Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil

Abioye, O. Peter Institute of Biological Sciences, University of Malaya, Kuala Lumpur Malaysia

1. Introduction

Contamination of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper (2003). Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger et al., 1997). These hydrocarbon pollutants usually caused disruptions of natural equilibrium between the living species and their natural environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Das and chandran, 2010).

Heavy metals contaminated soil from industrial waste; electronic wastes etc. on the other hand pose a serious threat to both man and animals in the environment if not properly remediated to the innocuous level. Environmental pollution by heavy metals which are released into the environment through various anthropogenic activities such as mining, energy and fuel production, electroplating, wastewater sludge treatment and agriculture is one of the world's major environmental problem. Heavy metals or trace metals refer to a large group of trace elements which are both industrially and biologically important. Initially, heavy metals are naturally present in soils as natural components but as of now, the presence of heavy metals in the environment has accelerated due to human activities. This is a widespread problem around the world where excessive concentration of heavy metals such as Pb, Zn, Cr, Cu, Cd, Hg, and As can be found in soils.

Soil contamination by heavy metals is consequently the most critical environmental problems as it poses significant impacts to the human health as well as the ecosystems. The contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. Heavy metals in the soil subsequently enter the human food web through plants and they constitute risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Heavy metals are discovered in various food chains where the results are usually detrimental to microorganisms, plants, animals and humans alike.

Many techniques of remediation of contaminated soil have been developed, such as physical, chemical degradation, photodegradation. However, most of these methods have some drawbacks in completely remediating hydrocarbon contaminated soil. Some of these methods leave behind daughter compounds which are more toxic to the environment than the parent compounds. Biological treatment offers the best environmental friendly method for remediating hydrocarbon and heavy metal contaminated soil because it utilized the capability of the indigenous microorganisms in the soil environment to break down the hydrocarbons and heavy metals into innocuous substances.

Biological remediation, a process defined as the use of microorganisms or plants to detoxify or remove organic and inorganic xenobiotic compounds from the environment is a remediation option that offers green technology solution to the problem of environmental degradation. This process relied upon microbial enzymatic activities to transform or degrade the contaminants from the environment (Philp et al., 2005). It offers a cost effective remediation technique, compared to other remediation methods, because it is a natural process and does not usually produce toxic by-products. It also provides a permanent solution as a result of complete mineralization of the contaminants in the environment (Perelo, 2010). Advantages of biological remediation compared to other treatment methods include (Okoh and Trejo-Hernandez, 2006):

- i. Destruction rather than transfer of the contaminants to another medium.
- ii. Minimal exposure of workers to the contaminants.
- iii. Longtime protection of public health.
- iv. Possible reduction in the duration of the remediation process.

2. Impact of hydrocarbon and heavy metals contamination on environment and human health

Hydrocarbon spills in the form of petroleum products both on land and in water, have been a problem since discovery of oil as a fuel source. They can have devastating effects on the biota of an environment. Oil spills and oil waste discharged into the sea from refineries, factories or shipping contain poisonous compounds that constitutes potential danger to plants and animals. The poisons can pass through the food web of an area and may eventually be eaten by humans (Gibson and Parales, 2000).

Environmental contamination by hydrocarbons and petroleum products constitute nuisance to the environment due to their persistent nature and tendency to spread into ground and surface waters. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Oil spillages into the environment have become one of the major problems. Used motor oils such as diesel or jet fuel contaminate natural environment with hydrocarbon (Husaini, et. al 2008). The hydrocarbons spread horizontally on the groundwater surface thereby causing extensive ground waters contamination (Plohl et al. 2002). Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater) especially by PAHs has drawn public concerns because many PAHs are toxic, mutagenic, and carcinogenic (Bumpus 1989; Clemente et al. 2001; Cerniglia and Sutherland 2001). Aromatic hydrocarbons are considered to be the most acute toxic component of petroleum products, and are also associated with chronic and carcinogenic effects (Anderson, et al., 1974). Aromatics are often distinguished by the number of rings they possess, which may range from one to five (Anderson, et al., 1974). Lighter, mono-aromatics (one ring) compounds include benzene, toluene, ethylbenzene, and xylenes (NOAA, 1995). Aromatics with two or more rings are referred to as polycyclic aromatic hydrocarbons (PAHs) (Anderson, et al., 1974). Used lubricating oil contains several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo(a)pyrene (a PAH). Upshall et al (1992) reported that motor oil had a density of 0.828 g/ml and contained 14% aromatics and 65.4% aliphatics (by weight). In their study, the sum of 26 individual PAHs represented 0.17% of the oil, or 1.2% of the aromatic fraction.

The main threats to human health from heavy metals are related with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid but is usually classified as a heavy metal). Heavy metals have been utilised by humans for thousands of years. Exposure to heavy metals continues although several adverse health effects of heavy metals have been known for a long time. For example, mercury is still used in gold mining in many parts of Latin America. Arsenic is still common in wood preservatives, and tetraethyl lead remains a common additive to petrol, although this use has decreased dramatically in the developed countries. Waste-derived fuels are especially prone to contain heavy metals which should be a central concern in the consideration for their use. Since the mid 19th century, production of heavy metals increased abruptly for more than 100 years, with associated emissions to the environment, particularly in less developed countries though emissions have lessened in most developed countries over the last century.

Some heavy metals are dangerous to health or to the environment (e.g. mercury, cadmium, lead, chromium), some may cause corrosion (e.g. zinc, lead), some are harmful in other ways (e.g. arsenic may pollute catalysts). Some of these elements are actually necessary for humans in minute amounts (cobalt, copper, chromium, manganese, nickel) while others are carcinogenic or toxic, affecting, among others, the central nervous system (manganese, mercury, lead, arsenic), the kidneys or liver (mercury, lead, cadmium, copper) or skin, bones, or teeth (nickel, cadmium, copper, chromium). One of the largest problems associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification causing heavier exposure for some organisms than is present in the environment alone. Through precipitation of their compounds or by ion exchange into soils and muds, heavy metal pollutants can localize and lay dormant. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation.

3. Remediation techniques for hydrocarbon and heavy metal contaminated soil

Internationally, petroleum contamination is widespread, posing serious environmental risks including surface and groundwater contamination (Balasubramaniam et al. 2007). The environment can potentially be affected by numerous operations in petroleum exploration, production and transportation, with common sources of contamination being leaking underground storage tanks (Nadim et al., 2000). Contamination poses serious environmental risks, including surface and groundwater contamination, and risks to human health and safety (Balasubramaniam et al. 2007). Remediation of contaminated soil is an essential practice. Some of the different techniques used in remediating contaminated soil are discussed below.

3.1 Physical and chemical remediation techniques

3.1.1 In situ soil vapour extraction

Volatile and some semi-volatile organic compounds (VOCs and Semi-VOCs) can be removed from unsaturated soils by a process known as soil vapour extraction (SVE). SVE as

an in situ clean-up process allows contaminated soil to be remediated without disturbance or excavation (Nadim et al. 2000).

Soil vapor extraction (SVE) is an in situ unsaturated (vadose) zone soil remediation technology in which a vacuum is applied to the soil to induce the controlled flow of air and remove volatile and some semi-volatile contaminants from the soil. The gas leaving the soil may be treated to recover or destroy the contaminants. The drawback in the use of SVE for remediation of contaminated site is that SVE can not remove heavy oils, metals, PCBs, or dioxins from contaminated soil; it is only effective for remediation of soil contaminated with VOCs and Semi-VOCs. Because the process involves the continuous flow of air through the soil, however, it often promotes the in situ biodegradation of low volatility organic compounds that may be present.

3.1.2 In situ steam injection vapour extraction

Cold soil vapour extraction is a common technique for remediating volatile organic compounds from the unsaturated subsurface. Limitations in efficiency can be overcome by using thermal enhancement, e. g. steam as a fluid heat transport medium to speed up the process (Sleep and Ma, 1997).

In situ steam extraction is a new technology and has had limited use across the globe. Steam extraction can be used in two different systems; mobile and stationary. The mobile system has a unit that volatilizes contaminants in small areas in a sequential manner by injecting steam and hot air through rotating cutter blades that pass through the contaminated medium. The stationary system uses steam injection as a means to volatilize and displace contaminants from the undisturbed subsurface soil. In both systems, steam (at 200°C) and compressed air (at 135°C) are forced through the soil medium and the mixture of air; vapor and chemicals are collected by extraction wells (Nadim et al. 2000).

3.1.3 Air sparging

Air sparging is an in situ technology in which air is injected through a contaminated aquifer. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that removes contaminants by volatilization (EPA, 2001). Air sparging can also be explained as a method of site remediation that introduces air (or other gases) into the saturated zone contaminated with VOCs. In addition to volatilization of VOCs, air sparging promotes the growth of aerobic bacteria in saturated zones and may oxidize reduced chemical species (Nadim et al 2000). Air sparging has been shown to be effective in removing several types of contaminants such as the lighter petroleum compounds (C_3 - C_{10}) and chlorinated solvents (Marley et al. 1992; Reddy et al. 1995).

3.1.4 Excavation

Excavation (removal) is a fundamental remediation method involving the removal of contaminated soil/media, which can be shipped off-site for treatment and/or disposal, or treated on-site when contaminants are amenable to reliable remediation techniques. Excavation is generally utilized for localized contamination and point source and is also used for the removal of underground structures that are out of compliance or have been identified as a potential or actual point source of contamination. The limiting factor for the use of excavation is often represented by the high unit cost for transportation and final off-site disposal. EPA (1991) further stated some limiting factors that may limit the applicability and effectiveness of the process to include:

- i. Generation of fugitive emissions may be a problem during operations.
- ii. The distance from the contaminated site to the nearest disposal facility will affect cost.
- iii. Depth and composition of the media requiring excavation must be considered.

iv. Transportation of the soil through populated areas may affect community acceptability. In this respect, the on-site removal and treatment can often yield significant savings and, in addition, the treated soil may have beneficial secondary use (e.g. as construction fill or road base material) at the same site.

3.2 Bioremediation techniques

Bioremediation is one of the most viable options for remediating soil contaminated by organic and inorganic compounds considered detrimental to environmental health. Bioremediation is a process defined as the use of microorganisms/plants to detoxify or remove organic and inorganic xenobiotics from the environment. It is a remediation option that offers green technology solution to the problem of hydrocarbon and heavy metals contamination. The main advantage of bioremediation is its reduced cost compared to conventional techniques. Besides cost-effectiveness, it is a permanent solution, which may lead to complete mineralization of the pollutant. Furthermore, it is a non-invasive technique, leaving the ecosystem intact (Perelo, 2010). Bioremediation can deal with lower concentration of contaminants where the cleanup by physical or chemical methods would not be feasible. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Vidali, 2001).

Hydrocarbon considered to be one of the major sources of energy supply across the world usually constitutes major contaminants to both aquatic and terrestrial ecosystems. Various techniques has been employed to remediate soil environment contaminated by hydrocarbons, ranging from physical, to chemical and mechanical forms of treating or removing the contaminants. Bioremediation offers a better technique for treatment and removal of these contaminants into an innocuous substance. Effective bioremediation of hydrocarbons in the soil environment can be achieved by either or both of the following techniques: Biostimulation and Bioaugmentation.

Microorganisms play a significant and vital role in bioremediation of heavy metal contaminated soil and wastewater. Though when microorganisms especially bacteria are exposed to higher concentration of metal, it may have cidal effects on them. Hence, microorganisms are effective only at low metal concentration in the soil. Microorganisms are usually used for the removal of heavy metals. Microorganisms can interact with metals and radionuclides via many mechanisms, some of which may be used as the basis for potential bioremediation strategies (Lloyd et al., 2005). Mechanisms by which microorganisms act on heavy metals includes biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) (Lloyd, 2002). Biosorption seems to be the most common mechanisms (Haferburg and Knothe, 2007). It is the only option when dead cells are applied as bioremediation agent. However, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via

different mechanisms (Malik et al., 2004). On the other hand, living cells shows higher sensitivity to environmental conditions and demand nutritional and energetic sources.

Many genera of microbes like *Bacillus, Enterobacter, Escherichia, Pseudomonas* and also some yeasts and moulds help in bioremediation of metal and chromium-contaminated soil and water by bio-absorption and bioaccumulation of chromium (Kotas and Stasicka, 2000). The heavy metal removal by the bacteria *Pseudomonas* was attributed to the cellular growth of these organisms (Ray and Ray, 2009).

3.2.1 Biostimulation

Biostimulation of indigenous microbes is a bioremediation strategy mostly used for remediation of contaminated soil. This involves addition of nutrients, either organic or inorganic, to enhance the activities of indigenous microbes. Input of large quantities of carbon sources such as crude oil, used lubricating oil, diesel oil etc. tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P. Levels of N and P added to stimulate biodegradation at contaminated sites are often estimated from C/N ratios (Sang-Hwan et al. 2007).

Biostimulation aims at enhancing the activities of indigenous microorganisms that are capable of degrading pollutant from soil environment, it is often been applied to the bioremediation of oil-contaminated soil. Nutrient enrichment, also called fertilization is a bioremediation approach in which fertilizer similar to phosphorus and nitrogen that are applied to plant in farms are added to contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants (Thieman and Palladino 2009). Microorganisms need an abundance of key elements such as carbon, hydrogen, nitrogen, oxygen and phosphorus for building macromolecules, addition of fertilizer provides these microbes with essential elements to reproduce and thrive. In some instances, manure, wood chips and straw may provide microbes with the sources of carbon as a fertilizer. The concept of biostimulation is that, by adding more nutrients, microorganisms replicate, increase in number and grow rapidly and thus increase the rate of biodegradation (Thieman and Palladino 2009). Addition of inorganic nutrients do act as fertilizer to stimulate biodegradation by autochthonous microorganisms in some cases; in other cases, it is the intentional stimulation of resident xenobiotic-degrading bacteria by use of electron acceptors, water, nutrient addition, or electron donors (Widada, et al., 2002). Combinations of inorganic nutrients often are more effective than single nutrients (Sutherland, et al., 2000). Laboratory-based respiration experiments by Liebeg and Cutright (1999) showed that a low level of macronutrients and a high level of micronutrients were required to stimulate the activities of indigenous microbes. The greatest stimulation was recorded with a solution consisting of 75% sulphur, 3% nitrogen and 11% phosphorus.

Addition of a carbon source as a nutrient in contaminated soil is known to enhance the rate of pollutant degradation by stimulating the growth of microorganisms responsible for biodegradation of the pollutant. It has been suggested that the addition of carbon in the form of pyruvate stimulates the microbial growth and enhances the rate of PAH degradation (Lee, et al., 2003). Biostimulation can also be achieved by the use of composting bioremediation technologies. Composting bioremediation strategy relies on mixing the primary ingredients of compost with the contaminated soil, such that as the compost matures, the pollutants are degraded by the active microflora within the mixture (Semple, et al., 2001). Mushroom compost and spent mushroom compost (SMC) are also applied in treating organopollutant contaminated sites (Eggen, 1999, Trejo-Hernandez et al., 2001).

Addition of SMC results in enhanced PAH-degrading efficiency (82%) as compared to the removal by sorption on immobilized SMC (46%). It was observed that the addition of SMC to the contaminated medium reduced the toxicity, added enzymes, microorganisms, and nutrients for the microorganisms involved in degradation of PAHs (Lau, et al., 2003). Organic wastes like banana skin, spent mushroom compost and brewery spent grain in earlier studies were found to enhance the biodegradation of used lubricating oil up to 90% loss of oil within the period of 3 months (Abioye, et al., 2009b, 2010). Also the results of our studies revealed the potential of melon shell to stimulate 75% crude oil degradation in soil contaminated with crude oil within the period of 28 days (Abioye, et al., 2009a).

Depending on the nature of the contaminated soil, some of these nutrients could become limiting, hence the additions of nutrients are necessary to enhance the biodegradation of oil pollutants (Choi et al., 2002; Kim et al., 2005). Pelletier et al. (2004) assessed the effectiveness of fertilizers for crude oil bioremediation in sub-Antarctic intertidal sediments over a oneyear and observed that chemical, microbial and toxicological parameters demonstrated the effectiveness of various fertilizers in a pristine environment. Frederic et. al., (2005), observed that addition of commercial oleophilic fertilizers containing nitrogen and phosphorus to hydrocarbon contaminated soil increased the hydrocarbon-degrading microbial abundance and total petroleum hydrocarbon degradation, and also reported 77 - 95% loss of total alkanes and 80% of PAHs in hydrocarbons contaminated soil within the period of 180days. In another study using poultry manure as organic fertilizer in contaminated soil, biodegradation was reported to be enhanced in the presence of poultry manure alone, but the extent of biodegradation was influenced by the incorporation of alternate carbon substrates or surfactants (Okolo et al., 2005). However, excessive nutrient concentrations can inhibit the biodegradation activity (Challain et al., 2006), and several authors have reported the negative effect of high NPK levels on the biodegradation of hydrocarbons (Oudot et al., 1998; Chaineau et al., 2005) and more especially on the aromatics (Carmichael and Pfaender, 1997).

3.2.2 Bioaugmentation

This is an approach that involves introduction of microorganisms that possessed biodegradation potential into the contaminated environment to assist the indigenous microbes with biodegradative processes. This may sometimes involved addition of genetically engineered microorganisms suited for biodegradation of the hydrocarbon contaminants into the contaminated soil. Bioaugmentation is a promising and low-cost bioremediation strategy in which an effective bacterial isolate(s) or microbial consortium capable of degrading xenobiotics is administered to contaminated sites (Gentry et al., 2004). Successful bioremediation of soil contaminated with hydrocarbon sources through bioaugmentation has been reported by various authors. Bagherzadeh et al., (2008) evaluated the efficiency of pollutant removal by selected microorganisms and reported thus: Five mixed cultures and 3 single bacteria strains, Pseudomonas sp., Arthrobacter sp. and Mycobacterium sp. were isolated from hydrocarbon-contaminated soils by enrichment on either crude oil or individual hydrocarbons, as the sole carbon sources. The strains were selected based on their ability to grow in medium containing crude oil, used engine oil or both. Their ability to degrade hydrocarbon contaminants in the environment was investigated using soil samples contaminated with used engine oil. The mixed starter culture #1 degraded 66 % of aliphatic compounds in the engine oil, after 60 days of incubation. The mixed starter culture #5 removed 47 % of aromatic compounds during 60 days of incubation. Bento et al. (2005) reported 72.7% light TPH fraction and 75.2% heavy TPH fraction degradation in diesel contaminated soil bioaugmented with bacterial consortium of *Bacillus cereus, Bacillus sphaericus, Bacillus fusiformis, Bacillus pumilus Acinetobacter junii* and *Pseudomonas* sp. Ying et al. (2010) augmented a PAH-contaminated soil with *Paracoccus* sp. strain HPD-2 and observed 23.2% decrease in soil total PAH concentrations after 28 days, with a decline in average concentration from 9942 to 7638 µg kg⁻¹ dry soil. They discovered percentage degradation of 3-, 4- and 5(+6)-ring PAHs was 35.1%, 20.7% and 24.3%, respectively.

The soil environment is very complicated and the degrading ability of exogenously added microorganisms tends to be affected by the physicochemical and biological features of the soil environment. Sometimes, the administration of petroleum degrading microorganisms leads to a failure of bioaugmentation (Vogel 1996; Gentry et al., 2004). Bioaugmentation is not always an effective solution for remediation of contaminated soil because in some cases laboratory strains of microorganisms rarely grow and biodegrade xenobiotics compared to the indigenous microbes (Thieman and Palladino 2009). Also Bioaugmentation is yet to gain public acceptance, most especially the use of genetically engineered microbes due to the believe that these microbes when seeded into contaminated soil may alter the ecology of the environment as well as pose risk to the environmental health if they persist after the remediation of the contaminated soil.

3.3 Phytoremediation of hydrocarbon and metal-contaminated soil

Phytoremediation is a remediation method that utilizes plants to remove, contain or detoxify environmental contaminants (Palmroth, 2006). Phytoremediation appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil (Wenzel, 2009). Some major advantages and disadvantages of phytoremediation are shown in Table 1. The most common plant species used in phytoremediation of organic and inorganic compounds includes willows, poplar and different types of grasses. Comprehensive list of plants that has recorded positive results in remediation of organic compounds are listed in Table 2.

On-site phytoremediation of petroleum hydrocarbons and heavy metals can be enhanced by employing a combination of common agronomic practices (e.g. fertilizer application, tillage and irrigation), this is because available nutrient reserves can be quickly depleted as the microbial community begins to degrade the contaminants (Farrell and Germida, 2002). Therefore fertilizer applications may enhance the degradation of petroleum hydrocarbons in soil by reducing competition for limited nutrients. Cutright (1995) reported that increasing the amount of nitrogen and phosphorus in soil under aerobic conditions increased the degradation of PAHs by the soil fungus *Cunninghamella echinulata* var. *elegans*. Brown, (1998) also observed loss of 2- and 3- rings of aromatic hydrocarbons from soil contaminated with weathered petroleum compounds when the soil was amended with sludge compost high in nitrogen compared to no amendment or low nitrogen amendment. Palmroth et al. (2002) recorded 60% loss of diesel fuel in 30 days in diesel-contaminated soil planted with pine tree and amended with NPK fertilizer. Also, Vouillamoz and Milke (2009) observed that compost addition combined with phytoremediation, increases the rate of removal of diesel

Advantages	Disadvantages
Relatively low cost	Longer remediation time
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fate might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant materials	
Costs 10 – 20% of mechanical treatments	Slower than mechanical treatments
Faster than natural attenuation	Only effective for moderately hydrophobic compounds
High public acceptance	Toxicity and bioavailability of biodegradation products is not known.
Fewer air and water emissions	Contaminants may be mobilized into the ground water
Conserves natural resources	Influenced by soil and climate conditions of the site.
(Susarla et al., 2002;	Kamath, et al., 2004)

fuel in soil. Agamuthu et al., (2010) recorded appreciable degradation of used lubricating oil in soil when the growth of *Jatropha curcas* was enhanced with brewery spent grain.

Table 1. Advantages and disadvantages of phytoremediation over traditional technologies.

3.3.1 Mechanisms of phytoremediation

Variety of pollutant attenuation mechanisms possessed by plants makes their use in remediating contaminated land and water more feasible than physical and chemical remediation (Glick, 2003; Huang et al., 2004, 2005; Greenberg, 2006; Gerhardt et al., 2009). As a result of their sedentary nature, plants have evolved diverse abilities for dealing with toxic compounds in their environment. Plants act as solar-driven pumping and filtering systems as they take up contaminants (mainly water soluble) through their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered, or volatilized (Greenberg et al., 2006; Abhilash, 2009). Plants utilize different types of mechanisms for dealing with environmental pollutants in soil. The mechanisms of phytoremediation include biophysical and biochemical processes like adsorption, transport and translocation, as well as transformation and mineralization by plant enzymes (Meagher, 2000). Plants have been shown to be able to degrade halogenated compounds like TCE by oxidative degradation pathways, including plant specific dehalogenases (Nzengung, et al., 1999). Dehalogenase activity was observed to be maintained after the plants death. Enzymes can become bound to the organic matrix of the sediment as plants die, they decay and they are buried in the sediment, thus contributing to the dehalogenase activity observed in organic-rich sediments (Nzengung, et al., 1999).

Plant used	Contaminants	Results	Reference
Jatropha curcas	Coal fly ash, lead, cadmium, arsenic and chromium	Enhanced heavy metals uptake by 117% in root, 62% in stem and 86% in leaves when EDTA was applied at 0.3g/kg to fly ash. Jatropha accumulated Cd and Pb in the shoot. It shows increase bioaccumulation potential of As and Cr with increase in metal concentration in soil system.	Santosh et al., (2009) Jamil et al., (2009) Mangkoediharjo and Surahmaida (2008)
Carex exigua, Panicum virgatum Tripsacum dactyloides	Petroleum hydrocarbons	70% loss of total petroleum hydrocarbons was recorded after one year growth of these plants in contaminated soil.	Euliss et al., (2008)
Vicia faba	Crude petroleum oil	47% of total petroleum hydrocarbon was degraded in 60 davs.	Diab (2008)
Populus tremula	Cadmium and Zinc	Both Cd and Zn accumulated in the leaves with maximum foliar concentration of 35 and 2400mg/kg	Hassinen et al., (2009)
Ditch reed and Alfalfa	Liquid bitumen agar (mainly paraffins & naphtenes) 70.9g/kg and soil containing PAHs 80mc/ke	82% removal was achieved in 27 months with 50 both plants.	Muratova et al., (2003)
Tall fescue	PAHs in creosote contaminated soil.	Removal of acenaphtene and fluorine in 36 months was slightly higher in the presence of tall fescue than in unveetated soil.	Robinson et al., (2003)
Rye grass and Sweet clover	Aged PAHs from manufacture gas plant.	PAHs removal in 12 months was higher in the presence of plants, 9% to 24% compared to 5% without plant.	Parish et al., (2004)

Table 2. Examples of plants used for phytoremediation of organic contaminants.

www.intechopen.com

Variety of contaminant-degrading enzymes can be found in plants. These include peroxidases, dioxygenases, P450 monooxygenases, laccases, phosphatases, dehalogenases, nitrilases, and nitroreductases (Susarla et al., 2002; Singer et al., 2004 Chaudhry et al., 2005). Phytoremediation is based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms, such as transpiration, photosynthesis, metabolism, and mineral nutrition. Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances; roots can stabilize and bind substances on their external surfaces, and when they interact with microorganisms in the rhizosphere (Marmiroli et al., 2006). Uptaken substances may be transported, stored, converted, and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules (Marmiroli et al., 2006). A series of six phytotechnologies have been identified by Interstate Technology and Regulatory Cooperation (ITRC, 2001) which may address different contaminants in different substrates, and which rely on one or more of the plant properties.

- 1. Phytotransformation, ideal for organic contaminants in all substrates
- 2. Rhizosphere bioremediation, applied to organic contaminants in soil
- 3. Phytostabilisation, for organic and inorganic contaminants in soil
- 4. Phytoextraction, useful for inorganic contaminants in all substrates
- 5. Phytovolatilisation, which concerns volatile substances
- 6. Evapotranspiration, to control hydraulic flow in the contaminated environment

4. Conclusion

Remediation of hydrocarbon contaminated soil is a necessity in order to have a safe and healthy environment that will in turn results in healthy lifestyle across the globe. Biological remediation of hydrocarbon and metal contaminated soil offers a better and more environmentally friendly technique that if properly and thoroughly explored can bring our environment into a better place for both plant and animals well being due to its enormous advantages over other treatment methods. However, despite these enormous advantages of biological treatment method, its potential is yet to be fully utilized in restoration of contaminated soil. This is possibly due to the fact that it takes a longer period of time for the complete restoration of the environment; this limitation can however be overcome through nutrient addition and introduction of microbes with biodegradative capability to degrade hydrocarbon and heavy metals in the environment. Future research and developments will requires focus on the use of cheap, environmental friendly and widely available nutrients that can be used to enhance the microbial and plant activities in mineralizing hydrocarbons and heavy metals in soil environment.

5. References

Abhilash, P.C., Jamil, S., Singh, N. (2009) Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics, Biotechnology Advance 27: 474 – 488.

- Abioye, O. P, Abdul Aziz, A, Agamuthu, P. (2009b). Stimulated biodegradation of used lubricating oil in soil using organic wastes. Malaysian Journal of Science. 28(2): 127 – 133.
- Abioye, O. P, Abdul Aziz, A, Agamuthu, P. (2010). Enhanced Biodegradation of Used Engine Oil in Soil Amended with Organic Wastes. Water Air and Soil Pollution. 209: 173 – 179.

- Abioye, O.P, Alonge, O.A, Ijah, U.J.J. (2009a) Biodegradation of crude oil in soil amended with melon shell. Assumption University Journal of Technology 13(1): 34 38.
- Agamuthu, P., Abioye, O.P., Abdul Aziz, A. (2010). Phytoremediation of soil contaminated with used lubricating oil using *Jatropha curcas*. Journal of Hazardous Materials 179: 891 – 894.
- Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem and G.M. Hightower. (1974). Characteristics of dispersions and watersoluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Marine Biology 27:75-88.
- Bagherzadeh N. A, Shojaosadati S. A, Hashemi N. S (2008) Biodegradation of used engine oil using mixed and isolated cultures. International Journal of Environmental Research 2(4): 431 – 440.
- Balasubramaniam, A., Boyle, A.R., Voulvoulis, N. (2007). Improving petroleum contaminated land remediation decision making through the MCA weighting process. Chemosphere 66: 791 – 798.
- Bento, F.M., Camargo, F.O.A., Okeke, B.C., Frankenberger, W.T., (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresource Technology 96: 1049–1055.
- Brown, J.L., Syslo, J., Lin, Y.H., Getty, S., Vemuri, R. Nadeau, R. (1998) On-site treatment of contaminated soils: An approach to bioremediation of weathered petroleum compounds. Journal of Soil Contamination 7: 773 – 800.
- Bumpus J.A (1989) Biodegradation of polycyclic aromatic hydrocarbons by Phanerochaete chrysosporium. Applied Environmental Microbiology 55:154–158
- Carmichael, L.M, Pfaender F.K (1997). The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. Biodegradation 8: 1-13.
- Cerniglia CE, Sutherland JB (2001) Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. In: Gadd GM (ed) Fungi in bioremediation. Cambridge University Press, Cambridge, pp 136–187.
- Chaillan F, Chaineau C.H, Point V, Saliot A, Oudot J (2006). Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. Environmental Pollution. 144:228 237
- Chaineau CH, Rougeux G, Yepremian C, Oudot J (2005). Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. Soil Biology and Biochemistry 37:1490-1497.
- Chaudhry, Q, Blom-Zandstra, M, Gupta, S, Joner, E. J. (2005) Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environmental Science Pollution Research 12: 34 – 48.
- Choi S-C, Kwon KK, Sohn JH, Kim S-J (2002). Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mescocosms. Journal of Microbiology and Biotechnology 12:431-436.
- Clemente A.R, Anazawa T.A, Durrant L.R (2001) Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Brazilian Journal of Microbiology 32:255–261.
- Cutright, T.J. (1995) Polycyclic aromatic hydrocarbon biodegradation and kinetics using *Cunninghamella echinulata* var. *elegans*. International Journal of Biodeterioration and Biodegradation 35(4): 397 408.
- Diab, E. (2008) Phytoremediation of oil contaminated desert soil using the rhizosphere effects. Global Journal of Environmental Research 2(2): 66 73.

- Eggen, T. (1999). Application of fungal substrate from commercial mushroom production-*Pleurotus ostreatus*- for bioremediation of creosote contaminated soil, International. Biodeterioration and Biodegradation. 44: 117–126.
- EPA, (1991) Survey of Materials-Handling Technologies Used at Hazardous Waste Sites, EPA, ORD, Washington, DC, EPA/540/2-91/010.
- EPA, (2001). A citizen's guide to monitored natural attenuation. EPA 542-F-01-004.
- Euliss, K, Ho, C, Schwab, A. P, Rock, S, Banks, M. K. (2008) Greenhouse and field assessment of phytoremediation for petroleum contaminants in a riparian zone. Bioresource Technology 99:1961 – 1971.
- Farrell, R.E., Germida, J.J. (2002) Phytotechnologies: Plant-based systems for remediation of oil impacted soils. www.esaa-events.com/remtech/2002/pdf/09Farrellpaper.pdf. (Accessed on 5th July, 2010).
- Frederic, C., Emilien, P., Lenaick, G and Daniel, D. (2005). Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere 58: 1439 1448.
- Gentry T.J, Rensing C, Pepper I.L (2004) New approaches for bioaugmentation as a remediation technology. Critical Review in Environmental Science and Technology 34:447–494.
- Gerhardt, K. E, Huang, X. D, Glick, B. R, Greenberg, B. M. (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Science 176: 20 – 30.
- Gibson, D. T., Parales, R. (2000) Aromatic hydrocarbon dioxygenases in environmental biotechnology. Current Opinion in Biotechnology 11: 236 243.
- Glick, B. R. (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnology Advances 21: 239 244.
- Greenberg, B. M. (2006) Development and field tests of a multi-process phytoremediation system for decontamination of soils. Canadian Reclamation 1: 27 29.
- Haferburg G, Kothe E (2007). Microbes and metals: Interactions in the environment. Journal of Basic Microbiology 47: 453-467.
- Hassinen, V, Vallinkoski, V. M, Issakainen, S, Tervahauta, A, Kärenlampi, S, Servomaa, K. (2009) Correlation of foliar MT2b expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula x tremuloides*) grown in contaminated soil. Environmental Pollution 157:922 – 930.
- Holliger, C., Gaspard, S., Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R.P., Vazquez, F. (1997) Contaminated environment in the subsurface and bioremediation: Organic contaminants. FEMS Microbiology Reviews 20(3-4): 517 – 523.
- Huang, X. D, El-Alawi, Y. S, Gurska, J, Glick, B. R, Greenberg, B. M. (2005) A multiprocess phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchemistry Journal 81:139–47.
- Huang, X. D, El-Alawi, Y. S, Penrose, D, Glick, B. R, Greenberg, B. M. (2004) A multiprocess phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution 130:465–76.
- Husaini, A., Roslan, H.A., Hii, K.S.Y. Ang, C.H. (2008). Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. World Journal of Microbiology and Biotechnology 24: 2789 2797.
- Interstate Technology and Regulatory Cooperation (ITRC), (2001) Phytotechnology Technical and Regulatory Guidance Document. Available on Internet at www.itrcweb.org.

- Jamil, S., Abhilash, P.C., Singh, N., Sharma, P. N. (2009) Jatropha curcas: A potential crop for phytoremediation. Journal of Hazardous Materials 172: 269 275.
- Kamath, R., Rentz, J.A., Schinoor, J.L., Alvarez, P.J.J. (2004) Phytoremediation of hydrocarbon-contaminated soils: Principles and applications. In: Petroleum biotechnology development and perspectives. Vazquez-Duhalt, R and Quintero-Ramirez, R. (eds). Elsevier, Amsterdam, pp 447 – 478.
- Kim S, Choi D.H, Sim D.S, Oh Y. (2005). Evaluation of bioremediation effectiveness on crude oil-contaminated sand. Chemosphere 59: 845 852.
- Kotas J and Stasicka Z. (2000) Chromium occurrence in the environment and methods of its speciation. Environmental pollution 107: 263-283.
- Kvenvolden, K.A., Cooper, C.K. (2003) Natural seepage of crude oil into the marine environment. Geo-Marine Letters 23(3-4): 140 146.
- Lau, K.L., Tsang, Y.Y., Chiu, S.W. (2003). Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52 (9), 1539–1546.
- Lee, K., Park, J.W. and Ahn, I. S. (2003). Effect of additional carbon source on naphthalene biodegradation by *Pseudomonas putida* G7, Journal of Hazardous Materials. 105: 157–167.
- Liebeg, E. W. and Cutright, T. J. (1999). The investigation of enhanced bioremediation through the addition of macro and micro nutrients in a PAH contaminated soil. International Biodeterioration and Biodegradation. 44: 55 – 64.
- Lloyd J R. (2002). Bioremediation of metals: The application of microorganisms that make and break minerals. Microbiology Today 29: 67-69.
- Lloyd, J.R., Anderson, R.T, Macaskie, L.E. (2005) Bioremediation of metals and radionuclides. In: Bioremediation: Applied microbial solutions for real world environmental cleanup. Atlas, R.M and Philp, J.C (eds). ASM Press, ISBN 1-55581-239-2, Washington, D.C. pp 294.
- Malik A (2004). Metal bioremediation through growing cells. Environment International 30: 261-278.
- Mangkoedihardjo, S., Surahmaida, (2008) *Jatropha curcas* L. for phytoremediation of lead and cadmium polluted soil, World Applied Science Journal 4(4): 519 522.
- Marley, M.C., Hazebrouck, D.J., Walsh, M.T. (1992). The application of in situ air sparging as an innovative soils and groundwater remediation technology, Groundwater Monitor and Remediation 12 (2): 137–144.
- Marmiroli, N., Marmiroli, M., Maestri, E. (2006). Phytoremediation and phytotechnologies: A review for the present and the future. In: Twardowska, I., Allen, H.E, and Haggblom, M.H. (eds). Soil and water pollution monitoring, protection and remediation. Springer, Netherland.
- Meagher, R.B. (2000). Phytoremediation of toxic elemental and organic pollutants, Current Opinion on Plant Biology 3: 153–162.
- Muratova, A. Y, Turkovskaya, O. V, Hubner, T, Kuschk, P. (2003) Studies of the efficacy of alfalfa and reed in the phytoremediation of hydrocarbon polluted soil. Applied Biochemistry and Microbiology 39:599 605.
- Nadim, F., Hoag, G.E., Liu, S., Carley, R.J., Zack, P. (2000). Detection and remediation of soil aquifer systems contaminated with petroleum products: an overview. Journal of Petroleum Science and Engineering 26: 169 – 178.
- National Oceanic and Atmospheric Administration (NOAA). Damage Assessment and Restoration Program. August 1995 .Injury Guidance Document for Natural Resources and Services under the Oil Pollution Act of 1990 (Public Review Draft). Prepared by M.T. Huguenin, D.H. Haury, and J.C. Weiss

- Nzengung, V.A., Wolfe, L.N., Rennels, D.E., McCutcheon, S.C., Wang, C. (1999). Use of aquatic plants and algae for decontamination of waters polluted with chlorinated alkanes. International Journal of Phytoremediation 1: 203–226.
- Okoh, A.I., Trejo-Hernandez, M.R. (2006) Remediation of petroleum polluted systems: Exploiting the bioremediation strategies. African Journal of Biotechnology 5(25): 2520 – 2525.
- Okolo J.C, Amadi E.N, Odu C.T.I. (2005). Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. Applied Ecology and Environmental Research 3(1): 47-53.
- Oudot J., Merlin F.X., Pinvidic P. (1998). Weathering rates of oil components in a bioremediation experiment in estuarine sediments. Marine Environmental Research 45: 113-125.
- Palmroth M. R. T, Koskinen P. E. P, Pichtel J, Vaajasaari K, Joutti A, Tuhkanen A. T, Puhakka A. J. (2006) Field-scale assessment of phytotreatment of soil contaminated with weathered hydrocarbons and heavy metals. Journal of Soil and Sediments 6(3): 128 – 136.
- Palmroth, M.R.T., Pichtel, J. Puhakka, J.A. (2002) Phytoremediation of subarctic soil contaminated with diesel fuel. Bioresource Technology 84: 221 228.
- Parish, Z. D, Banks, M. K, Schwab, A. P. (2004) Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil. International Journal of Phytoremediation 6:119 – 137.
- Pelletier E., Delille D, Delille B (2004). Crude oil bioremediation in sub- Antarctic intertidal sediments: chemistry and toxicity of oiled residues. Marine Environmental Research 57: 311-327.
- Perelo L. W. (2010) Review: In situ and bioremediation of organic pollutants in aquatic sediments. Journal of Hazardous Materials 177: 81 89.
- Philp, J. C., Atlas, R. M. (2005). Bioremediation of contaminated soil and aquifers. In: *Bioremediation: Applied Microbial Solution for Real- World Environmental Clean Up.* Atlas, R. M., and Jim, C. P. (ed.) ASM Press, ISBN 1-55581-239-2, Washington, D.C., pp.139.
- Plohl, K., Leskovs'ek , H., Bricelj, M. (2002) Biological degradation of motor oil in water. Acta Chim Slov 49:279–289
- Ray, S.A., Ray, M.K. (2009) Bioremediation of heavy metal toxicity with special reference to chromium. Al Ameen Journal of Medical Sciences 2(2): 57 63.
- Reddy, K.R., Kosgi, S. Zhou, J. (1995). A review of in situ air sparging for the remediation of VOC-contaminated saturated soils and groundwater, Journal of Hazardous Materials 12 (2): 97–118.
- Robinson, S. L, Novak, J. T, Widdowson, M. A, Crosswell, S. B, Fetterolf, G. D. (2003) Field and laboratory evaluation of the impact of tall fescue on polyaromatic hydrocarbon degradation in an aged creosote-contaminated surface soil. Journal of Environmental Engineering ASCE 129:232 – 240.
- Sang-Hwan L, Seokho L, Dae-Yeon K, Jeong-gyu K. (2007) Degradation characteristics of waste lubricants under different nutrient conditions. Journal of Hazardous Materials 143: 65 – 72.
- Santosh, K. V., Juwarkar, A. A., Kumar, G. P., Thawale, P. R., Singh, S. K., Chakrabarti, T. (2009) Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by *Jatropha curcas* L.: Impact of dairy sludge and biofertilizer, Bioresource Technology 100: 4616 – 4622.

- Semple, K.T., Reid, B.J., Fermor, T.R. (2001) Impact of composting strategies on the treatment of soils contaminated with organic pollutants: a review. Environmental Pollution 112: 269–283.
- Singer, A. C, Thompson, I. P, Bailey, M. J. (2004) The tritrophic trinity: a source of pollutant degrading enzymes and its implication for phytoremediation. Current Opinion in Microbiology 7: 239 – 244.
- Sleep, B. E., Ma, Y. (1997): Thermal variation of organic fluid properties and impact on thermal remediation feasibility. Journal of Soil Contamination 6(3): 281-30
- Susarla, S, Medina, V. F, McCutcheon, S. C (2002) Phytoremediation: an ecological solution to organic chemical contamination. Ecological Engineering 18:647–658
- Sutherland, T. D., Horne, I., Lacey, M. J., Harcourt, R. L., Russell, R. J. and Oakeshott, J. G. (2000). Enrichment of an endosulfan-degrading mixed bacterial culture. Applied Environmental Microbiology 66: 2822 – 2828.
- Thieman, W.J., Palladino, M.A. (2009). Introduction to biotechnology, 2nd edition. Pearson, New York, pp 209 222.
- Trejo-Hernandez, M.R., Lopez-Munguia, A. R. and Ramirez, Q. (2001). Residual compost of Agaricus bisporus as a source of crude laccase for enzymatic oxidation of phenolic compounds. Process Biochemistry. 36: 635–639.
- Upshall, C., J.F. Payne and J. Hellou. (1992). Induction of MFO enzymes and production of bile metabolites in rainbow trout (Oncorhynchus mykiss) exposed to waste crankcase oil. Reprinted with permission from Environmental Toxicology and Chemistry, .12: 2105-2112.
- Vidali, M. (2001) Bioremediation: An overview. Pure and Applied Chemistry 73(7): 1163 1172.
- Vogel TM (1996) Bioaugmentation as a soil bioremediation approach. Current Opinion in Biotechnology 7:311–316.
- Vouillamoz, J., Milke, M.W. (2009) Effect of compost in phytoremediation of dieselcontaminated soils. Water Science Technology 43(2): 291 – 295.
- Wenzel, W. W (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321: 385 408.
- Widada, J., Nojiri, H., and Omori, T. (2002). Recent development in molecular techniques for identification and monitoring of xenobiotic-degrading bacteria and their catabolic genes in bioremediation. Applied Microbiology and Biotechnology 60: 45 – 49.
- Ying T, Yongming L, Mingming S, Zengjun L, Zhengo L, Peter C. (2010) Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbon from aged contaminated soil. Bioresource Technology 101: 3437 – 3443.



Soil Contamination Edited by MSc Simone Pascucci

ISBN 978-953-307-647-8 Hard cover, 168 pages Publisher InTech Published online 12, September, 2011 Published in print edition September, 2011

Soil contamination has severely increased over the last decades, mainly due to petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals from industrial wastes and human activities. The critical point regarding contaminated soil monitoring is the intrinsic difficulty in defining fixed monitoring variables and indicators as the establishment of any a priori criterion and threshold for soil quality can be still considered subjective. This book is organized into eight chapters and presents the state-of-the art and new research highlights in the context of contaminated soil monitoring and remediation strategies, including examples from South America, Europe and Asia. The chapters deal with the following topics: - monitoring of dioxin, furan, hydrocarbons and heavy metals level in soils - bioindicators and biomarkers for the assessment of soil toxicity - use of reflectance spectroscopy for soil contaminants and waste material detection - remediation technologies and strategies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

O. Peter Abioye (2011). Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil, Soil Contamination, MSc Simone Pascucci (Ed.), ISBN: 978-953-307-647-8, InTech, Available from: http://www.intechopen.com/books/soil-contamination/biological-remediation-of-hydrocarbon-and-heavy-metals-contaminated-soil



InTech Europe

University Campus STeP Ri Slavka Krautzeka 83/A 51000 Rijeka, Croatia Phone: +385 (51) 770 447 Fax: +385 (51) 686 166 www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai No.65, Yan An Road (West), Shanghai, 200040, China 中国上海市延安西路65号上海国际贵都大饭店办公楼405单元 Phone: +86-21-62489820 Fax: +86-21-62489821 © 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the <u>Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License</u>, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.