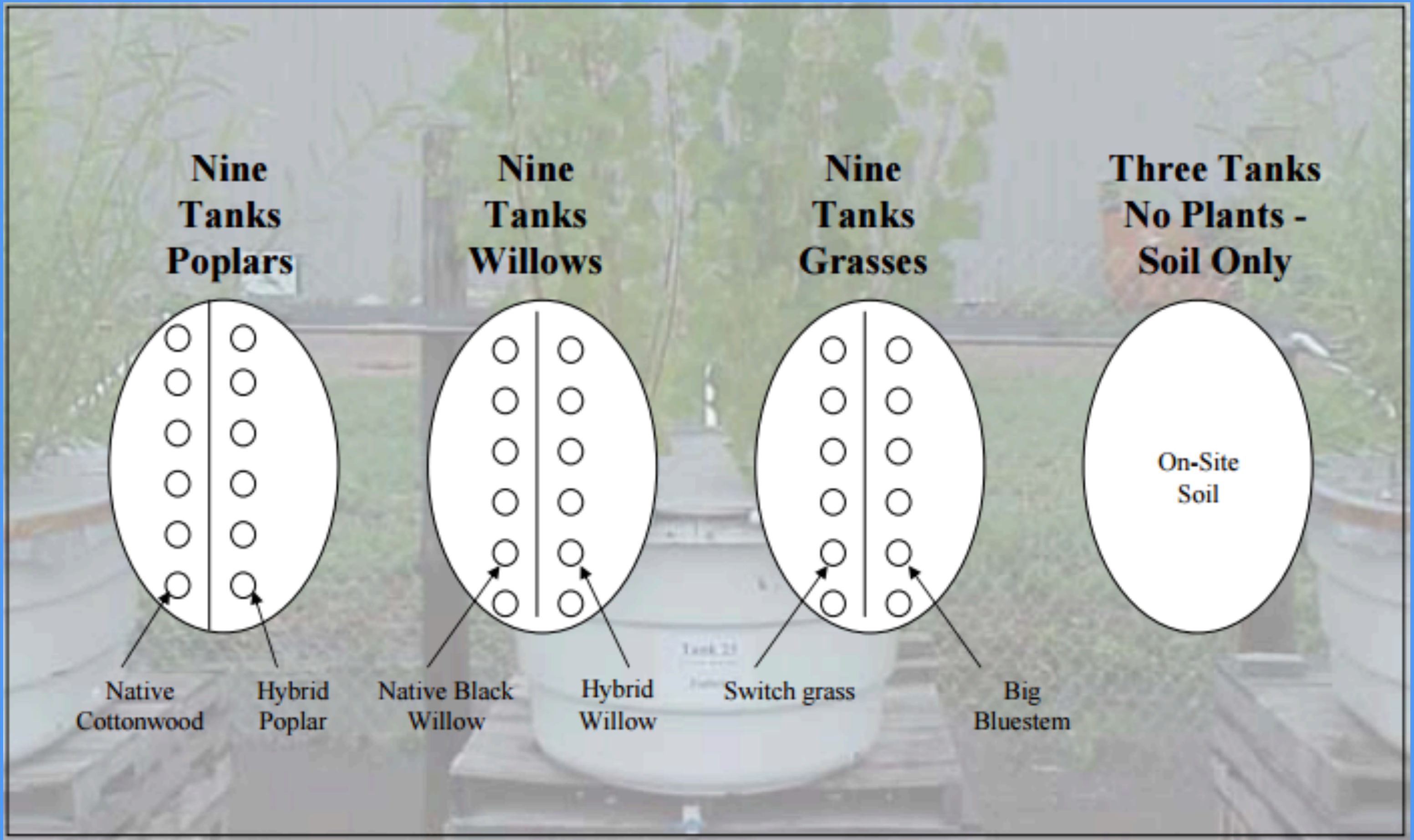
Phytoremediation Case Study

- Performed by SEH in 2004
- Looked at use of phytoremediation to reduce VOCs in the root zone
- Evapotranspiration of the volatiles
- Phytoremediation used for:
    - Hydraulic uptake and control
    - Water quality improvement
    - Develops a beneficial natural resource

Phase I

- Examine the ability of the plants to improve hydraulic control and removal of VOCs
- Test hybrid and natural species of poplars, willows, and grasses
- Determine which is the best plant to achieve the goal

Phase I


Nine Tanks Poplars
Nine Tanks Willows
Nine Tanks Grasses
Three Tanks No Plants - Soil Only
On-Site Soil
Native Cottonwood
Hybrid Poplar
Native Black Willow
Hybrid Willow
Switch grass
Big Bluestem

Phase I

- Inspect the plants
- Water balance 3 times a week
- Sampling and analysis to look at VOC reduction
- Measure the plant height and diameter

Phase II and III

- Create conditions more like what field conditions are like
- Get more data on uptake and VOC reduction
- Side by side tank study and in ground plantation
- Same operations as Phase I

Results

- Excellent plant growth
- Low mortality
- Effective hydraulic control
- VOC removal or reduction was found to be effective
- Improved the area through use of natural resources

Recommendations

- Expand the in-ground treatability study with the established plants
- Start a full scale implementation of this practice

Applicability and limitations

Applicability

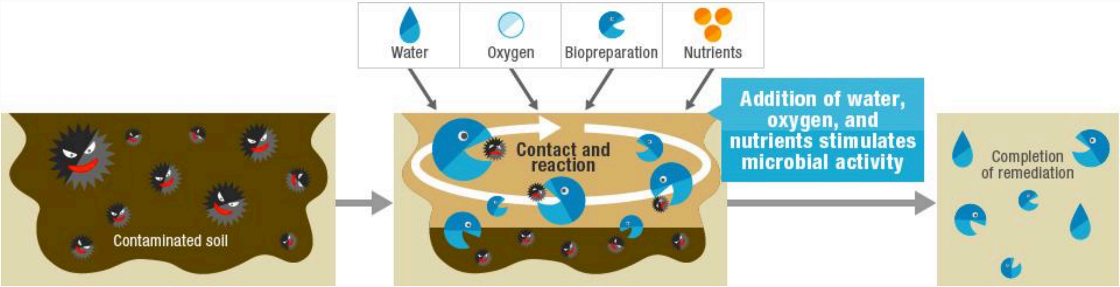
- pH
- Presence of Metals
- Permeability
- Moisture Content
- Temperature
- Heterogeneity of Soil
- Presence of peroxide
- Inorganics present
- Hydraulic Conductivity

Parameters	Condition required for microbial activity
Soil moisture	25–28% of water holding capacity
Soil pH	5.5–8.8
Oxygen content	Aerobic, minimum air-filled pore space of 10%
Nutrient content	N and p for microbial growth
Temperature (°C)	15–45
Contaminants	Not too toxic
Heavy metals	Total content 2000 ppm
Type of soil	Low clay or silt content

Applicability Continued

Aerobic vs. Anaerobic

- Heterogeneity of soil
- Free Phase or aqueous phase
- Location of contaminant
- Nutrient Demands
- Hydraulic Conductivity
- Type of Contamination
- Concentration of Contamination



Electron Acceptor Condition

Compound(s)	Aerobic	Anaerobic
Acetone	1	1
BTEX	1	2 to 4
PAH's	1	3 to 4
PCB's		
highly substituted	4	2
minimally substituted	2	4
Chlorinated ethenes		
PCE	4	1 to 2
TCE	3	1 to 2
DCEs	3	2 to 3
Vinyl chloride	1 to 2	3 to 4

1 Highly biodegradable
2 Moderately biodegradable
3 Slow biodegradation
4 Not biodegraded

Limitations of Anaerobic

- Timeframe
- Intermediate transformation products may be toxic
- Remediation of DNAPL Sources
- Decline of water quality

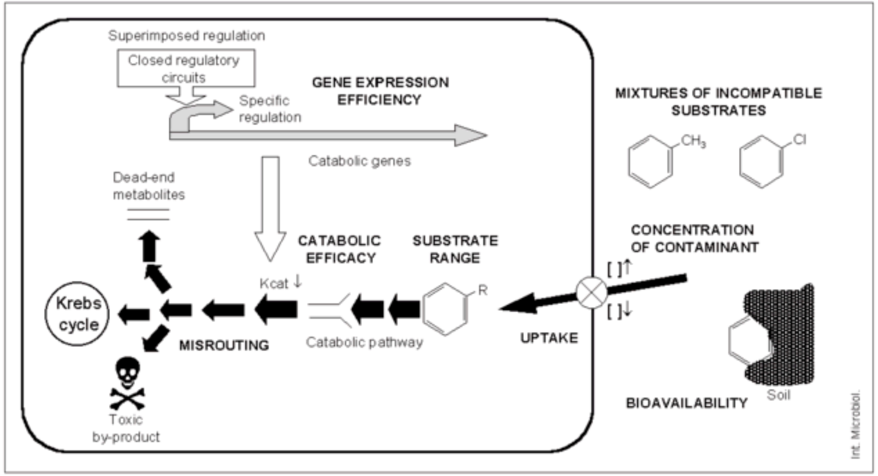
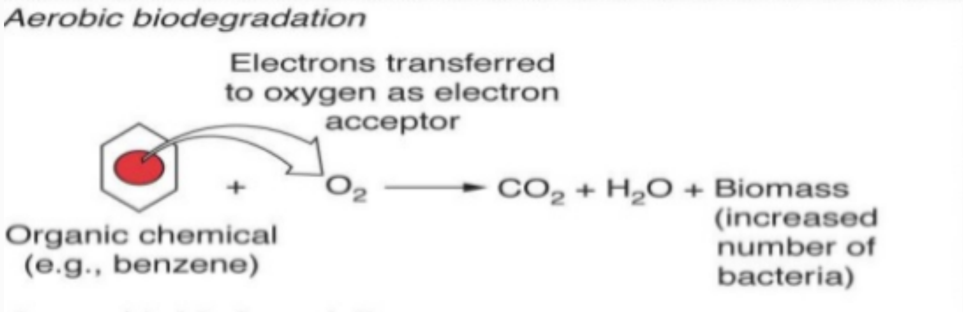


Fig. 5. Potential limitations of biological treatment approaches. The major problems that challenge biocatalyst performance are indicated by capital letters. **Gray arrows**, genes; **black arrows**, proteins.

Limitations of Aerobic

- Timeframe
- Change aquifer geochemistry
- Long term monitoring
- DNAPL Sources
- Nutrient/ oxygen injection may be required



Cost and availability

Availability

- This is highly dependant on the geographical location, geology, and contaminant chemistry.
- This method is most successful when it is paired with other remediation techniques.
- Companies specialize in biological remediation, but also the methods that are most effectively paired with this method.
- These companies also focus on wastewater and soil treatment because of the similarities in the biological analysis.

Cost Factors

- Area of Contamination
- Type of Contaminants
- Depth of Contamination
- Population Density
- Duration of Remediation
- Construction Required
- Pilot Testing

Cost: Biological Treatment Analysis

Annual Cost per GPM treated: $5,750

Annual Cost per Extraction Well: $87,560

The report that was used for this data also concluded that biological treatment of the contaminant was never the only method employed for that site.

Cost: Ar

Williams Property
Swainton, Middle Township, NJ (Region 2)
CERCLIS ID NJD980529945

Contact Information

RPM Ferdinand Cataneo 290 Broadway New York City, NY 10007-1866 212-637-4428 (phone) 212-637-4393 (fax) cataneo.fred@epa.gov	State Regulator Steve Wohleb NJDEP P.O. Box 413 Trenton, NJ 08625-0413 609-633-3970 (phone) 609-292-1975 (fax) swohleb@dep.state.nj.us	Contractor Richard Talbot TurnKey Env. Services, Inc 24 South Newton Street Road, Suite 1B Newton Square, PA 19073 610-356-3790 (phone) 610-356-4780 (fax) TurnKeyEnv@aol.com

System Information and Data

Type of Fund-lead Site:	State-lead w/ Fund $	Number of extraction wells:	2
Date original ROD was signed:	9/29/87	Date of construction completion:	1/1995
Date of last modification to ROD:		Date of operational and functional:	1/1995
Type of ROD:	Final	Expected date of turnover to state:	1/2001
Status of P&T system:	Operational	Expected date of completion:	12/2002
Primary goal of system:	Restoration	Approximate downtime per year:	0 weeks
Presence of NAPLs	Not present	Number of monitoring wells used:	18
Approximate annual O&M costs:	$350,000	Frequency of sampling:	2 times per year
Costs related to monitoring:		Is plume migration controlled?	Yes
Approximate pumping rate:	80 gpm	Progress of aquifer restoration:	more than 80% restored
Result of previous evaluation of peformance/effectiveness:	Evaluated and found sufficient	Difficulty (due to social/political factors) of implementing minor/major changes:	minor/minor

Contaminants of Concern:

Bis(2-chloroethyl) ether
Trichlorethylene (TCE)/Tetrachloroethylene (PCE)
Acetone
Isophorone
Methyl ethyl ketone (MEK)
Methyl isobutyl ketone (MIBK)
Xylene

Treatment Processes:

Metals precipitation	
Air stripping	
Biological treatment	yes
UV oxidation	yes
Carbon adsorption	yes
Filtration	
Ion Exchange	
Reverse Osmosis	
Off-gas treatment	
other/not sure	

Cost: Case Analysis #1

- Bioventing Process
- \$10 - 15/yd^3
- 25000 gallons of Jet Fuel
- Total reduction of contaminants - 98%

Cost: Case Analysis #2

- Train Derailment Site
- In-situ bioremediation
- $145/yd^3
- 1600 yd^3 of soil over an area of 1 acre
- Contaminant concentration: 38000 ppm of butyl benzyl phthalate

Cost: Case Analysis #3

- New York Department of Conservation
- Leaking UST
- $274,000 (1990) for total cost
- Installed 6 monitoring wells
- Nutrients and H_2O_2 were pumped into the ground to promote remediation
- Contaminant Concentration: 10 ppm BTEX in groundwater

A. SOIL, SEDIMENT, AND SLUDGE

1.a. BIOLOGICAL TREATMENTS: *IN SITU*

IN SITU BIOREMEDIATION

Treatment Technology	Cost Elements	Cost	Site Characteristics/Comments	Reference
In situ bioremediation	Total treatment cost (including subcontractor)	$50,000 *($13/yd^3)*	Biota Division of CET Environmental Services, California Media: 4000 yd^3 soil Contaminants: TPH from 1200 ppm to 45,000 ppm; highest concentration at 20 ft. below surface Details: Remediation of an industrial bottling plant and truck garage containing USTs and fuel pumps; Action included initial biotreatability investigation, bench-scale assessment, and on-site field implementation	8, (1994)
In situ bioremediation	Total cost/unit (including comprehensive biotreatability investigation, pilot-scale test, and full-scale treatment)	$145/yd^3	Biota Division of CET Environmental Services, Arizona Media: 1600 yd^3 soil over a one acre area Contaminants: up to 38,000 ppm butyl benzyl phthalate Details: Train derailment site; Reduced phthalate to an average level of >90 ppm	9, (1994)
In situ bioremediation	Total treatment cost	$274,000 (1990)	New York State Department of Conservation UST Site Media: soil, groundwater Contaminants: 10 ppm BTEX in groundwater Details: Leaking UST; 6 monitoring wells installed to track movement of plume; Infiltration gallery constructed at former UST location to flush hydrocarbons out of aquifer; Nutrients and H_2O_2 added to promote in situ biodegradation; Remediated to below detect	10, (1994)

Cost: Case Analysis #4

- Nitrate - mediated bioremediation
- UST site on US Coast Guard Michigan base (4 leaking tanks)
- 2,640,000 gallons of groundwater requiring treatment
- $84/gal of JP-4
- $200/m^3 JP-4 contaminated groundwater
- $17/m^3 groundwater down to confining layer

Cost: Case Analysis #5

- In-situ bioremediation of water plus solid phase bioremediation
- Railroad Superfund Site in Montana
- Total Cost: $11 million
- 70,000 yd^3 of soil; 12,000 yd^3 of soil excavated
- Contaminants: PAHs, zinc and phenol
- Total Project Duration: 5 - 10 years