Chapter

7

Polluted Soils Restoration

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1 BACKGROUND FOR EFFECTIVE REMEDIATION TECHNOLOGIES

1.1 Definition

Any unwanted substance introduced into the environment is referred to as a ‘contaminant’. Deleterious effects or damages by the contaminants lead to ‘pollution’, a process by which a resource (natural or man-made) is rendered unfit for use, more often than not, by humans. Pollutants are present since time immemorial, and life on the earth as we define now has always evolved amongst them. With pollutant analogues from geothermal and volcanic activities, comets, and space dust which are about 100 t of organic dust per day, the earth is forever a polluted planet. Relative to the pre-industrialization era, industrialization and intensive use of chemical substances such as petroleum oil, hydrocarbons (e.g., aliphatic, aromatic, polycyclic aromatic hydrocarbons (PAHs), BTEX (benzene, toluene, ethylbenzene, and xylenes), chlorinated hydrocarbons like polychlorinated biphenyls (PCBs), trichloroethylene (TCE), and perchloroethylene, nitroaromatic compounds, organophosphorus compounds) solvents, pesticides, and heavy metals are contributing to environmental pollution. Large-scale pollution due to man-made chemical substances and to some extent by natural substances is of global concern now. Seepage and run-offs due to the mobile nature, and continuous cycling of volatilization and condensation of many organic chemicals such as pesticides have even led to their presence in rain, fog and snow. Every year, about 1.7 to 8.8 million metric tons of oil is released into the world’s water. More than 90% of this oil pollution is directly related to accidents due to human failures and activities including deliberate waste disposal.

PAHs are present at levels varying from 1 µg to 300 g kg^{-1} soil, depending on the sources of contamination like combustion of fossil fuels, gasification and liquefaction of coal, incineration of wastes, and wood treatment processes (Bamforth and Singleton, 2005). Incomplete combustion of organic substances gives out about 100 different PAHs which are the ubiquitous pollutants. Except for a few PAHs used in medicines, dyes, plastics and pesticides, they are rarely of industrial use (US EPA, 1998). Some PAHs and their epoxides are highly toxic, and mutagenic even to microorganisms. About six specific PAHs are listed among the top 126 priority pollutants by the US Environmental Protection Agency. PCBs, used in hydraulic fluids, plasticizers, adhesives, lubricants, flame retardants and dielectric fluids in transformers are toxic, carcinogenic, and degrade slowly. Polychlorinated dibenzodioxins and dibenzofurans are recalcitrant chemicals and some of the congeners with lateral chlorine substitutions at positions 2,3,7 and 8 are carcinogenic to humans (Kaiser, 2000). Many solvents such as TCE and carbon tetrachloride pollute the environments due to large-scale industrial production and anthropogenic uses. Pesticides are regularly used in agricultural- and public health-programs worldwide. In many cases, the environmental effects of these chemical substances outweigh the benefits they accrue to humans and necessitate the need of their degradation after the intended uses.

The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (cometabolism). Because of the ubiquitous nature of microorganisms, their numbers and large biomass relative to other living organisms in the earth (Curtis et al., 2002), wider diversity and capabilities in their catalytic mechanisms (Chen et al., 1999 and Paul et al., 2005), and their ability to function even in the absence of oxygen and other extreme conditions (Mishra et al., 2001 and Watanabe, 2001), the search for pollutant-degrading microorganisms, understanding their genetics and biochemistry, and developing methods for their application in the field have become an important human endeavor. The recent advances in metagenomics and whole genome sequencing have opened up new avenues for searching the novel pollutant degradative genes and their regulatory elements from both culturable and non-culturable microorganisms from the environment (Golyshin et al., 2003 and Zhao and Poh, 2008). Compared to other living organisms which can degrade organic pollutants as well as the cost-intensive physical and chemical methods for the cleanup, microorganisms are preferred agents. Their capabilities to degrade organic chemical compounds can be made use of to attenuate the polluted sites.

Environmental remediation deals with the removal of pollution or contaminants from environment (mostly from soil, groundwater, sediment or surface water) for the general protection of human health and the environment or from a industrial site intended for restoration. Remediation is generally subject to regulatory requirements, and also can be based on assessments of human health and ecological risks where no legislated standards exist or where standards are advisory.

In the USA the most comprehensive set of Preliminary Remediation Goals (PRGs) is from the Environmental Protection Agency (EPA) Region 9. A set of standards used in Europe exists and is often called the Dutch standards. The European Union is rapidly moving towards Europe-wide standards, although most of the industrialised nations in Europe have their own standards at present. In Canada, most standards for remediation are set by the provinces individually, but the Canadian Council of Ministers of the Environment provides guidance at a federal level in the form of the Canadian Environmental Quality Guidelines and the Canada-Wide Standards (Canada-Wide Standard for Petroleum Hydrocarbons in Soil).

Once a site is suspected of being contaminated there is a need to assess the contamination. Often the assessment begins with preparation of relevant documents. The historical use of the site and the materials used and produced on site will guide the assessment strategy and type of sampling and chemical analysis to be done. Often nearby sites owned by the same company or which are nearby and have been reclaimed, levelled or filled are also contaminated even where the current land use seems innocuous. For example, a car park may have been levelled by using contaminated waste in the fill. Also important is to consider off site contamination of nearby sites often through decades of emissions to soil, groundwater, and air. Ceiling dust, topsoil, surface and groundwater of nearby properties should also be tested, both before and after any remediation. This is a controversial step as:

1. No one wants to have to pay for the clean up of the site;
2. If nearby properties are found to be contaminated it may have to be noted on their property title, potentially affecting the value;
3. No one wants to pay for the cost of assessment.

In the US there has been a mechanism for taxing polluting industries to form a Superfund to remediate abandoned sites, or to litigate to force corporations to remediate their contaminated sites. Other countries have other mechanisms and commonly sites are rezoned to “higher” uses such as high density housing, to give the land a higher value so that after deducting clean up costs there is still an incentive for a developer to purchase the land, clean it up, redevelop it and sell it.

There are several tools for mapping these sites and which allow the user to view additional information. One such tool is TOXMAP, a Geographic Information System (GIS) from the Division of Specialized Information Services of the United States National Library of Medicine (NLM) that uses maps of the United States to help users visually explore data from the United States Environmental Protection Agency’s (EPA) Superfund and Toxics Release Inventory programs.

1.2 Participatory approach in community consultation

In preparation for any significant remediation there should be extensive community consultation. The proponent should both present information to and seek information from the community. The proponent needs to learn about “sensitive” future uses like childcare, schools, hospitals, and playgrounds as well as community concerns and interests information. Consultation should be open, on a group basis so that each member of the community is informed about issues they may not have individually thought about. An independent chairperson acceptable to both the proponent and the community should be engaged (at proponent expense if a fee is required). Minutes of meetings including questions asked and the answers to them and copies of presentations by the proponent should be available both on the internet and at a local library (even a school library) or community centre.

1.3 Health risk

Incremental health risk is the increased risk that a receptor (normally a human being living nearby) will face from (the lack of) a remediation project. The use of incremental health risk is based on carcinogenic and other (e.g., mutagenic, teratogenic) effects and often involves value judgements about the acceptable projected rate of increase in cancer. In some jurisdictions this is 1 in 1,000,000 but in other jurisdictions the acceptable projected rate of increase is 1 in 100,000. A relatively small incremental health risk from a single project is not of much comfort if the area already has a relatively high health risk from other operations like incinerators or other emissions, or if other projects exist at the same time causing a greater cumulative risk or an unacceptably high total risk. An analogy often used by remediators is to compare the risk of the remediation on nearby residents to the risks of death through car accidents or tobacco smoking.

1.4 Emissions standards

Standards are set for the levels of dust, noise, odour, emissions to air and groundwater, and discharge to sewers or waterways of all chemicals of concern or chemicals likely to be produced during the remediation by processing of the contaminants. These are compared against both natural background levels in the area and standards for areas zoned as nearby areas are zoned and against standards used in other recent remediations. Just because the emission is emanating from an area zoned industrial does not mean that in a nearby residential area there should be permitted any exceedances of the appropriate residential standards.

Monitoring for compliance against each standards is critical to ensure that exceedances are detected and reported both to authorities and the local community.

Enforcement is necessary to ensure that continued or significant breaches result in fines or even a jail sentence for the polluter.

Penalties must be significant as otherwise fines are treated as a normal expense of doing business. Compliance must be cheaper than to have continuous breaches.

1.5 Transport and emergency safety assessment

Assessment should be made of the risks of operations, transporting contaminated material, disposal of waste which may be contaminated including workers' clothes, and a formal emergency response plan should be developed. Every worker and visitor entering the site should have a safety induction personalised to their involvement with the site.

1.6 Impacts of funding

The rezoning is often resisted by local communities and local government because of the adverse effects on the local amenity of the remediation and the new development. The main impacts during remediation are noise, dust, odour and incremental health risk. Then there is the noise, dust and traffic of developments. Then there is the impact on local traffic, schools, playing fields, and other public facilities of the often vastly increased local population.

2 TYPES OF REMEDIATION TECHNOLOGIES FOR SOILS AND GROUNDWATER

Remediation technologies are many and varied but can be categorised into **ex-situ** and **in-situ** methods. Ex-situ methods involve excavation of affected soils and subsequent treatment at the surface, In-situ methods seek to treat the contamination without removing the soils. The more traditional remediation approach (used almost exclusively on contaminated sites from the 1970s to the 1990s) consists primarily of soil excavation and disposal to landfill “dig and dump” and groundwater “pump and treat”. In situ technologies include Solidification and Stabilization and have been used extensively in the USA.

2.1 Thermal desorption

Thermal desorption is a technology for soil remediation. During the process a desorber volatilizes the contaminants (e.g. oil, mercury or hydrocarbon) to separate them from especially soil or sludge. After that the contaminants can either be collected or destroyed in an offgas treatment system.

2.2 Excavation or dredging

Excavation processes can be as simple as hauling the contaminated soil to a regulated landfill, but can also involve aerating the excavated material in the case of volatile organic compounds (VOCs). Recent advancements in bioaugmentation and biostimulation of the excavated material have also proven to be able to remediate semi-volatile organic compounds (SVOCs) onsite. If the contamination affects a river or bay bottom, then dredging of bay mud or other silty clays containing contaminants may be conducted. Recently, ExSitu Chemical oxidation has also been utilized in the remediation of contaminated soil. This process involves the excavation of the contaminated area into large bermed areas where they are treated using chemical oxidation methods.

2.3 Surfactant enhanced aquifer remediation (SEAR)

Also known as Solubilization and recovery, the Surfactant Enhanced Aquifer Remediation process involves the injection of hydrocarbon mitigation agents or specialty surfactants into the subsurface to enhance desorption and recovery of bound up otherwise recalcitrant non aqueous phase liquid (NAPL).

In geologic formations that allow delivery of hydrocarbon mitigation agents or specialty surfactants, this approach provides a cost effective and permanent solution to sites that have been previously unsuccessful utilizing other remedial approaches. This technology is also successful when utilized as the initial step in a multi faceted remedial approach utilizing SEAR then In situ Oxidation, bioremediation enhancement or soil vapor extraction (SVE).

2.4 Pump and treat

Pump and treat involves pumping out contaminated groundwater with the use of a submersible or vacuum pump, and allowing the extracted groundwater to be purified by slowly proceeding through a series of vessels that contain materials designed to adsorb the contaminants from the groundwater. For petroleum-contaminated sites this material is usually activated carbon in granular form. Chemical reagents such as flocculants followed by sand filters may also be used to decrease the contamination of groundwater. Air stripping is a method that can be effective for volatile pollutants such as BTEX compounds found in gasoline.

For most biodegradable materials like BTEX, MTBE and most hydrocarbons, bioreactors can be used to clean the contaminated water to non-detectable levels. With fluidized bed bioreactors it is possible to achieve very low discharge concentrations which will meet or exceed discharge standards for most pollutants.

Depending on geology and soil type, pump and treat may be a good method to quickly reduce high concentrations of pollutants. It is more difficult to reach sufficiently low concentrations to satisfy remediation standards, due to the equilibrium of absorption (chemistry)/desorption processes in the soil. However, pump and treat is typically not the best form of remedation. It is expensive to treat the groundwater, and typically is a very slow process to cleanup a release with pump and treat. It is best suited to control the hydraulic gradient and keep a release from spreading further. Better options of in-situ treatment often include air sparge/soil vapor extraction (AS/SVE) or dual phase extraction/multiphase extraction(DPE/MPE). Other methods include trying to increase the dissolved oxygen content of the groundwater to support microbial degradation of the compound (especially petroleum) by direct injection of oxygen into the subsurface, or the direct injection of a slurry that slowly releases oxygen over time (typically magnesium peroxide or calium oxy-hydroxide).

2.5 Solidification and stabilization

Solidification/stabilization work has a reasonably good track record but also a set off serious deficiencies related to durability of solutions and potential longterm effects. In addition CO_2 emissions due to the use of cement are also becoming a major obstacle to its widespread use in solidification/stabilization projects.

Stabilization/solidification is a remediation/treatment technology that relies on the reaction between a binder and soil to stop/prevent or reduce the mobility of contaminants.

- **Stabilization** - involves the addition of reagents to a contaminated material (e.g. soil or sludge) to produce more chemically stable constituents; and
- **Solidification** - involves the addition of reagents to a contaminated material to impart physical/dimensional stability to contain contaminants in a solid product and reduce access by external agents (e.g. air, rainfall).

Conventional Stabilization/solidification is an established remediation technology for contaminated soils and treatment technology for hazardous wastes in many countries in the world. However, the uptake of Stabilization/solidification technologies has been relatively modest, and a number of barriers have been identified including:

- the relatively low cost and widespread use of disposal to landfill;

- the lack of authoritative technical guidance on Stabilization/solidification;
- uncertainty over the durability and rate of contaminant release from Stabilization/solidification -treated material;
- experiences of past poor practice in the application of cement stabilization processes used in waste disposal in the 1980s and 1990s (ENDS, 1992);
- residual liability associated with immobilized contaminants remaining on-site, rather than their removal or destruction.

2.6 In situ oxidation

New in situ oxidation technologies have become popular, for remediation of a wide range of soil and groundwater contaminants. Remediation by chemical oxidation involves the injection of strong oxidants such as hydrogen peroxide, ozone gas,potassium permanganate or persulfates.

Oxygen gas or ambient air can also be injected to promote growth of aerobic bacteria which accelerate natural attenuation of organic contaminants. One disadvantage of this approach is the possibility of decreasing anaerobic contaminant destructionnatural attenuation where existing conditions enhance anaerobic bacteria which normally live in the soil prefer a reducing environment. In general though, aerobic activity is much faster than anaerobic and overall destruction rates are typically greater when aerobic activity can be successfully promoted.

The injection of gases into the groundwater may also cause contamination to spread faster than normal depending on the site's hydrogeology. In these cases, injections downgradient of groundwater flow may provide adequate microbial destruction of contaminants prior to exposure to surface waters or drinking water supply wells.

Migration of metal contaminants must also be considered whenever modifying subsurface oxidation-reduction potential. Certain metals are more soluble in oxidizing environments while others are more mobile in reducing environments.

2.7 Soil vapor extraction

Soil vapor extraction (SVE) is an effective remediation technology for soil. “Multi Phase Extraction” (MPE) is also an effective remediation technology when soil and groundwater are to be remediated coincidentally. SVE and MPE utilize different technologies to treat the off-gas volatile organic compounds (VOCs) generated after vacuum removal of air and vapors (and VOCs) from the subsurface and include granular activated carbon (most commonly used historically), thermal and/or catalytic oxidation and vapor condensation. Generally, carbon is used for low (<500ppmV) VOC concentration vapor streams, oxidation is used for moderate (up to 4,000 ppmV) VOC concentration streams, and vapor condensation is used for high (>4,000 ppmV) VOC concentration vapor streams. Below is a brief summary of each technology.

1. Granular activated carbon (GAC) is used as a filter for air or water. Commonly used to filter tap water in household sinks. GAC is a highly porous adsorbent material, produced by heating organic matter, such as coal, wood and coconut shell, in the absence of air, which is then crushed into granules. Activated carbon is positively charged and therefore able to remove negative ions from the water such as organic ions, ozone, chlorine, fluorides and dissolved organic solutes by adsorption onto the activated carbon. The activated carbon must be replaced periodically as it may become saturated and unable to adsorb (i.e. reduced absorption efficiency with loading). Activated carbon is not effective in removing heavy metals.

2. Thermal oxidation (or incineration) can also be an effective remediation technology. This approach is somewhat controversial because of the risks of dioxins released in the atmosphere through the exhaust gases or effluent off-gas. Controlled, high temperature incineration with filtering of exhaust gases however should not pose any risks. Two different technologies can be employed to oxidize the contaminants of an extracted vapor stream. The selection of either thermal or catalytic depends on the type and concentration in parts per million by volume of constituent in the vapor stream. Thermal oxidation is more useful for higher concentration (~4,000 ppmV) influent vapor streams (which require less natural gas usage) than catalytic oxidation at ~2,000 ppmV.

- Thermal oxidation which uses a system that acts as a furnace and maintains temperatures ranging from 732 to 800 °C.

- Catalytic oxidation which uses a catalyst on a support to facilitate a lower temperature oxidation. This system usually maintains temperatures ranging from 300 to 400 °C.

3. Vapor condensation is the most effective off-gas treatment technology for high (>4,000 ppmV) VOC concentration vapor streams. The process involves cryogenically cooling the vapor stream to below 40 degrees Celsius such that the VOCs condensate out of the vapor stream and into liquid form where it is collected in steel containers. The liquid form of the VOCs is referred to as dense non-aqueous phase liquids (DNAPL) when the source of the liquid consists predominantly of solvents or light non-aqueous phase liquids (LNAPL) when the source of the liquid consists predominantly of petroleum or fuel products. This recovered chemical can then be reused or recycled in a more environmentally sustainable or green manner than the alternatives described above. This technology is also known as cryogenic cooling and compression (C3-Technology).

2.8 Other technologies

The treatment of environmental problems through biological means is known as bioremediation and the specific use of plants for example by using phytoremediation. Bioremediation is sometimes used in conjunction with a pump and treat system. In bioremediation, either naturally occurring or specially bred bacteria are used to consume contaminants from extracted groundwater. This is sometimes referred to as a bio-gac system. Many times the groundwater is recycled to allow for continuously flowing water and enhanced bacteria population growth. Occasionally the bacteria can build up to such a point that they can affect filtration and pumping. The vessel should then be partially drained. Care must be taken to ensure that a sharp change in the groundwater chemistry does not kill the bacteria (such as a sudden change in pH).

Dual-phase extraction utilizes a soil vapor extraction system that produces a high vacuum resulting in the extraction of both contaminated vapors as well as a limited amount of contaminated groundwater. This method is somewhat inefficient due to large amount of energy required by pulling water by vacuum compared to pushing water with a submersible pump.

Mycoremediation is a form of bioremediation, the process of using fungi to return an environment (usually soil) contaminated by pollutants to a less contaminated state.

In an experiment conducted in conjunction with Batelle, a major contributor in the bioremediation industry, a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelial-inoculated plots. It appears that the natural microbial community participates with the fungi to break down contaminants into carbon dioxide and water. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain persistent pesticides; Battelle, 2000). Hair mats inoculated with oyster mushrooms were successfully employed in the clean-up of the San Francisco Bay area oil spill in 2007.

The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have also been reported to successfully degrade the nerve gases VX and sarin. Mycofiltration is a very similar process, using mycelial mats to filter toxic waste and microorganisms from polluted water.

3 BIOLOGICAL DEGRADATION

As much as the diversity in sources and chemical complexities in organic pollutants exists, there is probably more diversity in microbial members and their capabilities to synthesize or degrade organic compounds. Microbial populations even contribute to naturally-occurring hydrocarbons by diagenesis of bacteriohopanetetrol (a membrane constituent) into the formation of hopanoic acids and hydrocarbons such as hopane (Stout et al., 2001). The microbial diversity is larger than what is known from the cultured members (Curtis et al., 2002). However, the metabolic diversity of culturable microorganisms for degrading organic pollutants may be insufficient to protect the earth from the anthropogenic pollution. This is largely due to recalcitrant chemicals with substituent or structural elements, which seldom occur in nature (Pieper and Reineke, 2000). But, Singer et al. (2004) were of the opinion that the naturally-occurring tritrophic trinity of microbe–plant–insect interactions has capabilities to produce hundreds of thousands of different chemicals to attract, defend, antagonize, monitor and misdirect one another among these members and only negligible numbers are truly novel chemicals of anthropogenic origin. Hence, there is a fortuitous evolution of xenobiotic-degrading enzymes from the interactions of microbe–plant–insect.

The potential to degrade organic pollutants varies among microbial groups or different guilds (group of species that exploit the same class of environmental resources in a similar way) and is dose-dependent. For example, mycobacteria are excellent candidates for remediating aged PAH-polluted sites as these organisms have lipophilic surfaces, suitable for uptake of bound pollutants from soil particles and have catabolic efficiency towards PAHs up to five benzene rings (Bogan et al., 2003). Higher doses of PAHs are phytotoxic to algae including microalgae. The inhibitory or toxic components of the pollutant mixture can attenuate the potential of microbial degradation and are important stressors. Organic compounds such as toluene are toxic to microorganisms because they disrupt cell membranes. Providentially, several bacteria develop resistance to solvents by the ***cis*** to ***trans*** isomerization of fatty acids, increased synthesis of phospholipids, low cell-surface hydrophobicity (modification of the lipopolysaccharide or porines of the outer membrane), and the presence of solvent efflux pump. In the soils polluted by aromatic hydrocarbons, the solvent-tolerant microorganisms are the first to colonize and become predominant in the removal of pollutants (Huertas et al., 1998). Either bioaugmentation with the solvent-tolerant bacteria or modifying these bacteria with an appropriate catabolic potential will provide advantages in bioremediation programs.

The microbial populations of soil or aquatic environments are composed of diverse, synergistic or antagonistic communities rather than a single strain. In the natural environments, biodegradation involves transferring the substrates and products within a well coordinated microbial community, a process referred to as metabolic cooperation (Abraham et al., 2002). It still remains very challenging to introduce all the genes required for degradation for many organic pollutants or stable maintenance of even a single gene or a desired trait such as enhanced degradative capacity in a single organism. Hence, the microbial consortia of ecologically relevant candidate taxa which are known to degrade the chemical pollutants and respond to different environmental stimuli are desired, rather than the single isolate for augmentation (Supaphol et al., 2006).

The reductionist approach to studying biodegradation processes has been very useful so far for understanding individual genes, enzymes and organisms, but the systems biology approach is necessary to examine the complex web of metabolic and regulatory interactions even within a single organism (Pazos et al., 2003 and Trigo et al., 2009). Pazos et al. (2003) considered the biodegradation process as a single interconnecting network (metabolic cooperation), with metabolic activities and substrates and intermediate compounds flowing freely in the environment and less boundaries existing between bacterial species. The 'network theory,' thus forms a basis for studying the functional properties and mechanisms involved in the organization of biological systems and predicting their responses to environmental (both internal and external) variations (Feist and Palsson, 2008).

Formalization and categorization of many biodegradation reactions and pathways have been done in the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD) (Ellis et al., 2006). With information on about 900 compounds, 600 enzymes, 1000 reactions and 350 microbial entries, the UM-BBD is useful for applying the system biology approaches. The most likely metabolic pathway for any given compound is predictable using the 'reaction rules' for particular functional groups (Ellis et al., 2008). Pazos et al. (2005) developed a database, 'Metarouter', based on the information available at the UM-BBD. Using the Metarouter, Gomez et al. (2007) showed the existence of a correlation between the frequency of 149 chemical triads (chemotypes) common in organo-chemical compounds and the global capacity of microorganisms to metabolize them. These authors developed a predictive tool (http://www.pdg.cnb.uam.es/BDPSERVER) which can provide the biodegradative outcome of the compounds as biodegradable or recalcitrant, depending on the type of environmental fate defined. Trigo et al. (2009) suggested that (i) the central metabolism of the global biodegradation networks involves transferases, isomerases, hydrolases and ligases, (ii) linear pathways converging on particular intermediates form a funnel topology, (iii) the novel reactions exist in the exterior part of the network, and (iv) the possible pathway between compounds and the central metabolism can be arrived at by considering all the required enzymes in a given organism and intermediate compounds.

Biodegradation in the natural environment is beyond the 'complete system' of a single cell, where the 'system' is extremely complex involving multiple biotic and abiotic components. Nevertheless, there exists the coordination of microbial communities to mediate and transfer substrates and products between species and communities (Abraham et al., 2002). The application of molecular site assessment (Fleming et al., 1998 and Sayler et al., 1995) and molecular ecological techniques for community profiling (Malik et al., 2008), soil metagenomics using isotope distribution analysis (Villas-Boas and Bruheim, 2007), and functional genomics and proteomics (Zhao and Poh, 2008) can help in identifying the partners and the patterns of responses to external stimuli within the network and the 'system complexities' of contaminated sites. Stable isotope probing (SIP) analyses, either DNA-SIP (Winderl et al., 2010) or RNA-SIP (Bombach et al., 2010), provide opportunities to link microbial diversity with function and identify those culturable as well as yet-to-be cultured organisms which are involved in biodegradation in the field (Cupples, 2011). Likewise, the high-throughput approaches such as DNA microarrays, metagenomics, metatranscriptomics, metaproteomics, metabolomics, and whole cell-based biosensors are useful to characterize the contaminated sites, identify new degradative activities and monitor bioremediation efficiency.

4 BIOREMEDIATION WITH BACTERIA, FUNGUS, ENZYMES

4.1 Basics

Bioremediation, which is defined as a process that uses microorganisms, green plants or their enzymes to treat the polluted sites for regaining their original condition (Glazer and Nikaido, 1995), has considerable strength and certain limitations. Remediation, whether by biological, chemical or a combination of both means, is the only option as the problem of pollution has to be solved without transferring to the future. As the knowledge demand and complexities vary for different bioremediation treatments, a better understanding of the premises together with the limitations of bioremediation aids in maximizing the benefits and minimizing the cost of treatments.

The process of bioremediation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability (Antizar-Ladislao, 2010). There is a considerable debate in the literature on "what constitutes the bioavailable fraction" and the methods of its measurements (Alexander, 2000 and Vasseur et al., 2008). Following entry into the soil environment, pollutants rapidly bind to the mineral and organic matter (solid phases) via a combination of physical and chemical processes. Sorption, complexation and precipitation constitute the pollutant–soil interaction. The ability of soils to release (desorb) pollutants determines its susceptibility to microbial degradation, thereby influencing effectiveness of the bioremediation process. In soil aggregates which are the smallest 'composite units' in the heterogeneous soil environment, bioavailability is limited by transport of the pollutant molecule to a microbial cell, i.e., diffusion of pollutant out of a soil aggregate to the cell attached to the external surface of the aggregate.

Sorption which influences the bioavailability of a contaminant is a critical factor, yet a poorly understood process in bioremediation. There are two schools of thought concerning bioavailability and the consequent biodegradation of organic contaminants (Singh et al., 2008): (i) the pre-requisite release of contaminant from sorbed phase to aqueous phase for its degradation by microorganisms (Harms and Zehnder, 1994 and Shelton and Doherty, 1997), and (ii) biodegradation of the contaminant in the sorbed phase, without being desorbed, by the enzymes (Singh et al., 2003). The degradation of sorbed contaminants can presumably occur via microbially-mediated desorption of contaminants through production of biosurfactants and the development of a steep gradient between solid phase and interfacial contaminant (Tang et al., 1998). Thus, these reports suggest that bioavailability is even species specific (i.e., the ability of certain species to desorb the contaminant and then degrade). The organic contaminants can also be degraded without prior desorption. Singh et al. (2003) demonstrated that a soil bacterium, ***Brevibacterium*** sp. degraded the pesticide fenamiphos which was intercalated into the cationic-surfactant modified montmorillonite clay (CTMA–Mt–fenamiphos complex). The interlayer space is otherwise inaccessible to the bacterium due to its size of several orders lower than that of the bacteria. The scanning electron microscope analysis showed the surface attachment of bacteria to the surface of the CTMA–Mt–fenamiphos complex, suggesting the involvement of extracellular enzyme in the degradation of fenamiphos, without its prior desorption. The degradation of sorbed contaminants depends on the enrichment and isolation procedures used for obtaining the culturable bacteria. As against the conventional approach of providing the contaminant as a sole carbon source in aqueous medium, the provision of phenanthrene sorbed on a polyacrylic porous resin to the bacterial cultures led to faster degradation of phenanthrene than those isolated by the conventional technique (Grosser et al., 2000 and Tang et al., 1998).

Aqueous solubility, volatility or reactivity of organic pollutants varies greatly, and all of them may influence their bioavailability in water and soils. On a mass basis, no relationship exists between the chemical pollutant in soil and its biological effect. The dissolved form of contaminants in pore water is considered to be bioavailable, compared to the bound chemical which does not exert direct biological effects. This has led to the ‘pore water hypothesis.’ The equilibrium partitioning theory is applied to estimate the dissolved fraction of pollutant in pore water and to remove the ***soil to soil*** differences in toxicological effects. The basic assumption of equilibrium partitioning theory is that the partitioning of an ionic chemical between the mineral and organic matter in soil or sediment and the pore water is at equilibrium, and in each phase the chemical potential which controls its biological activity is the same. The performance of chemical extraction data of nonionic organic chemicals can be improved by organic matter normalization in order to predict the occurrence of toxicity effects.

For highly hydrophobic chemical pollutants which have higher octanol–water partition coefficient ($\boldsymbol{K}_{ow}$) with log $\boldsymbol{K}_{ow}$ values more than 4, the measured concentration in the pore water is the sum of the free chemical and the fraction sorbed to dissolved organic matter (DOM). To account for the sorbed fraction to DOM, the separation methods for DOM are required (Landrum et al., 1984). The soil–chemical contact time determines the usefulness of pore water hypothesis in measuring bioavailability and predicting the biological effects or the fraction which can be degraded, but not immediately after contamination. There are also variations in bioavailability due to the nature of chemical pollutants, soil types, and other factors such as water content and temperature. Toxicity testing of a pollutant to microorganisms (Ronday et al., 1997) or the use of extracts such as the mild hydroxypropyl-β-cyclodextrin for PAHs (Ling et al., 2010) or the matrix solid-phase microextraction for DDTs (1,1,1-trichloro-2,2-bis(***p***-chlorophenyl) ethane and its metabolites) (Fang et al., 2010) can provide direct measures of bioavailability. Cornelissen et al. (1998) demonstrated that microbial factors, not bioavailability, were responsible for the persistence of rapidly desorbing fractions of the nondegraded PAHs, and these fractions were found to be substantial (up to 55%) and remained unchanged during remediation. For the purpose of bioremediation and regulatory measures, the bioavailability in the initial rapid phase and the ensuing slow phase in the biphasic degradation profile of an organic pollutant is to be monitored.

The sequestration of pollutants over time may occur due to the contact and interaction of soil with pollutant molecules. Factors such as organic matter, cation exchange capacity, micropore volume, soil texture and surface area affect the pollutant sequestration (Chung and Alexander, 2002). Sequestration and reduced bioavailability of phenanthrene were reported for a Gram-negative bacterial isolate (strain PS5-2) when the hydrophobic compound entered into nanopores having hydrophobic surfaces (Nam and Alexander, 1998).Sharer et al. (2003) observed that aging caused an increase in sorption for some organic compounds (e.g., 2,4-dichlorophenoxyacetic acid) but not for others (chlorobenzene, ethylene dibromide) on a common soil type. Even a weakly sorbed and easily degraded carbamate insecticide, carbaryl, can be effectively sequestrated in soil with aging, thereby rendering it partly inaccessible to microorganisms and affecting the bioavailability (Ahmad et al., 2004). Hence, the generalizations about the effects of aging on the sorption–desorption behavior of different organic chemicals are difficult to achieve. Some pertinent issues that need to be considered include: (a) bioavailability and toxicity of parent molecules and their residues in soils, (b) standardized protocols for different pollutants and their use across the sites, (c) assessment on remobilization of pollutants during the post-remediation period, and (d) determination of environmentally acceptable pollutant end-points in the bioremediated soils. The 'pollutant (or contaminant) sequestration' due to the prolonged contact between soil particles and chemical molecules, however, poses less risk and threat to the environmental health. In general, difficulties with analytical measurements for determining low levels of new organic pollutants in soils, the absence of base-line values related to their compositional, geographical and distribution patterns, and the complexities in their toxicological interactions (Mas et al., 2010) make the bioavailability measurements of organic pollutants exigent.

Application of surfactants to polluted soils has been used as one of the treatment strategies for increasing the mass transfer of hydrophobic organic contaminants. The surfactants are amphiphilic molecules that contain hydrophilic and hydrophobic moieties; hydrophilic groups can be anionic, cationic, zwitter ionic, and nonionic. The synthetic surfactants contain sulfate, sulfonate or carboxylate group (anionic); quaternary ammonium group (cationic); polyoxyethylene, sucrose, or polypeptide (nonionic) and the hydrophobic parts of paraffins, olefins, alkylbenzenes, alkylphenols, or alcohols. The common chemical surfactants such as Triton X-100, Tween 80 and sodium dodecyl sulphate are petroleum-derived products. The zwitter ionic surfactants (e.g., ***N***-dodecyl betaine) which contain both anionic and cationic groups have low critical micelle concentration (CMC) values, more surface active, and high solubilization capacity. Increased desorption rates of sorbed pollutants from soils by the application of surfactants make the pollutants available for remediation (Fu and Alexander, 1995). Solubilization of hydrophobic contaminants is attributed to the incorporation of the molecule into the hydrophobic core of micelles in solution (Guha and Jaffe, 1996). The salient mechanisms which are involved in the surfactant-amended remediation are: (i) lowering of interfacial tension, (ii) surfactant solubilization of hydrophobic organic compounds, and (iii) the phase transfer of organic compounds from soil-sorbed to pseudo-aqueous phase (Laha et al., 2009).

Surfactants enhance mobilization and biodegradation of PAHs in soils (Tiehm et al., 1997). Enhanced rates of degradation of naphthalene and phenanthrene in the presence of some nonionic surfactants at applications below their CMC were observed by Aronstein et al. (1991). Similarly, significant solubility enhancements of

DDT in Triton and Brij 35 surfactants were noticed by Kile and Chiou (1989) below their CMC. Factors such as cost, effectiveness at concentrations lower than 3%, low toxicity to humans, animals and plants, low adsorption to soil, low soil dispersion, and low surface tension determine the selection of surfactants for field application (Mulligan et al., 2001). Toxicities of surfactants to soil biota can prevent the biodegradation of pollutants and disturb the balanced ecological functions.

The food-grade surfactants (T-MAZ 28, T-MAZ 10, and T-MAZ 60), the plant-based surfactants (e.g., fruit pericarp from ***Sapindus mukurossi***) or the natural surfactants such as humic acids may be preferred to the synthetic surfactants due to high biodegradability, low toxicity, and higher public acceptance. Microorganisms also produce surfactants (surface-active amphiphilic metabolites such as glycolipids, phospholipids, lipopeptides, lipoproteins, and lipopolysaccharides). These low- and high-molecular weight biosurfactants find their uses in food processing, cosmetic and pharmaceutical industries, in addition to bioremediation efforts (Christofi and Ivshina, 2002). The classes of biosurfactant and microbial species which can produce them are numerous, leading to continuous search for the novel biosurfactants (Satpute et al., 2010). However, the ***in situ*** application of surfactants to enhance bioavailability of persistent organic pollutants requires careful planning and selection based on the prior information about the fate and behavior of the surfactant and the target pollutant. Caution is required to prevent groundwater contamination via leaching and consequent toxicity to microorganisms. Hence, a good strategy will be to select bacteria that are capable of not only catabolizing the target contaminant but also producing surfactant. More knowledge on the mechanisms of pollutant–surfactant interactions with regard to diffusion, in and out of the micelles, and modeling of pollutant's transport at the field site can help to design efficient remediation strategy.

4.2 In situ and ex situ bioremediation

Bioremediation approaches are generally classified as ***in situ*** or ***ex situ***. ***In situ*** bioremediation involves treating the polluted material at the site while ***ex situ*** involves the removal of the polluted material to be treated elsewhere (Aggarwal et al., 1990). ***In situ*** bioremediation can be described as the process whereby organic pollutants are biologically degraded under natural conditions to either carbon dioxide and water or an attenuated transformation product. It is a low-cost, low maintenance, environment-friendly and sustainable approach for the cleanup of polluted sites. With the need for excavation of the contaminated samples for treatment, the cost of ***ex situ*** bioremediation approaches can be high, relative to ***in situ*** methods. In addition, the rate of biodegradation and the consistency of the process outcome differ between the ***in situ***- and ***ex situ*** bioremediation methods. While the methods of both ***in situ*** and ***ex situ*** remediation depend essentially on microbial metabolism, the ***in situ*** bioremediation methods are preferred to those of ***ex situ*** for ecological restoration of contaminated soil and water environments (Jorgensen, 2007). Three different types of ***in situ*** bioremediation process are (i) bioattenuation which depends on the natural process of degradation, (ii) biostimulation where intentional stimulation of degradation of chemicals is achieved by addition of water, nutrient, electron donors or acceptors, and (iii) bioaugmentation where the microbial members with proven capabilities of degrading or transforming the chemical pollutants are added (Madsen, 1991). The suitability of a particular bioremediation technology is determined by several factors, such as site conditions, indigenous population of microorganism, and the type, quantity and toxicity of pollutant chemical species present.

4.2.1 Bioattenuation

During bioattenuation (natural attenuation), the pollutants are transformed to less harmful forms or immobilized. Such transformation and immobilization processes are largely due to biodegradation by microorganisms (Smets and Pritchard, 2003), and to some extent by the reactions with naturally-occurring chemicals and sorption on the geologic media. The natural attenuation processes are contaminant-specific, accepted as methods for treating fuel components (e.g., BTEX) (Atteia and Guillot, 2007), but not for many other classes. The time required for natural attenuation varies considerably with site conditions. Many polluted sites may not require an aggressive approach to remediation, and bioattenuation is efficient and cost-effective (Davis et al., 1994 and Mulligan and Yong, 2004). In fact, a variety of bioremediation techniques have been successfully employed at over 400 cleanup sites throughout the USA, at costs which are approximately 80–90% lower than other cleanup technologies, based on the physical and chemical principles. With minimal site disturbance, the post-cleanup costs are also substantially reduced. Consequently, the global demand for bioremediation along with phytoremediation technologies is valued to be about US $1.5 billion per annum (Singh et al., 2009). Industrial and environmental biotechnologies also prefer newer paths, resulting in processes with 'clean technologies', with maximum production and fewer residues. Bioattenuation alone becomes inadequate and protracted in many cases since many soils are oligotrophic in nature or lack appropriate microorganisms.

4.2.2 Biostimulation

The acceleration of microbial turnover of chemical pollutants generally depends on the supply of carbon, nutrients such as N and P, temperature, available oxygen, soil pH, redox potential, and the type and concentration of organic pollutant itself (Carberry and Wik, 2001). To stimulate microbial degradation, nutrients in the form of fertilizers (water soluble (e.g., KNO_3, $NaNO_3$, NH_3NO_3, K_2HPO_4 and $MgNH_4PO_4$), slow release (e.g., customblen, IBDU, max-bac), and oleophilic (e.g., Inipol EAP22, F1, MM80, S200)) are added (Nikolopoulou and Kalogerakis, 2008). As a thumb rule for oil spill remediation, around 1–5% N by weight of oil with a ratio of N:P between 5 and 10:1 is applied (Swannell et al., 1996). These additions may be insufficient or inaccurate for polluted sites with different types of pollutants. Formulation of nutrient-treatment strategies and maintenance of control on the degradation rates and the outcomes of degradation need to be tailored to specific site/pollutant combinations. Limitations of nutrients such as nitrogen and phosphorus on microbial decomposition of organic matter and the possible ecological implications of these effects for carbon flow through natural ecosystems are well known (Sterner and Elser, 2002). Wolicka et al. (2009)optimized the C:N:P ratio (at the level of 100:9:2, 100:10:1 or 250:10:3) before commencing ***in situ*** remediation of BTEX.

The 'ecological stoichiometry' is concerned with the supplies of nutrients, and their elemental stoichiometry relative to the nutritional demands of the cell's innate physiology. It also exemplifies the effects of resource (nutrient) supply rates and supply ratios on the structure and function of microbial communities (Smith, 2002). Smith et al. (1998) applied the resource-ratio theory to hydrocarbon degradation and demonstrated that the changes in nitrogen and phosphorus supply ratios not only altered the biodegradation rates of hydrocarbons (hexadecane and phenanthrene) but also the microbial community composition significantly. In addition, the changes in absolute nutrient supply levels, at constant supply ratio, were found to alter total hydrocarbon degrader biomass, with altered rates of hydrocarbon degradation. The 'resource-ratio approach' to gain information on the ecophysiological status of pollutant-degrading microorganisms has many practical implications. Basically, it provides the theoretical framework for optimizing nutrient formulation and application in biostimulation approaches.

4.2.3 Bioaugmentation

Often, the biological response lags behind, up to weeks or months, in the polluted sites with no exposure history. The 'soil activation,' a concept which is based on the cultivation of biomass from a fraction of a contaminated soil and the subsequent use as an inoculum for bioaugmentation for the same soil was attempted by Otte et al. (1994) for degradation of PCP and PAHs. The soils with microbiota, adapted by prior exposure to degradation of organic pollutants such as hydrocarbons can be a source of microorganisms for remediating soils freshly contaminated with hydrocarbons. Priming with 2% bioremediated soil was found to increase biodegradation of PAH constituents of a fuel oil-treated soil (Lamberts et al., 2008). Similar priming effect of exhaustively bioremediated soils for hydrocarbon degradation was observed by Greenwood et al. (2009). Exposure history and adaptive status of microbial degraders thus determine the lag period of degradation. In addition, ascertaining the history of exposure of chemical pollutants in the contaminated sites has even become significant in the environmental forensics such as the 1989 Exxon Valdez oil spill case (Peters et al., 2005) and for ecological engineering such as the 2010 Gulf of Mexico oil spill case (Mitsch, 2010).

Pre-adaptation of catabolic bacteria to the target environment, prior to inoculation, improves survival, persistence and degradative activities, leading to enhanced remediation of the polluted soil (Megharaj et al., 1997). ***Sphingomonas*** sp. RW1 which contained a mini transposon Tn-5 ***lacZ*** was pre-adapted to soil by growing in the soil extract medium. The pre-adapted bacterium exhibited better survival and efficient degradation of dibenzo-***p***-dioxin and dibenzofuran in the polluted soil, compared to the unadapted bacterium, grown only in the nutrient-rich medium. Sudden exposure to stresses in soil (oligotrophic conditions that generally exist in soils, starvation or susceptibility/resistance, etc.) determines the physiological response of bacteria and their subsequent survival and activities.

Pre-exposure and subsequent re-exposure of a chemical pollutant enhances the metabolic potential of microorganisms (Reddy and Sethunathan, 1983). The phenomenon of retaining specific metabolic capacity after pre-exposure over long periods of time is referred to as 'soil memory.' The soil memory makes a contribution to the subsequent natural attenuation. Now, in a typical bioaugmentation approach, microorganisms are amended to a polluted site to hasten detoxification and/or degradation. There are many reports on bioaugmentation for treatment of soils containing organic pollutants (Brunner et al., 1985). Gilbert and Crowley (1998) found that the repeated application of carvone-induced bacteria enhanced biodegradation of PCBs in soil. To improve efficiency of bioaugmentation, microorganisms of different physiological groups and of different divisions can also be brought together. Bender and Phillips (2004) suggested the use of microbial mats which occur in nature as stratified communities of cyanobacteria and bacteria to remediate organic contaminants by degrading and completely mineralizing the contaminants. Wolicka et al. (2009)applied aerobic microbial communities, selected from those adapted to utilize one type of BTEX compound, for bioremediation of soil contaminated with BTEX.

A successful strategy for in situ bioremediation can be the combination, in a single bacterial strain or in a syntrophic bacterial consortium, of different degrading abilities with genetic traits that provide selective advantages in a given environment (Diaz, 2004). The present strain selection procedures dwell on isolating 'superbugs' with high resilience to environmental stresses, those harboring catabolically superior enzymes, and those species that are not human pathogens (Singer et al., 2005). Most laboratory strains which are capable of degrading organic pollutants constitute a fraction of culturable microorganisms, making only small contributions to bioaugmentation (Watanabe, 2001). Paul et al. (2005) also pointed out that only a fraction of total microbial diversity has been harnessed so far while the genetic resource for degradation of recalcitrant and xenobiotic pollutants is vast.

Bioaugmentation efforts are met with failures more often due to lesser efficiency, competitiveness and adaptability, relative to the indigenous members of natural communities. For example, the well known bacteria capable of degrading PCBs in laboratory culture media survived poorly in natural soils, and when these strains were inoculated to remediate PCB-contaminated soils, the resultant was the failure of bioaugmentation (Blasco et al., 1995). Further investigations revealed that formation of an antibiotic compound, protoanemonin, from 4-chlorocatechol via the classical 3-oxoadipate pathway by the native microorganisms was the reason for poor survival of the introduced specialist PCB-degrading strains. Indeed, bioaugmentation itself is undesirable in all the environmentally sensitive locations, especially those protected from the introduction of exotic flora or fauna. Scott et al. (2010) proposed a new strategy of using a free enzyme-based product to remediate water bodies contaminated with atrazine. The ecological or environmental issues associated with degrading organisms can be circumvented by this strategy. The soils do have exoenzymes (cell-free enzymes) which include proteases, and the presence of proteases along with other inhibitors may limit the longevity of free enzymes applied for bioremediation. The cell-free approach can only be used for viable and efficient enzymes that are not dependent on diffusible co-factors such as NAD (particularly hydrolases), and cannot be applied in cases where the enzyme activity (e.g., most oxygenases) is lost when the cells are broken (Scott et al., 2008). Orica Watercare (Australia) has commercialized for the first time a free-enzyme for phosphotriester insecticides under the trade name LandGuardTM which was proven to be successful and cost effective. Nevertheless, the technical feasibility of such strategy needs careful evaluation for many contaminants or their mixtures. Immobilizing enzymes on suitable carriers will make them more stable and resistant to changes in pH, temperature and substrate concentrations (Gainfreda and Rao, 2004 and Kandelbauer et al., 2004). Other limitations for enzymes include: (a) expensive production costs for pure enzymes, (b) reduced activity due to sorption in soils requiring repeated doses, and (c) the issues with delivery of enzymes, immobilized enzymes in particular, to come in contact with the pollutant in the contaminated site. Selection of suitable carrier materials for immobilizing enzymes will not only help to increase their longevity but also allow their re-use thus making them more cost-effective. Further research into cheap nutrient sources for growing microorganisms may lower production costs of pure enzymes. Also, more research is required into the mechanisms of delivery of enzymes for their in situ application.

Most of the biosurfactants are anionic or nonionic; the structure is a characteristic of the microorganism producing the surfactant under the specific growth conditions (Mulligan and Gibbs, 1993 and Zhang and Miller, 1995). Relative to a synthetic surfactant (Tween-80), the biosurfactant (rhamnolipid) was found to enhance the solubility and the subsequent degradation of phenanthrene by Sphingomonas sp. (Pei et al., 2010). The biosurfactants can be toxic or even utilized preferentially by the pollutant-degrading microorganisms. But, the application of biosurfactant-producing and pollutant-degrading microorganisms offers dual advantages of a continuous supply of biodegradable surfactant and the ability to degrade pollutant(s) (Moran et al., 2000 and Rahman et al., 2002). In a recent report, Hua et al. (2010) demonstrated that a salt-tolerant Enterobacter cloacae mutant could be used as an agent for bioaugmentation of petroleum- and salt-contaminated soil due to increased K+ accumulation inside and exopolysaccharide level outside the cell membrane.

Microorganisms respond differently to various kinds of stresses and gain fitness in the polluted environment. This process can be accelerated by applying genetic engineering techniques. The recombinant DNA and other molecular biological techniques have enabled (i) amplification, disruption, and/or modification of the targeted genes that encode the enzymes in the metabolic pathways, (ii) minimization of pathway bottlenecks, (iii) enhancement of redox and energy generation, and (iv) recruiting heterologous genes to give new characteristics (Liu et al., 2006, Shimizu, 2002 and Timmis and Piper, 1999). Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation (Pieper and Reineke, 2000). New information on the metabolic routes and bottlenecks of degradation is still accumulating, requiring the need to reinforce the available molecular toolbox (Stegmann, 2001). Nevertheless, the introduced genes or enzymes, even in a single modified organism, need to be integrated within the regulatory and metabolic network for proper expression (Cases and Lorenzo, 2005).

There are some drawbacks with the field release of genetically engineered microorganisms (GEMs), which include the decreased levels of fitness and the extra energy demands imposed by the presence of foreign genetic material in the cells (Saylor and Ripp, 2000 and Singh et al., 2011). More importantly, there remains a great risk of mobile genetic elements entering the environment and being acquired by undesirable organisms. The biotechnological innovations for making GEMs are numerous. According to Pandey et al. (2005), the advances such as the programmed cell death based on the principle of killer–anti-killer gene(s) after detoxification can help to develop 'suicidal genetically engineered microorganisms' (S-GEMs) that can lead to safe and efficient bioremediation. Few GEMs have been used for field application because of strict regulations for the release of GEMs into the environment (Ezezika and Singer, 2010). The only GEM approved for field testing in the USA for bioremediation was Pseudomonas fluorescens HK44, possessing a naphthalene catabolic plasmid (pUTK21), mutagenized by transposon insertion of lux genes (Ripp et al., 2000). The transition of genetically engineered microorganisms from the laboratory to the field environments is hampered due to the lack of information on the population dynamics of introduced genetically engineered microorganisms in the field and poor physiological control of catabolic gene expression in the engineered organisms under nutrient and other stresses (Cases and Lorenzo, 2005). The bioengineering and environmental release of those engineered microorganisms has to overcome several obstacles which include inconsistencies in risk assessment procedures and public health concerns before their effective application in the field. Selecting an indigenous bacterium able to grow rapidly and withstand the local stressful conditions for genetic engineering to enhance the biodegradation capabilities will be more advantageous over other bacterial strains. We hope, in 5 to 10 years from now, research into the field release of GEMs will help in designing them for alleviation or prevention of any perceived risks and eventually gaining public and regulatory acceptance in bioremediation of contaminated sites.

4.3 Bioremediation technologies

Bioremediation technologies based on the principles of biostimulation and bioaugmentation include bioventing, land farming, bioreactor, and composting. From these technologies which are at different stages of development in terms of experimentation and acceptance, the choice of technology option can be made considering many factors which include the class of organic contaminants and the cost of operation. Sebate et al. (2004) proposed a protocol for biotreatability assays in two phases, for the successful application of bioremediation technology. In the first phase, the type and metabolic activity of indigenous microorganisms at the polluted site and the presence of possible inhibitors are to be assayed to know whether bioremediation itself is appropriate. In the second phase, the influences of nutrients, surfactant, and specialized inocula amendment are to be evaluated in microcosms to identify the appropriate treatment for the polluted site. Recently, Bento et al. (2005) reiterated the need for a detailed site-specific characterization studies since the soil properties and the indigenous soil microbial population affect the degree of biodegradation. These conclusions were drawn from a comparative study on natural attenuation, biostimulation and bioaugmentation on degradation of total petroleum hydrocarbons (TPHs) in contaminated soils collected from Long Beach, California, USA and Hong Kong, China. Improvements in reliability, cost efficiency and speed of remediation can be achieved by the use of various methods ranging from minimal intervention (bioattenuation), through in situ introduction of nutrients and/or bacterial inocula, improvements of physicochemical conditions or development of novel methods (Romantschuk et al., 2000).

The fate of pollutants is largely influenced by the competing processes of degradation and sorption which refers to both adsorption, occurring on surfaces (e.g., between a charged compound and clay) and absorption, i.e., the sorption beyond the surface into a separate portion defined by the surface (e.g., partitioning into organic matter). The soil sorption of neutral, and hydrophobic compounds is dependent on soil and sediment organic content; one of the useful parameters for describing sorption of neutral, and hydrophobic compounds is the organic carbon partition coefficient (Koc) that is correlated to its Kow(Karickhoff et al., 1979). For successful bioremediation treatment, the pollutants as substrates must be available and accessible either to microorganisms or their extracellular enzymes for metabolism to occur. Another important limiting factor is microbial movement. Because of low bioavailability and accessibility of pollutants, biphasic ('hockey stick') kinetics of biodegradation, consisting of an initial period of fast degradation, followed by a second, much slower phase, is commonly observed in soils and sediments during bioremediation (Semple et al., 2004). These constraints drive a constant demand for developing innovative treatment methods.

4.3.1 Composting

Traditionally, the practice of composting is intended to reduce volume and water content of vegetable wastes, to destroy pathogens, and to remove odor-producing compounds. This technology is now applied for handling polluted soil or sediments by two chief ways: (i) composting of polluted soils for efficient degradation, and (ii) addition of composted materials. Additions of composted material were found to improve degradation of two herbicides, benthiocarb (S-4-chlorobenzyl diethylthiocarbamate) and MCPA (4-chloro-2-methylphenoxyacetic acid) in soil (Duah-Yentumi and Kuwatsuka, 1980). Van Gestel et al. (2003) reported that the impact of diesel on the composting process was negligible when soil was spiked with diesel oil and mixed with biowaste (vegetable, fruit and garden waste) at a 1:10 ratio (fresh weight) and composted in a monitored composting bin system. The spent mushroom waste from ***Pleurotus ostreatus*** was found to degrade and mineralize DDT in soil (Purnomo et al., 2010). On the contrary, Alvey and Crowley (1995)observed that additions of compost suppressed soil mineralization of atrazine relative to rates in unamended soils or in soils amended with starch or rice hulls, probably due to the high nitrogen content of the compost.

The critical parameters for composting depend on the type of contaminants and waste materials to be used for composting. The composting efficiency essentially depends on temperature and soil/waste amendment ratio as the two important operating parameters for bioremediation (Antizar-Ladislao et al., 2005). According to Baheri and Meysami (2002), the increase in the bulking agents such as peat moss, pine wood shavings, bran flakes, or a mixture of these agents from 6 to 12% led to an increase of 4–5% in the biodegradation of total petroleum hydrocarbons. In another study, the soil amendment with sludge-only or compost-only in a ratio of 1:0.1, 1:0.3, 1:0.5, and 1:1 (soil/amendment, wet weight basis) increased the rates, but higher mix ratios did not increase the degradation rates of total petroleum hydrocarbons correspondingly (Namkoong et al., 2002). For the optimum removal of aged PAH during composting, Guerin (2000) recommended to keep moisture and amendment ratio constant. During the composting-bioremediation, not only the contaminant but also the waste amendment and the operating conditions will determine the rate of biodegradation.

Organic pollutants can be degraded during the first phase of rapid decomposition during composting. Heat which is generated by microbial metabolism is trapped in the compost matrix and most of the microbial decomposition and biomass formation occur during the thermophilic stage of composting. The mixing of remediated soil with contaminated soil can increase the effectiveness of composting because the remediated soil with acclimated microorganisms significantly influences pollutant degradation in the composting process (Hwang et al., 2001). The mineralization may be only a small fraction of pollutant degradation, with other prominent fates being partial degradation to secondary compounds, volatilization, and adsorption to compost (Buyuksonmez et al., 1999). In the composting matrices, microorganisms can degrade pollutants into innocuous compounds, transform pollutants into less toxic substances and/or aid in locking up the chemical pollutants within the organic matrix, thereby reducing pollutant bioavailability. Even in the compost remediation strategy, the bioavailability and biodegradability of pollutants are the two most important factors which determine the degradation efficiency (Semple et al., 2001). Cai et al. (2007) showed that the efficiency of composting processes differed among the manually turned compost, inoculated manually turned compost, continuously aerated compost and intermittently aerated compost for bioremediating sewage sludge contaminated with PAHs, with the intermittently aerated compost treatment showing higher removal rate of high molecular weight PAHs. Composting or the use of composted materials can be applied to the bioremediation of polluted soils. However, the nature of waste or soil organic matter that consists of humic materials play an important role in binding of the contaminants such as PAHs and making them accessible to microbes for degradation. Plaza et al. (2009) reported that composting will induce significant modifications to the structural and chemical properties of the humic material fraction including loss of aliphatic materials, an increased polarity and aromatic polycondensation resulting in a decrease in PAH-binding. Recently, Sayara et al. (2010) demonstrated that stable composts in municipal solid wastes enhanced biodegradation of PAH particularly during the initial phase of composting. Humic material which accumulates with an increase in stability of the compost is known to act like a surfactant and plays an important role in releasing PAHs sorbed to the soil. PAH degradation mostly occurs during mesophilic stage of composting, while thermophilic stage is inhibitory for biodegradation (Antizar-Ladislao et al., 2004,Haderlein et al., 2006 and Sayara et al., 2009).

Similar to any other technology, composting has both advantages and limitations. Addition of compost to contaminated soil for bioremediation makes it a sustainable technology since the biodegradable organic waste in the compost is being utilized for beneficial activity. Also, composting improves the soil structure, nutrient status and microbial activity. During composting the contaminant can disappear via different mechanisms such as mineralization by microbial activity, transformation to products, volatilization, and formation of nonextractable bound residues with organic matter. The fate of nonextractable bound residues of contaminants in composting is another area of interest that requires more research into their release, behavior and risk. One of the critical knowledge gaps of composting is lack of sufficient knowledge about microorganisms involved during various stages of composting, the thermophilic stage in particular, which is almost like a blackbox. In fact, there are conflicting views about the role of the thermophilic stage of composting in bioremediation of contaminants. Added to this complexity is the fate of bound residues and whether or not they pose a risk in the future. Knowledge about (a) the nature and activity of microorganisms involved in various stages of composting, and (b) the degree of stability of compost and its humic matter content will greatly assist in better designing of composting as a bioremediation strategy for contaminated soils.

4.3.2 Electrobioremediation

Electrobioremediation as a hybrid technology of bioremediation and electrokinetics for the treatment of hydrophobic organic compounds is becoming popular. It involves passage through polluted soil of a direct current between appropriately distributed electrodes and uses microbiological phenomena for pollutant degradation and electrokinetic phenomena for the acceleration and orientation of transport of pollutants (or their derivatives) and the pollutant-degrading microorganisms (Chilingar et al., 1997 and Li et al., 2010). The electrokinetics is the use of weak electric fields of about 0.2 to 2.0 V cm^{-1} to soil (Saichek and Reddy, 2005) and the basic phenomena which make up electrokinetic remediation are diffusion, electrolysis, electroosmosis, electrophoresis, and electromigration. Since the present electrokinetic approaches mainly aim at pollutant extraction through transport over large distances, the impact of direct current on organism–soil interactions and organism–compound is often neglected (Wick et al., 2007). Shi et al. (2008) showed that direct current ($\boldsymbol{X}$ = 1 V cm^{-1}; $\boldsymbol{J}$ = 10.2 mA cm^{-2}) as typically used for electrobioremediation measures had no negative effect on the activity of a PAH-degrading soil bacterium (***Sphingomonas*** sp. LB126), and the DC-exposed cells exhibited up to 60% elevated intracellular ATP levels and yet remained unaffected on all other levels of cellular integrity and functionality. Information on the direct reduction or oxidation of the pollutant at the electrode and the changes in microbial community due to generation of hydrogen or oxygen at the electrode is limited.

Luo et al. (2005) developed the non-uniform electrokinetic system with periodic polarity-reversal to accelerate the movement and ***in situ*** biodegradation of phenol in a sandy loam soil. Although reversing the polarity of an electric field increased the consumption of electricity, a higher and more uniform removal of phenol from the soil was observed. The 2-dimensional (2-D) non-uniform electric field enhanced the ***in situ*** bioremediation process by promoting the mass transfer of organics to degrading bacteria. When tested at bench-scale with a sandy loam soil and 2,4-dichlorophenol (2,4-DCP) at bidirectional and rotational modes, the 2-D non-uniform electric field stimulated the desorption and the movement of 2,4-DCP. About 73.4% of 2,4-DCP was removed at the bidirectional mode and about 34.8% was removed at the rotational mode, which also maintained remediation uniformity in soil, in 15 days (Fan et al., 2007). In an electrochemical cell packed with an inert support, the application of low intensity electric current led to the degradation of hexadecane as well as higher biomass production by ***Aspergillus niger*** (Velasco-Alvarez et al., 2011).

During electrobioremediation, the transport of PAH-degrading bacteria, ***Sphingomonas*** sp. L138 and ***Mycobacterium frederiksbergense*** LB501 from the surface into the subsurface occurred due to electroosmosis (Wick et al., 2004). Niqui-Arroyo and Ortega-Calvo (2007) integrated biodegradation and electroosmosis for the enhanced removal of PAHs from the creosote-polluted soils. The residual concentrations of total biodegradable PAHs, remaining after bioremediation in soil slurries, were two-fold lower in electrokinetically pretreated soils than in untreated soils. The remediation rate of ***in situ*** bioremediation will be otherwise very slow due to limited mass transfer of pollutants to the degrading bacteria. Very recently, Maillacheruvu and Chinchoud (2011) demonstrated synergistic removal of contaminants by the electrokinetically transported aerobic microbial consortium.

There are limitations with electrobioremediation technology that need to be overcome, and these include: (i) solubility of the pollutant and its desorption from the soil matrix, (ii) the availability of the right type of microorganisms at the site of contamination, (iii) the ratio between target and nontarget ion concentrations, (iv) requirement of a conducting pore fluid to mobilize pollutants, (v) heterogeneity or anomalies found at sites, such as large quantities of iron or iron oxides, large rocks or gravel, and (vi) toxic electrode effects on microbial metabolism or dielectric cell membrane breakdown or changes in the physicochemical surface properties of microbial cells.

4.3.3 Microbe-assisted phytoremediation

Pollutant effects on plant growth are concentration-dependent and different plant species respond differently. Low doses of pollutant can increase plant weight while high doses can inhibit, a phenomenon referred to as 'hormesis' (Calabrese and Blain, 2009). In general, plants can promote dissipation of organic pollutants by immobilization, removal, and promotion of microbial degradation. Some organic compounds are transported across plant membranes, released through leaves via evapotranspiration (phytovolatilization) or extracted, transported and accumulated in plant tissues (phytoextraction) or degraded via enzymatic processes (phytodegradation). Some of the non-volatile compounds are sequestered ***in planta*** and are less bioavailable (phytostabilization). Several limitations of bioremediation such as the inability of degrading microorganisms to compete with indigenous microflora, insufficient microbial activities at sub-surface, poor support of native as well as pollutant-degrading microflora by available or limiting nutrients, heterogeneity of bioavailable contaminants, and toxic or inhibitory compounds in the pollutant mixture requires the union of phytoremediation and other bioremediation strategies (Gerhardt et al., 2009).

Plants have several kilometers of roots per hectare, suggesting the potential of pollutant degradation in the rhizosphere (Boyajian and Carreira, 1997). Sugars, organic acids, and larger organic compounds which constitute about 10–50% of plant's photosynthate are deposited in soils (Kumar et al., 2006), and the carbon cycling from CO_2 assimilation by plants to root exudation to incorporation to microbial biomass to microbial respiration takes about just 5 h (Ostle et al., 2003). In the rhizosphere which is dependent on morphology, proportion of fine roots, water and nutrient conditions, root exudation, and associated microbial communities, there may be either promotion or competition between the pollutant degraders and other microbial members.Ma et al. (2010) suggested from a meta-analysis that the activity of PAH decomposers in soil is more likely to be enhanced by root activities than to be inhibited by other microorganisms in the rhizosphere, despite the variations due to species, habitats, contamination types and doses. The complex aromatic compounds such as flavonoids and coumarins which aid microbial colonization of roots are structurally similar to PCBs, PAHs and PHC, providing opportunities as the analogue-enrichment for stimulating degradative pathways in microorganisms (Holden and Firestone, 1997). Rhizoremediation, an integral component of phytoremediation can occur naturally or can be triggered by introducing specific pollutant-degrading microbes or plant growth promoting microorganisms (Gerhardt et al., 2009). Since the root depth of herbaceous plants varies from plant to plant, from soil to soil, and season to season, the presence of contaminants in soils which is deeper than the root zone of plants requires excavation, other agronomic practices or selection of trees with deeper roots. Nevertheless, most of the recalcitrant organic contaminants are typically found in the top few cm of the soil. Dendroremediation, which is a type of phytoremediation using trees may be useful in attenuating certain pollutants such as 2,4,6-trinitrotoluene and trichloroethylene from soil and groundwater (Susarla et al., 2002).

Plants produce many secondary plant metabolites (SPMEs) which include allelopathic chemicals, root exudates, phytohormones/phytoalexins, phytosiderophores, and phytoanticipins and are derived from isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide pathways (Hadacek, 2002). Singer et al. (2004)argued that SPMEs are pollutant analogues within the network of suprametabolism, having implications for predicting the fate of pollutants. Gilbert and Crowley, 1998 and Kim et al., 2003 showed that SPMEs such as limonene, cymene, carvone and pinene enhanced degradation of PCBs. ***Pseudomonas putida*** PCL1444, isolated from the rhizosphere of ***Lolium multiflorum*** cv. Barmultra when grown in PAH-polluted soil degraded the PAHs and protected the plant from the pollutant, by efficient utilization of root exudates for growth and high transcription of naphthalene catabolic genes (Kupier et al., 2002). Narasimhan et al. (2003) applied the rhizosphere metabolomics-driven approach, which has been referred to profiling of root exudates for identification of targeted compounds for creating the nutritional bias, to degrade PCBs (2Cl-biphenyl, 4Cl-biphenyl and Aroclor 1254 at 53 µM) in the rhizosphere of ***Arabidopsis***. The growth of ***gfp***-tagged***Pseudomonas putida*** PML2 was increased due to the exudation of SPMEs such as phenylpropanoids and consequently PCB degradation was enhanced. The rhizosphere metabolomics-driven approach will become an important tool for engineering phytoremediation systems.

The activity and the numbers of the pollutant-degrading endophytes are both plant- and contaminant-dependent (Siciliano et al., 2001). Contaminants such as TCE and methyl ***tert***-butyl ether which are routinely assimilated in the transpiration pathways of plants may be degraded effectively by the pollutant-degrading endophytes. ***Methylobacterium*** sp. strain BJ001, a phytosymbiotic bacterium isolated from tissue culture plantlets of ***Populus deltoides*** × ***nigra*** DN34 was found to transform 2,4,6-trinitrotoluene and mineralize hexahydro-1,3,5-trinitro-1,3,5-triazine and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine to CO_2 (Van Aken et al., 2004). Barac et al. (2004) demonstrated that the engineered endophyte (***Burkholderia cepacia*** strain L.S.2.4 containing the toluene-degrading plasmid, pTOM), when applied to surface-sterilized yellow lupine seeds led not only to the protection against the phytotoxic effects of toluene but also decreased emissions from the transpiration stream of its host. The pollutant-degrading endophytes are relatively free from the competition for nutrients and water among the colonizers in the rhizosphere. Greater opportunities for employing the endophyte-assisted phytoremediation, either through naturally-occurring or engineered endophytes exist, especially for the mobile pollutants. Phytostimulation of pollutant degradation by microorganisms in the rhizosphere or inside the plants can offer many economic and environmental advantages compared to the conventional strategies employed in biostimulation. But, the disadvantages include hydrophobicity and chemical stability of pollutants that influence the phytostabilization and the rates of degradation by the associated microorganisms (Van Aken et al., 2010), and plant root exudation which modifies the structure and activities of pollutant-degrading microorganisms (Corgie et al., 2004). Besides, phytoremediation in the field is also challenged by many obstacles which include the inability to mitigate plant stress factors and non-availability of suitable methods for the assessment of phytoremediation (Gerhardt et al., 2009).

4.4 Bioremediation monitoring and efficacy testing

Monitoring and efficacy testing for bioremediation are essential for the purposes of efficiency and economics. There is a strong need to test the efficacy. The 'conservative biomarkers', the internal markers such as dimethyl chrysene which are recalcitrant can be used to test the efficacy of bioremediation (Huang et al., 2005). The concentration of an individual pollutant can be normalized to the internal marker and the relative ratio of a specific pollutant to the internal marker should decrease during the remediation process. Indices such as the carbon preference index, average chain length and various ***n***-alkane/acyclic isoprenoid ratios which are used for the chemical fingerprinting in the environmental forensics can be applied to distinguish the plant- or microbe-derived hydrocarbons from the hydrocarbons of petrogenic or anthropogenic origin. The epicuticular waxes derived from leaf cuticles are generally abundant with odd-numbered ***n***-alkane peaks in the range of C_{25}–C_{31}, while the even-numbered carbon compounds are abundant in petroleum. The carbon preference indices indicating the ratio of odd-numbered to even-numbered carbon compounds provide information on the predominance of phytogenic or petrogenic hydrocarbon contamination (Jeng, 2006).

Several microbiological methods are currently employed for the general soil quality assessment. The monitoring and efficacy testing for bioremediation require a careful selection from them, besides using specific information on abundance of microbial members or genes, and microbial processes and activities since factors such as water content, temperature and many others determine the course of attenuation. The global regulatory networks in which sets of operons, scattered on the bacterial genome and representing disparate functions such as response to nutrient starvation are coordinately controlled in microorganisms (Gottesman, 1984). The signal transduction and effector proteins which are involved in the nitrogen regulation (Reitzer, 2003) or the involvement of ***cra***, ***crp*** and ***relA/spoT*** modulons and the accumulated levels of alarmone guanosine 3′,5′-bis(diphosphate) and cAMP (Hardiman et al., 2007) can be the basis of a means to monitor changes in nutrient limitation of microbial response during the bioremediation process. Many functional genes namely, ***nahAc***, ***alkB*** and ***xylE*** which are involved in the degradation of naphthalenes, ***n***-alkanes and toluene, respectively are known from the cultured microorganisms. With optimized assays, the functional gene abundances which seem to reflect the type as well as the actual degradation rates can be used to assess the efficacy of bioremediation (Salminen et al., 2008). Recently,Kao et al. (2010) used the culture-based method, real-time polymerase chain reaction of genes such as phenol hydroxylase, ring-hydroxylating toluene monooxygenase, naphthalene dioxygenase, toluene monooxygenase, toluene dioxygenase and biphenyl dioxygenase, and denaturing gradient gel electrophoresis fingerprinting analysis for microbial communities to evaluate the effectiveness of bioremediation of a petroleum contaminated site. Compound specific carbon isotope (CSI) analysis has emerged recently as a powerful tool to quantify and/or distinguish biodegradation from other abiotic processes such as sorption, volatilization etc. of contaminants like chlorinated solvents (TCE, PCE, DCE) and aromatic hydrocarbons (benzene, toluene, xylene, ethyl benzene, naphthalene, etc.) and to confirm intrinsic biodegradation during natural attenuation process in the contaminated aquifers (Fischer et al., 2007,Fischer et al., 2008, Hunkeler et al., 2005 and Meckenstock et al., 2004). In stable carbon isotope analysis, the lighter isotope is preferentially utilized by microorganisms leaving behind the heavier isotope thereby resulting in a distinct fractionation pattern among ^{12}C and ^{13}C.

Toxicity testing should be an integral part of the bioremediation program since a reduction in toxicity is a necessary characteristic of bioremediation process. The toxicity of a pollutant to microorganisms is also regarded as a direct measure of bioavailability (Ronday et al., 1997). Megharaj et al. (2000) suggested that chemical analysis in conjunction with bioassays were necessary for toxicological estimations. Despite the importance of toxicological assays, only few bioremediation studies have attempted to include one or two such assays. The toxicity of fuel spills followed by bioremediation treatment was assessed by Microtox measurements, seed germination and plant growth assays (Leung et al., 1997 and Wang and Bartha, 1990). Although the standardized toxicity test system such as Microtox which employs ***Vibrio fischeri***, a bioluminescent marine bacterium, has certain advantages, the ecological relevance of toxicity tests can be improved by use of ecologically relevant (aquatic or terrestrial) representatives from different trophic levels. The efficacy of bioremediation (bioaugmentation with ***Pseudomonas*** sp. strain ADP or biostimulation with citrate) of atrazine-contaminated soils was tested by ecotoxicological endpoints such as plant biomass production, earthworm reproduction, microalgae growth, and cladoceran reproduction (Chelinho et al., 2010). Since no single organism is consistently sensitive to all pollutants, it is pertinent to include a battery of bioassays, by involving members from different trophic levels of the food chain. Every bioremediation technology thus requires the use of experimental controls and performance indicators for both process optimization and implementation of regulatory decisions.

5 PHYTOREMEDIATION

Phytoremediation is an emerging green technology that uses plants to remediate soil, sediment, surface water, and groundwater environments contaminated with toxic metals, organics, and radionuclides (Pradhan et al., 1998).

Phytoremediation is an effective, non-intrusive, and inexpensive means of remediating soils. It is more cost-effective than alternative mechanical or chemical methods of removing hazardous compounds from the soil. Phytoremediation is a natural, aesthetically pleasing low-cost technology. It is socially accepted by surrounding communities and regulatory agencies as a potentially elegant and beautiful technology.

Although phytoremediation has been employed to remediate environments contaminated with metals, radionuclides, organics, etc. there appear to be many more works on the search for hyperaccumulators of metals than any other topic. In fact, most publications appear to deal with phytoextraction of metal contaminants. Besides, most phytoremediation review papers focus on the phytoextraction of metals and dedicate only a few paragraphs to the phytoremediation of organics.

As overwhelmingly positive results have become available regarding the ability of plants to degrade certain organic compounds, more and more people are getting involved in the phytoremediation of organic contaminants. Actually, the concept of using plants to remediate soils contaminated with organic pollutants is based on observations that disappearance of organic chemicals is accelerated in vegetated soils compared with surrounding nonvegetated bulk soils (Burken and Schnoor, 1996 and Cunningham and Berti, 1993).

Phytoremediation of organic contaminants has generally focused on three classes of compounds: chlorinated solvents, explosives and petroleum hydrocarbons. Nonetheless, in recent years, researchers have begun to address the potential of phytoremediation to treat other organic contaminants including polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).

Although correlations between agronomic performance and phytoremediation potential are yet to be determined, better agronomic performance of the plant may improve phytoremediation. Plants that are less affected by compounds in contaminated soils are healthier and more persistent and will produce healthier root systems and greater top growth.

There are two different strategies for the phytoremediation of organics: ***direct phytoremediation*** and ***phytoremediation explanta*** (Anderson et al., 1993, Cunningham et al., 1995 and Salt et al., 1998). The latter is based on the secretion by plants of their photosynthate in root exudates, which support the growth and metabolic activities of diverse fungal and bacterial communities in the rhizosphere. Some organic compounds in root exudates (i.e., phenolics, organic acids, alcohols, proteins) may serve as carbon and nitrogen sources for growth and long-term survival of microorganisms that are capable of degrading organic pollutants. Densities of rhizospheric bacteria can be as much as two to four orders of magnitude greater than populations in the surrounding bulk soils as well as displaying a greater range of metabolic.

The chemical composition of root exudates and rates of exudation differ considerably among plant species. This has led some research groups to screen for plant species that exude phenols capable of supporting PCB-degrading bacteria (Fletcher and Hedge, 1995 and Salt et al., 1998). Since not all plants produce and release the same types of phenolics (Rao, 1990), it would be expected that some plants may preferentially harbor PCB-degrading (polychlorinated phenols) bacteria in their rhizosphere (Wenzel et al., 1999). Fletcher and Hedge (1995) screened 17 different perennial plants for release of phenols that could support PCB-degrading microbes and found that mulberry (***Morus rubra*** L.) had many attributes that would favor its use in phytoremediation efforts.

Rhizospheric microorganisms may also accelerate remediation processes by volatilizing organics such as PAHs (polynuclear aromatic hydrocarbons) or by increasing the production of humic substances from organic pollutants (Cunningham et al., 1996 and Dec and Bollag, 1994). Similar experiments have been performed to remediate soils contaminated with TCE (trichloroethylene) and TNT (trinitrotoluene).

In addition to secreting organic compounds that support the growth and activities of rhizospheric microorganisms, plants also release a number of enzymes into soils and waters and these enzymes degrade organic contaminants. Soil enzymes derived from plant sources include laccases, dehalogenases, nitroreductases, nitrilases and peroxidases. Field tests of plant-derived nitroreductases and laccases showed significant degradation of ammunition wastes (TNT, dinitromono-aminotoluene, and mononitrodiaminotoluene) and triaminotoluene, respectively (Wolfe et al., 1993). Boyajian and Carreira (1997) reported on the ability of nitroreductase to degrade various additional nitroaromatic compounds. Similarly, other studies have examined the ability of a nitrilase to degrade 4-chlorobenzonitrile and of halogenases to metabolize hexachloroethane and TCE (Wenzel et al., 1999). The degree of enzyme release into soils and sediments remains poorly understood but the measured half-life of these enzymes suggests they may actively degrade soil contaminants for days following their release from plant tissues (Schnoor et al., 1995).

The presence of plant-derived enzymes capable of degrading environmentally problematic xenobiotics will no doubt be exploited for the development of future phytoremediation strategies (Salt et al., 1998).

By analogy with the phytoextraction of metals, direct uptake of organic contaminants is primarily limited by the availability of the target compound and uptake mechanisms (Salt et al., 1998). With a few notable exceptions, movement of organics into plants occurs via the liquid phase of the soil, which has been extensively investigated in plants for uptake of pesticides and herbicides (Briggs et al., 1982, Paterson et al., 1990 and Topp et al., 1986).

The primary factors that govern the uptake of xenobiotics are the physicochemical characteristics of the compound, i.e., the octanol-water partition coefficient, log K_{ow}, acidity constant, pK_a, concentration and others (Wenzel et al., 1999). Organics that are most likely to be taken up by plants are moderately hydrophobic compounds with octanol–water partition coefficients ranging from 0.5 to 3 (Briggs et al., 1982,Ryan et al., 1988 and Wenzel et al., 1999). In addition to factors that govern the bioavailability of organics for uptake, there appears to be a significant disparity in the uptake and translocation of organics among plant species, as observed for nitrobenzene (MacFarlane et al., 1990) and atrazine (Anderson and Walton, 1995 and Burken and Schnoor, 1996). Differences in evapotranspiration rates that are known to have a marked effect on the contaminant uptake could explain these differences.

As mentioned above, bioavailability of organics in soils appears to be a primary restriction for effective phytoremediation of organic pollutants (Cunningham et al., 1996, Schnoor et al., 1995 and Salt et al., 1998). The application of soil amendments has been considered a major breakthrough in the development of "induced" (as opposed to "continuous") metal phytoextraction strategies. Unfortunately, similar attempts have not been made in relation to organic uptake of plants. The use of synthetic (triton X-100, SDS) and naturally-produced biosurfactants (rhamnolipids) to enhance the apparent water solubility and bacterial degradation of organic contaminants is well documented (Bragg et al., 1994, Desai and Banat, 1997,Providenti et al., 1995, Van Dyke et al., 1993 and Zajic and Panchel, 1976). It has also been reported (Brusseau et al., 1997) that cyclodextrins increase the solubilities of both organics and metals. Potential advantages of using biosurfactants or cyclodextrins which have the ability to solubilize both organics and metals (Miller, 1995 and Nivas et al., 1996) could be instrumental in remediation of soils with mixed contaminants.

If plants are to be used for phytoextraction of organic contaminants it is essential to determine the fate of the parent compounds and their metabolites. The partitioning of organics between roots and aboveground tissues varies considerably depending on the chemical in question. Following uptake, organic compounds may have multiple fates: they may be translocated to other plant tissues and subsequently volatilized, they may undergo partial or complete degradation or they may be transformed to less toxic compounds and bound in plant tissues to nonavailable forms. Schroll et al. (1994) found that hexachlorobenzene (HBC) and octachlorodibenzo-p-dioxin (OCDD) could be taken up by roots or leaves but that no translocation from roots to shoots or vice versa was observed. On the other hand, root and foliar uptake of the herbicides chlorobenzene and trichloroacetic (TCA) was followed by translocation in both directions (Wenzel et al., 1999). Preferential concentration in the roots has also been documented for TNT and aniline and in shoots for hexahydro-1,3,5-trinitro-1,3,5-trizazine (RDX), phenol, and quinoline following uptake by roots (Cataldo et al., 1987, Fellows et al., 1996 and Wenzel et al., 1999).

Most organics appear to undergo some degree of transformation in plant cells before being sequestered in vacuoles or bound to insoluble cellular structures, such as lignin (Salt et al., 1998). However, few chemicals appear to be fully mineralized by plants to water and CO2, and where this does occur, it represents only a small percentage of the total parent compound (Newman et al., 1997). This property puts plants at a relative disadvantage compared with bacteria in degrading organic pollutants. In addition, the possibility that plant metabolites of pollutants may be more toxic than the original pollutants creates a difficult regulatory environment for remediation of organics.

6 METAL REMOVAL

6.1 Problem

Metals contamination is a persistent problem at many contaminated sites, approximately 75% of contaminated areas face that problem. The most commonly occurring metals at these sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), and mercury (Hg). Figure 1 summarizes the frequency with which these metals occur at restoration sites in USA.

The presence of metals in groundwater and soils can pose a significant threat to human health and ecological systems. The chemical form of the metal contaminant influences its solubility, mobility, and toxicity in ground-water systems. The chemical form of metals depends on the source of the metal waste and the soil and ground-water chemistry at the site. A detailed site characterization must be performed to assess the type and level of metals present and allow evaluation of remedial alternatives.

Typically metals are relatively immobile in subsurface systems as a result of precipitation or adsorption reactions. For this reason, remediation activities at metals-contaminated sites have focused on the solid-phase sources of metals, i.e., contaminated soils, sludges, wastes, or debris. A range of technologies is available for remediation of metals-contaminated soil and groundwater at contaminated sites. General approaches to remediation of metal contamination include isolation, immobilization, toxicity reduction, physical separation and extraction. These general approaches can be used for many types of contaminants but the specific technology selected for treatment of a metals-contaminated site will depend on the form of the contamination and other site-specific characteristics. One or more of these approaches are often combined for more cost-effective treatment. A number of the available technologies have been demonstrated in full-scale applications and are presently commercially available. A comprehensive list of these technologies is available (U.S. EPA, 1996). Several other technologies are being tested for application to metals-contaminated sites.

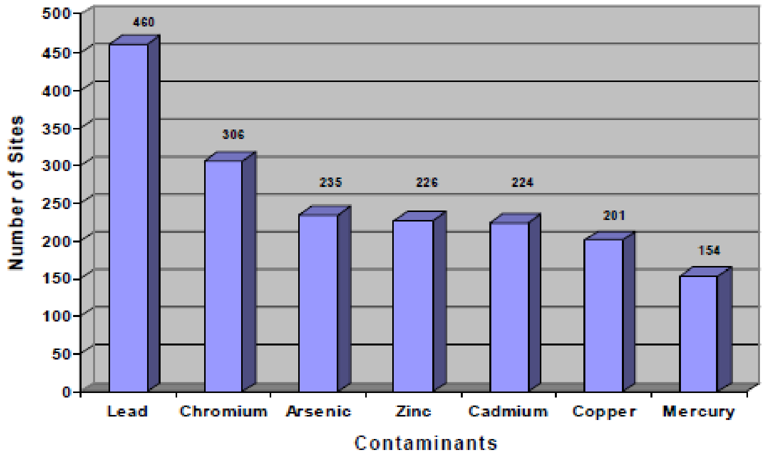

Figure 1. Metals Most Commonly Present in all Matrices at Superfund Sites (from u.s. EPA, 1996).

6.2 Sources of contaminants

Surface water and groundwater may be contaminated with metals from wastewater discharges or by direct contact with metals-contaminated soils, sludges, mining wastes, and debris. Metal-bearing solids at contaminated sites can originate from a wide variety of sources in the form of airborne emissions, process solid wastes, sludges or spills. The contaminant sources influence the heterogeneity of contaminated sites on a macroscopic and microscopic scale. Variations in contaminant concentration and matrix influence the risks associated with metal contamination and treatment options.

6.2.1 Airborne Sources

Airborne sources of metals include stack or duct emissions of air, gas, or vapor streams, and fugitive emissions such as dust from storage areas or waste piles. Metals from airborne sources are generally released as particulates contained in the gas stream. Some metals such as arsenic, cadmium, and lead can also volatilize during high-temperature processing. These metals will convert to oxides and condense as fine particulates unless a reducing atmosphere is maintained (Smith et al., 1995).

Stack emissions can be distributed over a wide area by natural air currents until dry and/or wet precipitation mechanisms remove them from the gas stream. Fugitive emissions are often distributed over a much smaller area because emissions are made near the ground. In general, contaminant concentrations are lower in fugitive emissions compared to stack emissions. The type and concentration of metals emitted from both types of sources will depend on site-specific conditions.

6.2.2 Process Solid Wastes

Process solid wastes can result from a variety of industrial processes. These metal-bearing solid wastes are disposed above ground in waste piles or below ground or under cover in landfills. Examples of process solid wastes include slags, fly ash, mold sands, abrasive wastes, ion exchange resins, spent catalysts, spent activated carbon, and refractory bricks (Zimmerman and Coles, 1992). The composition of the process waste influences the density, porosity, and leach resistance of the waste and must be considered in evaluating the contaminated matrix.

Because waste piles are above ground, they are exposed to weathering which can disperse the waste pile to the surrounding soil, water and air and can result in generation of leachate which infiltrates into the subsurface environment. The ability of landfills to contain process solid wastes varies due to the range of available landfill designs. Uncontained landfills can release contaminants into infiltrating surface water or groundwater or via wind and surface erosion.

6.2.3 Sludges

The composition of sludges depends on the original waste stream and the process from which it was derived. Sludges resulting from a uniform wastestream, such as wastewater treatment sludges, are typically more homogeneous and have more uniform matrix characteristics. Sludge pits, on the other hand, often contain a mixture of wastes that have been aged and weathered, causing a variety of reactions to occur. Sludge pits often require some form of pretreatment before wastes can be treated or recycled (Smith et al., 1995).

6.2.4 Soils

Soil consists of a mixture of weathered minerals and varying amounts of organic matter. Soils can be contaminated as a result of spills or direct contact with contaminated waste streams such as airborne emissions, process solid wastes, sludges, or leachate from waste materials. The solubility of metals in soil is influenced by the chemistry of the soil and groundwater (Sposito, 1989; Evans, 1989). Factors such as pH, Eh, ion exchange capacity, and complexation/chelation with organic matter directly affect metal solubility.

6.2.5 Direct Ground-Water Contamination

Groundwater can be contaminated with metals directly by infiltration of leachate from land disposal of solid wastes, liquid sewage or sewage sludge, leachate from mine tailings and other mining wastes, deep-well disposal of liquid wastes, seepage from industrial waste lagoons, or from other spills and leaks from industrial metal processing facilities (e.g., steel plants, plating shops, etc.). A variety of reactions may occur which influence the speciation and mobility of metal contaminants including acid/base, precipitation/dissolution, oxidation/ reduction, sorption or ion exchange. Precipitation, sorption, and ion exchange reactions can retard the movement of metals in groundwater. The rate and extent of these reactions will depend on factors such as pH, Eh, complexation with other dissolved constituents, sorption and ion exchange capacity of the geological materials, and organic matter content. Groundwater flow characteristics also influence the transport of metal contaminants.

6.3 Chemical fate and mobility

The fate and transport of a metal in soil and groundwater depends significantly on the chemical form and speciation of the metal (Allen et al., 1991). The mobility of metals in ground-water systems is hindered by reactions that cause metals to adsorb or precipitate, or chemistry that tends to keep metals associated with the solid phase and prevent them from dissolving. These mechanisms can retard the movement of metals and also provide a long-term source of metal contaminants (NRC, 1994). While the various metals undergo similar reactions in a number of aspects, the extent and nature of these reactions varies under particular conditions. In Figure 2, for example, the extent of sorption of several metal cations and anions onto iron oxide is shown as a function of pH for a particular background electrolyte composition. It may be seen there that lead sorbs extensively at much lower pH values than zinc or cadmium (Kinniburgh et al., 1976).

The chemical form and speciation of some of the more important metals found at contaminated sites are discussed below. The influence of chemical form on fate and mobility of these compounds is also discussed.

6.3.1 Pb

The primary industrial sources of lead (Pb) contamination include metal smelting and processing, secondary metals production, lead battery manufacturing, pigment and chemical manufacturing, and lead-contaminated wastes. Widespread contamination due to the former use of lead in gasoline is also of concern. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and leadmetal oxyanion complexes (Smith et al., 1995).

Lead occurs most commonly with an oxidation state of 0 or +II. Pb(II) is the more common and reactive form of lead and forms mononuclear and polynuclear oxides and hydroxides. Under most conditions Pb2+ and lead-hydroxy complexes are the most stable forms of lead (Smith et al., 1995). Low solubility compounds are formed by complexation with inorganic (Cl^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}) and organic ligands (humic and fulvic acids, EDTA, amino acids) (Bodek et al., 1988). Lead carbonate solids form above pH 6 and PbS is the most stable solid when high sulfide concentrations are present under reducing conditions.

Most lead that is released to the environment is retained in the soil (Evans, 1989). The primary processes influencing the fate of lead in soil include adsorption, ion exchange, precipitation, and complexation with sorbed organic matter. These processes limit the amount of lead that can be transported into the surface water or groundwater. The relatively volatile organolead compound tetramethyl lead may form in anaerobic sediments as a result of alkyllation by microorganisms (Smith et al., 1995).

The amount of dissolved lead in surface water and groundwater depends on pH and the concentration of dissolved salts and the types of mineral surfaces present. In surface water and ground-water systems, a significant fraction of lead is undissolved and occurs as precipitates ($PbCO_3$, Pb_2O, $Pb(OH)_2$, $PbSO_4$), sorbed ions or surface coatings on minerals, or as suspended organic matter.

6.3.2 Cr

Chromium(Cr) is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite, $FeCr_2O_4$. Major sources of Cr contamination include releases from electroplating processes and the disposal of chromium containing wastes (Smith et al., 1995).

Cr(VI) is the form of chromium commonly found at contaminated sites. Chromium can also occur in the +III oxidation state, depending on pH and redox conditions. Cr (VI) is the dominant form of chromium in shallow aquifers where aerobic conditions exist. Cr(VI) can be reduced to Cr(III) by soil organic matter, S^{2-} and Fe^{2+} ions under anaerobic conditions often encountered in deeper groundwater. Major Cr(VI) species include chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) which precipitate readily in the presence of metal cations (especially Ba^{2+}, Pb^{2+}, and Ag^+). Chromate and dichromate also adsorb on soil surfaces, especially iron and aluminum oxides. Cr(III) is the dominant form of chromium at low pH (<4). Cr^{3+} forms solution complexes with NH_3, OH^-, Cl^-, F^-, CN^-, SO_4^{2-}, and soluble organic ligands. Cr(VI) is the more toxic form of chromium and is also more mobile. Cr(III) mobility is decreased by adsorption to clays and oxide minerals below pH 5 and low solubility above pH 5 due to the formation of $Cr(OH)_3$(s) (Chrotowski et al., 1991).

Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content and the amount of organic matter present. Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form. Soluble and unadsorbed chromium complexes can leach from soil into groundwater. The leachability of Cr(VI) increases as soil pH increases. Most of chromium released into natural waters is particle associated, however, and is ultimately deposited into the sediment (Smith et al., 1995).

6.3.3 As

Arsenic (As) is a semimetallic element that occurs in a wide variety of minerals, mainly as As_2O_3, and can be recovered from processing of ores containing mostly copper, lead, zinc, silver and gold. It is also present in ashes from coal combustion. Arsenic exhibits fairly complex chemistry and can be present in several oxidation states (-III, 0, III, V) (Smith et al., 1995).

In aerobic environments, As(V) is dominant, usually in the form of arsenate (AsO_4^{3-}) in various protonation states: H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}. Arsenate, and other anionic forms of arsenic behave as chelates and can precipitate when metal cations are present (Bodek et al., 1988). Metal arsenate complexes are stable only under certain conditions.

As(V) can also coprecipitate with or adsorb onto iron oxyhydroxides under acidic and moderately reducing conditions. Coprecipitates are immobile under these conditions but arsenic mobility increases as pH increases (Smith et al., 1995).

Under reducing conditions As(III) dominates, existing as arsenite (AsO_3^{3-}) and its protonated forms: H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$. Arsenite can adsorb or coprecipitate with metal sulfides and has a high affinity for other sulfur compounds. Elemental arsenic and arsine, AsH_3, may be present under extreme reducing conditions.

Biotransformation (via methylation) of arsenic creates methylated derivatives of arsine, such as dimethyl arsine $HAs(CH_3)_2$ and trimethylarsine $As(CH_3)_3$ which are highly volatile.

Since arsenic is often present in anionic form, it does not form complexes with simple anions such as Cl^- and SO_4^{2-}. Arsenic speciation also includes organometallic forms such as methylarsinic acid $(CH_3)AsO_2H_2$ and dimethylarsinic acid $(CH_3)_2AsO_2H$. Many arsenic compounds sorb strongly to soils and are therefore transported only over short distances in groundwater and surface water. Sorption and coprecipitation with hydrousiron oxides are the most important removal mechanisms under most environmental conditions (Krause and Ettel, 1989; Pierce and Moore, 1982). Arsenates can be leached easily if the amount of reactive metal in the soil is low. As(V) can also be mobilized under reducing conditions that encourage the formation of As(III), under alkaline and saline conditions, in the presence of other ions that compete for sorption sites, and in the presence of organic compounds that form complexes with arsenic (Smith et al., 1995).

6.3.4 Zn

Zinc (Zn) does not occur naturally in elemental form. It is usually extracted from mineral ores to form zinc oxide (ZnO). The primary industrial use for Zinc is as a corrosion-resistant coating for iron or steel (Smith et al., 1995).

Zinc usually occurs in the +II oxidation state and forms complexes with a number of anions, amino acids and organic acids. Zn may precipitate as $Zn(OH)_2(s)$, $ZnCO_3(s)$, $ZnS(s)$, or $Zn(CN)_2(s)$. Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values. At higher pH values, zinc can form carbonate and hydroxide complexes which control zinc solubility. Zinc readily precipitates under reducing conditions and in highly polluted systems when it is present at very high concentrations, and may coprecipitate with hydrous oxides of iron or manganese (Smith et al., 1995).

Sorption to sediments or suspended solids, including hydrous iron and manganese oxides, clay minerals, and organic matter, is the primary fate of zinc in aquatic environments. Sorption of zinc increases as pH increases and salinity decreases.

6.3.5 Cd

Cadmium (Cd) occurs naturally in the form of CdS or $CdCO_3$. Cadmium is recovered as a by-product from the mining of sulfide ores of lead, zinc and copper. Sources of cadmium contamination include plating operations and the disposal of cadmium-containing wastes (Smith et al., 1995).

The form of cadmium encountered depends on solution and soil chemistry as well as treatment of the waste prior to disposal The most common forms of cadmium include Cd^{2+}, cadmium-cyanide complexes, or $Cd(OH)_2$ solid sludge (Smith et al., 1995). Hydroxide ($Cd(OH)_2$) and carbonate ($CdCO_3$) solids dominate at high pH whereas Cd^{2+} and aqueous sulfate species are the dominant forms of cadmium at lower pH (<8). Under reducing conditions when sulfur is present, the stable solid CdS(s) is formed. Cadmium will also precipitate in the presence of phosphate, arsenate, chromate and other anions, although solubility will vary with pH and other chemical factors.

Cadmium is relatively mobile in surface water and ground-water systems and exists primarily as hydrated ions or as complexes with humic acids and other organic ligands (Callahan et al., 1979). Under acidic conditions, cadmium may also form complexes with chloride and sulfate. Cadmium is removed from natural waters by precipitation and sorption to mineral surfaces, especially oxide minerals, at higher pH values (>pH 6). Removal by these mechanisms increases as pH increases. Sorption is also influenced by the cation exchange capacity (CEC) of clays, carbonate minerals, and organic matter present in soils and sediments. Under reducing conditions, precipitation as CdS controls the mobility of cadmium (Smith et al., 1995).

6.3.6 Cu

Copper (Cu) is mined as a primary ore product from copper sulfide and oxide ores. Mining activities are the major source of copper contamination in groundwater and surface waters. Other sources of copper include algicides, chromated copper arsenate (CCA) pressuretreated lumber, and copper pipes. Solution and soil chemistry strongly influence the speciation of copper in ground-water systems. In aerobic, sufficiently alkaline systems, $CuCO_3$ is the dominant soluble copper species. The cupric ion, Cu^{2+}, and hydroxide complexes, $CuOH^+$ and $Cu(OH)_2$, are also commonly present. Copper forms strong solution complexes with humic acids. The affinity of Cu for humates increases as pH increases and ionic strength decreases. In anaerobic environments, when sulfur is present CuS(s) will form.

Copper mobility is decreased by sorption to mineral surfaces. Cu^{2+} sorbs strongly to mineral surfaces over a wide range of pH values (Dzombak and Morel, 1990). The cupric ion (Cu^{2+}) is the most toxic species of copper. Copper toxicity has also been demonstrated for $CuOH^+$ and $Cu_2(OH)_2^{2+}$ (LaGrega et al., 1994).

6.3.7 Hg

The primary source of mercury is the sulfide ore cinnabar. Mercury (Hg) is usually recovered as a by-product of ore processing (Smith et al., 1995). Release of mercury from coal combustion is a major source of mercury contamination. Releases from manometers at pressure measuring stations along gas/oil pipelines also contribute to mercury contamination. After release to the environment, mercury usually exists in mercuric (Hg^{2+}), mercurous (Hg_2^{2+}), elemental (Hg^o), or alkyllated form (methyl/ethyl mercury). The redox potential and pH of the system determine the stable forms of mercury that will be present. Mercurous and mercuric mercury are more stable under oxidizing conditions. When mildly reducing conditions exist, organic or inorganic mercury may be reduced to elemental mercury, which may then be converted to alkyllated forms by biotic or abiotic processes. Mercury is most toxic in its alkyllated forms which are soluble in water and volatile in air (Smith et al., 1995).

Hg(II) forms strong complexes with a variety of both inorganic and organic ligands, making it very soluble in oxidized aquatic systems (Bodek et al., 1988). Sorption to soils, sediments, and humic materials is an important mechanism for removal of mercury from solution. Sorption is pH-dependent and increases as pH increases. Mercury may also be removed from solution by coprecipitation with sulfides (Smith et al., 1995).

Under anaerobic conditions, both organic and inorganic forms of mercury may be converted to alkyllated forms by microbial activity, such as by sulfur-reducing bacteria. Elemental mercury may also be formed under anaerobic conditions by demethylation of methyl mercury, or by reduction of Hg(II). Acidic conditions (pH<4) also favor the formation of methyl mercury, whereas higher pH values favor precipitation of HgS(s) (Smith et al., 1995).

6.4 Available technologies

6.4.1 Site characterization and establishment of remediation goals

The physical and chemical form of the metal contaminant in soil or water strongly influences the selection of the appropriate remediation treatment approach. Information about the physical characteristics of the site and the type and level of contamination at the site must be obtained to enable accurate assessment of site contamination and remedial alternatives.

The importance of adequate, well-planned site characterization to selection of an appropriate cost-effective remediation approach has been discussed many times (e.g., CII, 1995) but cannot be overemphasized. The contamination in the groundwater and soil should be characterized to establish the type, amount, and distribution of contaminants across different media.

Once the site has been characterized, the desired level of each contaminant in soil and groundwater must be determined. This is done by comparison of observed contaminant concentrations with soil and ground-water quality standards for a particular regulatory domain, or by performance of a site-specific risk assessment. Remediation goals for metals may be set as desired concentrations in groundwater, as total metal concentration in soil, as leachable metal in soil, or as some combination of these.

6.4.2 General remediation approaches

Several technologies exist for the remediation of metals-contaminated soil and water. These technologies are contained within five categories of general approaches to remediation: isolation, immobilization, toxicity reduction, physical separation and extraction. These are the same general approaches used for many types of contaminants in the subsurface (LaGrega et al., 1994). As is usually the case, combinations of one or more of these approaches are often used for more cost-effective treatment of a contaminated site.

Isolation

Isolation technologies attempt to prevent the transport of contaminants by containing them within a designated area. These technologies can be used to prevent further contamination of groundwater when other treatment options are not physically or economically feasible for a site. Contaminated sites may also be isolated temporarily in order to limit transport during site assessment and site remediation.

A) Capping

Capping systems are used to provide an impermeable barrier to surface water infiltration to contaminated soil for prevention of further release of contaminants to the surrounding surface water or groundwater. Secondary objectives include controlling gas and odor emissions, improving aesthetics, and providing a stable surface over a contaminated site.

Capping also eliminates risks associated with dermal contact and/or incidental ingestion of surface soils, but if this is the primary goal for the site and surface water infiltration is not a concern, a less expensive permeable cover may be preferred.

Capping provides a range of design options that includes simple single-layer caps and more complex multilayer systems (Rumer and Ryan, 1995; U.S. EPA, 1991). Design selection depends on site characteristics, remedial objectives and risk factors associated with the site. A variety of materials are available for use in capping systems and choice of materials is site specific because local soils are often incorporated into parts of the cap.

Synthetic membranes such as high-density polyethylene are also available for incorporation into capping systems. Surface water controls, such as ditches and dikes are usually included to help control drainage from the cap. Multilayered capping systems may also include a hard cover and/or a layer of topsoil to separate the underlying layers from the ground surface. Revegetation is promoted in order to reinforce the topsoil, to reduce soil erosion and runoff velocity, and to help remove water from the soil by evapotranspiration (Rumer and Ryan, 1995).

B) Subsurface Barriers

Subsurface barriers may be used to isolate contaminated soil and water by controlling the movement of groundwater at a contaminated site. These barriers are designed to reduce the movement of contaminated groundwater from the site, or to restrict the flow of uncontaminated groundwater through the contaminated site (Rumer and Ryan, 1995).

Vertical barriers are commonly used to restrict the lateral flow of groundwater. For effective isolation of the contaminated matrix, the barrier should extend and key into a continuous, low-permeability layer, such as clay or competent bedrock, below the contaminated area (U.S. EPA, 1985; Rumer and Ryan, 1995). If an impermeable layer is not available, a ground-water extraction system must be used to prevent transport of contaminants under the barrier. Vertical barriers may be installed upstream, downstream, or completely surrounding the site and are often implemented in conjunction with a capping system to control surface water infiltration. The use of circumferential barriers can prevent the escape of contamination from the site by using an infiltration barrier and collection system to create

a hydraulic gradient in the inward direction. Vertical barriers are often limited to depths achievable with backhoe excavation technology for trenches, i.e., to about 10 m (U.S. EPA, 1985).

Slurry walls are usually constructed in a vertical trench excavated under a slurry that is designed to prevent collapse and to form a filter cake on the walls of the trench to prevent the loss of fluids to the surrounding soil (Xanthakos, 1979). A vibrating beam method (Slurry Systems, Inc.) is also available in which the beam penetrates the ground and slurry materials are injected into the soil (with assistance from a high pressure/low volume jet if needed). Two options exist for the slurry composition. The soil-bentonite (SB) slurry wall is the most common type, and comprises a bentonite-water slurry that is mixed with a soil engineered to harden upon addition to the slurry (Rumer and Ryan, 1995). The trench can also be excavated under a portland cement-bentonite-water slurry that is left to harden and form a cement-bentonite (CB) slurry wall (LaGrega et al., 1994). Available technologies for installation of slurry walls allow installation to depths up to 40 m.

Slurry walls are the most common type of vertical barrier due to their low relative cost. The use of slurry walls can be limited by the topography, geology, and type of contamination at the site. For example, an SB slurry will flow unless the site and confining layer are nearly level. Also, some contaminants, such as concentrated organics and strong acids/bases, can degrade SB materials and prevent the application of SB slurry walls at some sites (Rumer and Ryan, 1995).

Other available vertical barriers include grout curtains and sheet piles. Grout curtains are constructed by drilling a borehole and injecting a fluid into the surrounding soil that is designed to solidify and reduce water flow through the contaminated region (U.S. EPA, 1985). The fluid is pressure-injected in rows of staggered boreholes that are designed to overlap once the fluid has permeated into the surrounding soil. Common materials used to construct grout curtains include cement, clays, alkali-silicate, and organic polymers (Rumer and Ryan, 1995). Clays are the most widely used grouting materials due to their low cost. This technique is more expensive than slurry walls and its use is therefore usually limited to sealing voids in existing rock.

Sheet piles usually comprise steel pilings that are driven into the formation to create a wall to contain the groundwater. Sheet piles are seldom used at contaminated sites due to concerns about wall integrity. This method is generally limited to isolation of shallow contamination (12-15 m) distributed over a relatively small area (U.S. EPA, 1985), or used in conjunction with a soil-bentonite slurry when site conditions prevent the use of conventional slurry walls (Rumer and Ryan, 1995).

Technologies for the construction of horizontal barriers are under investigation. Horizontal barriers would enable control of the downward migration of contaminants by lining the site without requiring excavation of the contaminated matrix. The technologies under investigation include grout injection by vertical boring and horizontal drilling. The vertical boring method is similar to the construction of grout curtains except that the grout is injected at a fixed elevation over a tightly spaced grid of vertical boreholes to create an impermeable horizontal layer. Problems with this method include soil compaction by the large drill rigs situated over the contaminated area. Also, the vertical boreholes would provide access to the deeper layers and may therefore increase vertical migration of contaminants. Horizontal drilling involves the use of directional drilling techniques to create the horizontal grout layer.

Horizontal barriers may also be used in conjunction with vertical barriers at sites where a natural aquitard is not present. In this case, the vertical barrier could key into the horizontal barrier to prevent the transport of contaminants under the vertical barrier (Smith et al., 1995).

Immobilization

Immobilization technologies are designed to reduce the mobility of contaminants by changing the physical or leaching characteristics of the contaminated matrix. Mobility is usually decreased by physically restricting contact between the contaminant and the surrounding groundwater, or by chemically altering the contaminant to make it more stable with respect to dissolution in groundwater. The aqueous and solid phase chemistry of metals is conducive to immobilization by these techniques. A variety of methods are available for immobilization of metal contaminants, including those that use chemical reagents and/or thermal treatment to physically bind the contaminated soil or sludge. Most immobilization technologies can be performed ex situ or in situ. In situ processes are preferred due to the lower labor and energy requirements, but implementation in situ will depend on specific site conditions.

A) Solidification/Stabilization

Solidification and stabilization (S/S) immobilization technologies are the most commonly selected treatment options for metals-contaminated sites (Conner, 1990). Solidification involves the formation of a solidified matrix that physically binds the contaminated material.

Stabilization, also referred to as fixation, usually utilizes a chemical reaction to convert the waste to a less mobile form. The general approach for solidification/stabilization treatment processes involves mixing or injecting treatment agents to the contaminated soils. Inorganic binders, such as cement, fly ash, or blast furnace slag, and organic binders such as bitumen are used to form a crystalline, glassy or polymeric framework around the waste. The dominant mechanism by which metals are immobilized is by precipitation of hydroxides within the solid matrix (Bishop et al., 1982; Shively et al., 1986).

S/S technologies are not useful for some forms of metal contamination, such as species that exist as anions (e.g., Cr(VI), arsenic) or metals that don't have low-solubility hydroxides (e.g., mercury). S/S may not be applicable at sites containing wastes that include organic forms of contamination, especially if volatile organics are present. Mixing and heating associated with binder hydration may release organic vapors. Pretreatment, such as air stripping or incineration, may be used to remove the organics and prepare the waste for metal stabilization/solidification (Smith et al., 1995). The application of S/S technologies will also be affected by the chemical composition of the contaminated matrix, the amount of water present, and the ambient temperature. These factors can interfere with the solidification/stabilization process by inhibiting bonding of the waste to the binding material, retarding the setting of the mixtures, decreasing the stability of the matrix, or reducing the strength of the solidified area (U.S. EPA, 1990b).

Cement-based binders and stabilizers are common materials used for implementation of S/S technologies (Conner, 1990). Portland cement, a mixture of Ca-silicates, aluminates, aluminoferrites, and sulfates is an important cement-based material. Pozzolanic materials which consist of small spherical particles formed by coal combustion (such as fly ash) and in lime and cement kilns, are also commonly used for S/S. Pozzolans exhibit cement-like properties, especially if the silica content is high. Portland cement and pozzolans can be used alone or together to obtain optimal properties for a particular site (U.S. EPA, 1989).

Organic binders may also be used to treat metals through polymer microencapsulation. This process uses organic materials such as bitumen, polyethylene, paraffins, waxes and other polyolefins as thermoplastic or thermosetting resins. For polymer encapsulation, the organic materials are heated and mixed with the contaminated matrix at elevated temperatures (120° to 200°C). The organic materials polymerize, agglomerate the waste and the waste matrix is encapsulated (U.S. EPA, 1989). Organics are volatilized and collected and the treated material is extruded for disposal or possible reuse (e.g., as paving material) (Smith et al., 1995). The contaminated material may require pretreatment to separate rocks and debris and dry the feed material. Polymer encapsulation requires more energy and more complex equipment than cement-based S/S operations. Bitumen (asphalt) is the cheapest and most common thermoplastic binder (U.S. EPA, 1989).

S/S is achieved by mixing the contaminated material with appropriate amounts of binder/stabilizer and water. The mixture sets and cures to form a solidified matrix and contain the waste. The cure time and pour characteristics of the mixture and the final properties of the hardened cement depend upon the composition (amount of cement, pozzolan, water) of the binder/stabilizer.

Ex situ S/S can be easily applied to excavated soils because methods are available to provide the vigorous mixing needed to combine the binder/stabilizer with the contaminated material. Pretreatment of the waste may be necessary to screen and crush large rocks and debris. Mixing can be performed via in-drum, in-plant or area mixing processes. In-drum mixing may be preferred for treatment of small volumes of waste or for toxic wastes. Inplant processes utilize rotary drum mixers for batch processes or pug mill mixers for continuous treatment. Larger volumes of waste may be excavated and moved to a contained area for area mixing. This process involves layering the contaminated material with the stabilizer/binder, and subsequent mixing with a backhoe or similar equipment. Mobile and fixed treatment plants are available for ex situ S/S treatment. Smaller pilot-scale plants can treat up to 100 tons of contaminated soil per day, while larger portable plants typically process 500 to over 1000 tons per day (Smith et al., 1995).

S/S techniques are available to provide mixing of the binder/stabilizer with the contaminated soil in situ. In situ S/S is less labor and energy intensive than ex situ process that require excavation, transport and disposal of the treated material. In situ S/S is also preferred if volatile or semi volatile organics are present because excavation would expose these contaminants to the air (U.S. EPA, 1990a). However the presence of bedrock, large boulders, cohesive soils, oily sands and clays may preclude the application of in situ S/S at some sites. It is also more difficult to provide uniform and complete mixing through in situ processes.

Mixing of the binder and contaminated matrix may be achieved using in-place mixing, vertical auger mixing or injection grouting. In-place mixing is similar to ex situ area mixing except that the soil is not excavated prior to treatment. The in situ process is useful for treating surface or shallow contamination and involves spreading and mixing the binders with the waste using conventional excavation equipment such as draglines, backhoes or clamshell buckets. Vertical auger mixing uses a system of augers to inject and mix the binding reagents with the waste. Larger (2-4 m diameter) augers are used for shallow (3-12 m) drilling and can treat 500-1000 m^3 per day (Ryan and Walker, 1992; Jasperse and Ryan, 1992). Deep stabilization/solidification (up to 45 m) can be achieved by using ganged augers (up to 1 m in diameter each) that can treat 150-400 m^3per day. Finally injection grouting may be performed to inject the binder containing suspended or dissolved reagents into the treatment area under pressure. The binder permeates the surrounding soil and cures in place (Smith et al., 1995).

B) Vitrification

The mobility of metal contaminants can be decreased by high-temperature treatment of the contaminated area that results in the formation of vitreous material, usually an oxide solid. During this process, the increased temperature may also volatilize and/or destroy organic contaminants or volatile metal species (such as Hg) that must be collected for treatment or disposal. Most soils can be treated by vitrification and a wide variety of inorganic and organic contaminants can be targeted. Vitrification may be performed ex situ or in situ, although in situ processes are preferred due to the lower energy requirements and cost (U.S. EPA, 1992a).

Typical stages in ex situ vitrification processes may include excavation, pretreatment, mixing, feeding, melting and vitrification, off-gas collection and treatment, and forming or casting of the melted product. The energy requirement for melting is the primary factor influencing the cost of ex situ vitrification. Different sources of energy can be used for this purpose, depending on local energy costs. Process heat losses and water content of the feed should be controlled in order to minimize energy requirements. Vitrified material with certain characteristics may be obtained by using additives such as sand, clay and/or native soil. The vitrified waste may be recycled and used as clean fill, aggregate, or other reusable materials (Smith et al., 1995).

In situ vitrification (ISV) involves passing electric current through the soil using an array of electrodes inserted vertically into the contaminated region. Each setting of four electrodes is referred to as a melt. If the soil is too dry, it may not provide sufficient conductance and a trench containing flaked graphite and glass frit (ground glass particles) must be placed between the electrodes to provide an initial flow path for the current. Resistance heating in the starter path melts the soil. The melt grows outward and down as the molten soil usually provides additional conductance for the current. A single melt can treat up to 1000 tons of contaminated soil to depths of 6 m, at a typical treatment rate of 3 to 6 tons per hour.

Larger areas are treated by fusing together multiple individual vitrification zones. The main requirement for in situ vitrification is the ability of the soil melt to carry current and solidify as it cools. If the alkali content (as Na_2O and K_2O) of the soil is too high (1.4 wt%) the molten soil may not provide enough conductance to carry the current (Buelt and Thompson, 1992).

Toxicity and/or Mobility Reduction

Chemical and/or biological processes can be used to alter the form of metal contaminants in order to decrease their toxicity and/or mobility.

A) Chemical Treatment

Chemical reactions can be initiated that are designed to decrease the toxicity or mobility of metal contaminants. The three types of reactions that can be used for this purpose are oxidation, reduction, and neutralization reactions. Chemical oxidation changes the oxidation state of the metal atom through the loss of electrons. Commercial oxidizing agents are available for chemical treatment, including potassium permanganate, hydrogen peroxide, hypochlorite and chlorine gas. Reduction reactions change the oxidation state of metals by adding electrons. Commercially available reduction reagents include alkali metals (Na, K), sulfur dioxide, sulfite salts, and ferrous sulfate. Changing the oxidation state of metals by oxidation or reduction can detoxify, precipitate, or solubilize the metals (NRC, 1994).

Chemical neutralization is used to adjust the pH balance of extremely acidic or basic soils and/or groundwater. This procedure can be used to precipitate insoluble metal salts from contaminated water, or in preparation for chemical oxidation or reduction.

Chemical treatment can be performed ex situ or in situ. However in situ chemical agents must be carefully selected so that they do not further contaminate the treatment area. The primary problem associated with chemical treatment is the nonspecific nature of the chemical reagents. Oxidizing/reducing agents added to the matrix to treat one metal will also target other reactive metals and can make them more toxic or mobile (NRC, 1994). Also, the long-term stability of reaction products is of concern since changes in soil and water chemistry might reverse the selected reactions.

Chemical treatment is often used as pretreatment for S/S and other treatment technologies. Reduction of Cr(VI) to Cr(III) is the most common form of chemical treatment and is necessary for remediation of wastes containing Cr(VI) by precipitation or S/S. Chromium in its Cr(III) form is readily precipitated by hydroxide over a wide range of pH values. Acidification may also be used to aid in Cr(VI) reduction. Arsenic may be treatable by chemical oxidation since arsenate, As(V), is less toxic, soluble and mobile than arsenite, As(III). Bench-scale work has indicated that arsenic stabilization may be achieved by precipitation and coprecipitation with Fe(III) (Smith et al., 1995).

B) Permeable Treatment Walls

Treatment walls remove contaminants from groundwater by degrading, transforming, precipitating or adsorbing the target solutes as the water flows through permeable trenches containing reactive material within the subsurface (Vidic and Pohland, 1996). Several methods are available for installation of permeable treatment walls, some of which employ slurry wall construction technology to create a permeable reactive curtain. The reactive zone can use physical, chemical and biological processes, or a combination of these. The ground-water flow through the wall may be enhanced by inducing a hydraulic gradient in the direction of the treatment zone or channeling ground-water flow toward the treatment zone (NRC, 1994).

Several types of treatment walls are being tried for arresting transport of metals in groundwater at contaminated sites. Trench materials being investigated include zeolite, hydroxyapatite, elemental iron, and limestone (Vidic and Pohland, 1996). Applications of elemental iron for chromium (VI) reduction and limestone for lead precipitation and adsorption are described below.

C) Elemental Iron

Trenches filled with elemental iron have shown promise for remediation of metalscontaminated sites. While investigations of this technology have focused largely on treatment of halogenated organic compounds, studies are being performed to assess the applicability to remediation of inorganic contaminants (Powell et al., 1994).

Low oxidation-state chemical species can serve as electron donors for the reduction of higher oxidation-state contaminants. This ability can be exploited to remediate metals that are more toxic and mobile in higher oxidation states, such as Cr(VI). Results of column experiments performed by Powell et al. (1994) and batch experiments performed by Cantrell et al. (1995) showed that chromate reduction was enhanced in systems containing iron filings in addition to the natural aquifer material. A field experiment has been initiated by researchers at the U.S. EPA National Risk Management Research Laboratory to investigate the use of zero-valent iron for chromium remediation at the U.S. Coast Guard air support base near Elizabeth City, North Carolina. Preliminary results indicate that the test barrier has reduced chromate in the groundwater to below detection limits (Wilson, 1995).

D) Limestone Barriers

The use of limestone treatment walls has been proposed for sites with metals contamination, in particular former lead acid battery recycling sites which have lead and acid contamination in groundwater and soil. In such cases, a limestone trench can provide neutralization of acidic groundwater. The attendant rise in pH promotes immobilization of any dissolved lead through precipitation and/or adsorption onto minerals. A limestone trench system is in design for implementation at the Tonolli Superfund site in Nesquehoning, Pennsylvania (U.S. EPA, 1992b).

There is some experience in the coal mining industry with use of limestone in the manner anticipated for the Tonolli site. Most of this experience has been acquired since 1990, when the concept of “anoxic limestone drains” was introduced (Turner and McCoy, 1990). Since that time, numerous limestone drain systems have been installed at Appalachian coal field sites (primarily in Kentucky, West Virginia, and Pennsylvania) in an attempt to control acid mine drainage. Summaries of installations and evolving design considerations are provided in Hedin and Nairn (1992), Hedin et al. (1994), and Hedin and Watzlaf (1994).

Design and operating guidelines for the anoxic limestone drains have for the most part been developed from trial and observation. Briefly, the systems in use employ fairly large, #3 or #4 (baseball size) limestone rocks. Anoxic mine water is directed to the limestone drain, which is installed with a soil cover to inhibit contact with air. Hedin and Nairn (1992) report that “some systems constructed with limestone powder and gravel have failed, apparently because of plugging problems.” Preliminary review of the literature on design of anoxic limestone drains indicates primary concern with maintenance of anoxic conditions in the drains. If high dissolved concentrations of Fe are present and aerobic conditions develop, insoluble ferric hydroxide can form and coat the limestone, rendering it ineffective. High concentrations of aluminum are also a concern, as aluminum hydroxide can precipitate and yield the same kind of coating problems. With use of large diameter stones, plugging is prevented even if precipitation occurs and the stones become coated with precipitate. Available operating data for anoxic limestone drains indicate that they can be effective in raising the pH of strongly acidic water. Hedin and Watzlaf (1994) reviewed operating data for 21 limestone drain systems. The data they compiled showed fairly consistent increases in pH of highly acidic mine drainage (at pH 2.3 to 3.5) to pH values in the range of 6.0 to 6.7.

Thus, there is clearly precedent for employing the limestone drain approach with some confidence of success in raising pH of highly acidic water. Long term (i.e., greater than 10 years) performance cannot be predicted with confidence as there has been relatively short duration operating experience. However, experience to date indicates clearly that limestone drain systems can operate effectively under appropriate conditions, especially anoxic or low-oxygen groundwater, for at least several years.

E) Biological Treatment

Biological treatment technologies are available for remediation of metals-contaminated sites. These technologies are commonly used for the remediation of organic contaminants and are beginning to be applied for metal remediation, although most applications to date have been at the bench and pilot scale (Schnoor, 1997). Biological treatment exploits natural biological processes that allow certain plants and microorganisms to aid in the remediation of metals. These processes occur through a variety of mechanisms, including adsorption, oxidation and reduction reactions, and methylation (Means and Hinchee, 1994).

F) Bioaccumulation

Bioaccumulation involves the uptake of metals from contaminated media by living organisms or dead, inactive biomass. Active plants and microorganisms accumulate metals as the result of normal metabolic processes via ion exchange at the cell walls, complexation reactions at the cell walls, or intra- and extracellular precipitation and complexation reactions.

Adsorption to ionic groups on the cell surface is the primary mechanism for metal adsorption by inactive biomass. Accumulation in biomass has been shown to be as effective as some ion exchange resins for metals removal from water (Means and Hinchee, 1994).

G) Phytoremediation

Phytoremediation refers to the specific ability of plants to aid in metal remediation. Some plants have developed the ability to remove ions selectively from the soil to regulate the uptake and distribution of metals. Most metal uptake occurs in the root system, usually via absorption, where many mechanisms are available to prevent metal toxicity due to high concentration of metals in the soil and water. Potentially useful phytoremediation technologies for remediation of metals-contaminated sites include phytoextraction, phytostabilization and rhizofiltration (U.S. EPA, 1996b).

H) Phytoextraction

Phytoextraction employs hyperaccumulating plants to remove metals from the soil by absorption into the roots and shoots of the plant. A hyperaccumulator is defined as a plant with the ability to yield 0.1% chromium, cobalt, copper or nickel or 1% zinc, manganese in the aboveground shoots on a dry weight basis. The aboveground shoots can be harvested to remove metals from the site and subsequently disposed as hazardous waste or treated for the recovery of the metals.

I) Phytostabilization

Phytostabilization involves the use of plants to limit the mobility and bioavailability of metals in soil. Phytostabilizers are characterized by high tolerance of metals in surrounding soils but low accumulation of metals in the plant. This technique may be used as an interim containment strategy until other remediation techniques can be developed, or as treatment at sites where other methods would not be economically feasible.

J) Rhizofiltration

Rhizofiltration removes metals from contaminated groundwater via absorption, concentration and precipitation by plant roots. This technique is use to treat contaminated water rather than soil and is most effective for large volumes of water with low levels of metal contamination. Terrestrial plants are more effective than aquatic plants because they develop a longer, more fibrous root system that provides a larger surface area for interaction. Wetlands construction is a form of rhizofiltration that has been demonstrated as a costeffective treatment for metals-contaminated wastewater.

K) Bioleaching

Bioleaching uses microorganisms to solubilize metal contaminants either by direct action of the bacteria, as a result of interactions with metabolic products, or both. Bioleaching can be used in situ or ex situ to aid the removal of metals from soils. This process is being adapted from the mining industry for use in metals remediation. The mechanisms responsible for bioleaching are not fully defined, but in the case of mercury bioreduction (to elemental mercury) is thought to be responsible for mobilization of mercury salts (Means and Hinchee, 1994).

L) Biochemical Processes

Microbially mediated oxidation and reduction reactions can be manipulated for metal remediation. Some microorganisms can oxidize/reduce metal contaminants directly while others produce chemical oxidizing/reducing agents that interact with the metals to effect a change in oxidation state. Mercury and cadmium have been observed to be oxidized through microbial processes, and arsenic and iron are readily reduced in the presence of appropriate microorganisms. The mobility of metal contaminants is influenced by their oxidation state. Redox reactions can therefore be used to increase or decrease metal mobility (Means and Hinchee, 1994).

Methylation involves attaching methyl groups to inorganic forms of metal ions to form organometallic compounds. Methylation reactions can be microbially mediated. Organometallic compounds are more volatile than inorganic metals and this process can be used to remove metals through volatilization and subsequent removal from the gas stream. However, organometallics are also more toxic and mobile than other metal forms and may potentially contaminate surrounding surface waters and groundwater (Means and Hinchee, 1994).

Physical Separation

Physical separation is an ex situ process that attempts to separate the contaminated material from the rest of the soil matrix by exploiting certain characteristics of the metal and soil. Physical separation techniques are available that operate based on particle size, particle density, surface and magnetic properties of the contaminated soil. These techniques are most effective when the metal is either in the form of discrete particles in the soil or if the metal is sorbed to soil particles that occur in a particular size fraction of the soil. Physical separation is often used as a form of pretreatment in order to reduce the amount of material requiring subsequent treatment (Rosetti, 1993). Several techniques are available for physical separation of contaminated soils including screening, classification, gravity concentration, magnetic separation and froth flotation.

Screening separates soils according to particle size by passing the matrix through a sieve with particular size openings. Smaller particles pass through the sieve and leave larger particles behind, however, the separation is not always complete. Screening may be performed as a stationary process or with motion using a wet or dry process stream (Smith et al., 1995).

Classification involves separation of particles based upon the velocity with which they fall through water (hydroclassification) or air (air classification). Hydroclassification is more common for soil separation and may be performed using a non-mechanical, mechanical or a hydraulic classifier (Rosetti, 1993).

Gravity concentration relies on gravity and one or more other forces (centrifugal force, velocity gradients, etc.) that may be applied to separate particles on the basis of density differences. Gravity concentration may be achieved through the use of a hydrocyclone, jig, spiral concentrator, or shaking table (Rosetti, 1993).

Froth flotation uses air flotation columns or cells to remove particles from water. In this process, air is sparged from the bottom of a tank or column that contains a slurry of the contaminated material. Some metals and minerals attach to the air bubbles due to particular surface properties, such as hydrophobicity. Froth flotation can be used to remove metals that attach to air bubbles, or to remove other minerals while the metal remains in the slurry (Rosetti, 1993).

Magnetic separation subjects particles to a strong magnetic field using electromagnets or magnetic filters and relies on differences in magnetic properties of minerals for separation. Low intensity wet magnetic separators are the most common magnetic separation devices. This process can recover a wide variety of minerals and is particularly successful for separating ferrous from nonferrous minerals (Allen and Torres, 1991).

Extraction

Metals-contaminated sites can be remediated using techniques designed to extract the contaminated fraction from the rest of the soil, either in situ or ex situ. Metal extraction can achieved by contacting the contaminated soil with a solution containing extracting agents (soil washing and in situ soil flushing) or by electrokinetic processes. The contaminated fraction of soil and/or process water is separated from the remaining soil and disposed or treated.

Soil Washing

Soil washing can be used to remove metals from the soil by chemical or physical treatment methods in aqueous suspension. Soil washing is an ex situ process that requires soil excavation prior to treatment. Chemical treatment involves addition of extraction agents that react with the contaminant and leach it from the soil (Elliot and Brown, 1989; Ellis and Fogg, 1985; Tuin and Tels, 1990). The liquid containing the contaminants is separated from the soil resulting in a clean solid phase. Physical treatment is achieved by particle size separation technologies adapted from mineral processing to concentrate the contaminant in a particular size fraction (Allen and Torres, 1991).

Fine particles (<63 :m) often contain the majority of contaminated material because they bind contaminants strongly due to their large and reactive surface area. Many current soil washing approaches attempt to separate the fine fraction from the remainder of the soil in order to reduce the amount of material for subsequent treatment or disposal (Rosetti, 1993).

Particle size separation techniques may not be successful if fine particle, e.g., metal oxide, coatings are present on particles in larger size fractions (Van Ben Schoten et al., 1994).

Preliminary Screening

After excavation, the soil undergoes preliminary screening and preparation in order to separate large rocks and debris from the contaminated matrix. Residual fines may be adhered to the surface of large rocks and are often washed off prior to return of the large rocks to the site (Rosetti, 1993).

Secondary Screening

Most soil washing processes employ secondary screening to segregate the particles into different size fractions, usually between 5 mm and 60 mm. Most secondary screening processes involve making an aqueous slurry of the soil stream and wet screening/sieving of the slurry. The particles in this size range are considered less contaminated than the finer fraction and may be returned to the site as clean soil after separation from the water (Rosetti, 1993).

Chemical Treatment

Chemical treatment may be used to solubilize contaminants from the most contaminated fraction of the soil. Chemical treatment is performed in an aqueous slurry of the contaminated material to which an extracting agent is added. The extraction is performed in a mixing vessel or in combination with the physical treatment stage. The type of extractant used will depend on the contaminants present and the characteristics of the soil matrix. Many processes manipulate the acid/base chemistry of the slurry to leach contaminants from the soil (Tuin and Tels, 1990). However, if a very low pH is required concerns about dissolution of the soil matrix may arise. Chelating agents (e.g., EDTA) selectively bind with some

metals and may be used to solubilize contaminants from the soil matrix (Elliot and Brown, 1989). Oxidizing and reducing agents (e.g., hydrogen peroxide, sodium borohydride) provide yet another option to aid in solubilization of metals since chemical oxidation/ reduction can convert metals to more soluble forms (Assink and Rulkens, 1989; Tuin et al., 1987). Finally, surfactants may be used in extraction of metals from soil (U.S. EPA, 1996b).

Physical Treatment

Physical treatment is used to separate the contaminated fraction, usually the fine materials, from the rest of the soil matrix. Physical separation may be performed alone or in conjunction with chemical treatment, as in most soil washing processes. The most common method for physical separation in soil washing uses rotary attrition scrubbers to isolate the contaminated particles. The rotation of the slurry causes contact between large particles, resulting in attrition of the larger particles which releases the contaminant and contaminated fines to the slurry. The contaminant remains suspended in solution or sorbs to the reactive fine particles. Vibration units are also available to perform similar separations (Rosetti, 1993).

Hydrocyclones are the most common method used to separate fines from the clean soil. Other options are available for fine particle separation, including mechanical classifiers, gravity classifiers, spiral concentrators, and magnetic separators (Rosetti, 1993). Froth flotation can be used to combine physical and chemical treatment processes into one step. For this method, extracting agent is added to the soil before it enters the froth flotation cell. The slurry is leached in the tanks to remove the contaminant and the fines (<50 :m) are then separated from coarse particles in the flotation unit (Rosetti, 1993).

A) Dewatering

After the contaminated fine particles are separated from the clean coarse particles, both fractions are dewatered. The fine fraction is usually dewatered using a belt filter or filter press and disposed of in a landfill. Larger particles are rinsed to remove residual extracting solution and contaminant and dewatered using belt and filter presses. This fraction is considered clean and can be returned to the site.

B) Water Treatment

The contaminated water from rinsing and dewatering steps is treated by manipulating the solution chemistry to separate the contaminant from the extractant if possible. Contaminants can then be removed from solution, most commonly by precipitation or sedimentation, and are dewatered before disposal with the contaminated fines. The extracting agent and process water can be recycled for reuse.

C) Pyrometallurgical Extraction

Pyrometallurgical technologies use elevated temperature extraction and processing for removal of metals from contaminated soils. Soils are treated in a high-temperature furnace to remove volatile metals from the solid phase. Subsequent treatment steps may include metal recovery or immobilization. Pyrometallurgical treatment requires a uniform feed material for efficient heat transfer between the gas and solid phases and minimization of particulates in the off-gas. This process is usually preceded by physical treatment to provide optimum particle size. Pyrometallurgical processes usually produce a metal-bearing waste slag, but the metals can also be recovered for reuse (U.S. EPA, 1996c).

D) In Situ Soil Flushing

In situ soil flushing is used to mobilize metals by leaching contaminants from soils so that they can be extracted without excavating the contaminated materials. An aqueous extracting solution is injected into or sprayed onto the contaminated area to mobilize the contaminants usually by solubilization. The extractant can be applied by surface flooding, sprinklers, leach fields, vertical or horizontal injection wells, basin infiltration systems or trench infiltration systems (U.S. EPA, 1996b). After being contacted with the contaminated material the extractant solution is collected using pump-and-treat methods for disposal or treatment and reuse. Similar extracting agents are used for in situ soil flushing and soil washing, including acids/bases, chelating agents, oxidizing/ reducing agents and surfactants/ cosolvents. Also, water can be used alone to remove water-soluble contaminants such as hexavalent chromium. The applicability of in situ soil flushing technologies to contaminated sites will depend largely on site-specific properties, such as hydraulic conductivity, that influence the ability to contact the extractant with contaminants and to effectively recover the flushing solution with collection wells (NRC,1994).

E) Electrokinetic Treatment

Electrokinetic remediation technologies apply a low density current to contaminated soil in order to mobilize contaminants in the form of charged species. The current is applied by inserting electrodes into the subsurface and relying on the natural conductivity of the soil (due to water and salts) to effect movement of water, ions and particulates through the soil.

Water and/or chemical solutions can also be added to enhance the recovery of metals by this process. Positively charged metal ions migrate to the negatively charged electrode, while metal anions migrate to the positively charged electrode. Electrokinetic treatment concentrates contaminants in the solution around the electrodes. The contaminants are removed from this solution by a variety of processes, including electroplating at the electrodes, precipitation/coprecipitation at the electrodes, complexation with ion exchange resins, or by pumping the water from the subsurface and treating it to recover the extracted metals (Smith et al, 1995).

Electrokinetic treatment is most applicable to saturated soils with low ground-water flow rates and moderate to low permeability. The efficiency of metal removal by this process will be influenced by the type and concentration of contaminant, the type of soil, soil structure, and interfacial chemistry of the soil.

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