Chapter

Plant-Microbe Interaction: Prospects and Applications in Sustainable Environmental Management

Ajuzieogu Chinyere Augusta, Ehis-Eriakha Chioma Bertha and Akemu Stephen Eromosele

Abstract

Plant-microbe interaction is mostly mutualistic although sometimes it can be negative. These interactions contribute to improving the environmental quality and health of all organisms. One significant aspect to this is application in sustainable environmental management. Plants are known to be involved in remediation of polluted environments through a mechanism known as phytoremediation and this process is usually more effective in collaboration with microorganism resident within the plant environment. These plants and microbes possess attributes that makes them great candidates for sustainable remediation of impacted environments. Different organic pollutants have been decontaminated from the environment using the phytoremediation approach. The plant-associated microbes possess certain traits that exert selective effect on the growth of plants which consequently perform the decontamination process through different mechanisms. Also, these microorganisms' harbour requisite genes charged with the responsibility of mineralization of different organic and inorganic compounds through several pathways to produce innocuous by-products. The limitations associated with this approach that prevents full-scale application such as contaminant-induced stress frequently leads to low/slow rates of seed germination, plant development and decreases in plant biomass have been solved by using plant growth promoting rhizobacteria. Phytoremediation is an emerging, cost-effective, eco-friendly and operational technology for the cleanup of polluted environment.

Keywords: environment, phytoremediation, pollution, plant, rhizobacteria

1. Introduction

The idea of phytoremediation of xenobiotics was birthed a few decades back as a result of the awareness that plants possess the capability of metabolizing toxic compounds. Since then, phytoremediation was adjudged a proven technology for the decontamination of environments polluted by a different of organic compounds such as polychlorinated biphenyls (PCBs), pesticides, polyaromatic hydrocarbons (PAHs), chlorinated solvents, dioxins and different approaches like rhizoremediation, combination of PGPR and specific contaminant-degrading bacteria, genetically engineered microbes, transgenic plants and enzyme technology can be used to improve the efficiency of bioremediation. Phytoremediation is an emerging technology that uses plants and associated bacteria for the treatment of contaminated environments by toxic pollutants. Although some challenges that have so far prevented full- scale application of phytoremediation technologies is that contaminant-induced stress frequently leads to low rates of seed germination, slow rates of plant development and decreases in plant biomass. However, this problem can be solved by using plant growth promoting rhizobacteria. Rhizobacteria that exert beneficial effect on the plant growth and development are called as plant growth promoting rhizobacteria (PGPR). The term rhizoremediation has been used to describe the combination of phytoremediation and bioaugmentation with contaminant

Petroleum is composed of various hydrocarbons including aromatics (e.g. polycyclic aromatic hydrocarbons [PAHs]), asphaltenes, aliphatics [i.e. n-alkanes (linear), branched, saturated & unsaturated], & others, which differ in chemical & physical composition based on the reservoir's origin [1, 2]. Petroleum hydrocarbons (PHCs) are organic in nature, comprising of hydrogen and carbon, and highly hydrophobic. In recent times, anthropogenic activities including industrial actions, petroleum & its products (fuel, diesel, kerosene and others) and partial oxidation of fossil fuels, have lead to build-up of PHCs in the environment [3–5]. Actually, petroleum & its products have impacted considerably, on both aquatic and terrestrial ecosystems contaminated by it. Owing to the fact that microorganisms participate directly in bio-geochemical cycles as major players of carbon and petroleum hydrocarbon breakdown (degradation), it is important to further understand petroleum biological degradation and its application [6, 7].

Microorganisms are capable of breaking down or producing hydrocarbons based on some kind of metabolic pathways, unique to each function in the underlying environment [7, 8]. There are key players in the biological degradation of several petroleum products like benzene, toluene, ethylbenzene & xylenes (BTEX), aliphatic & PAHs [2, 9, 10]. Efficient indicators of soil contamination levels are soil microorganisms, plants & biota. They have the ability to breakdown or retain approx. 100% of all kinds of soil pollutants & prevent them from gaining entrance to larger environment [11]. But, when there is severe pollution or contamination of the soil, it results in adverse impacts on soil biodiversity & soil quality. Soil functions (such as, fiber, food and fuel production) is also destroyed, and immediately the food chain is affected, this becomes a threat to public health [9, 12, 13]. Recording success in bio-degradation & bio-transformation of organics in PHC-contaminated soils to less-toxic by-products is usually dependent on the potential to build & sustain conditions that will favour & support microbe-driven degradation, bio-technologically & naturally. The interactions between microbes & plants in phyllosphere & soil are highly significant for both plant growth & productivity in agro-systems, natural systems &/or microbe-driven breakdown of soil PHCs. In field studies, the successful use of plant-microbe interactions in the biological remediation of PHC-contaminated soil relies basically on the native (autochthonous) microorganisms (rhizospheric & endophytic bacteria associated with plant) with the genes unique for bio-degradation of petroleum hydrocarbons [10].

Increasing research on ways to remediate or clean up contaminated environments is the outcome of increased awareness of the danger of soil pollution and its adverse effects on the entire ecological chain. Owing to the diverse nature of pollutants, no absolute remedy exists that is common to all kinds of soil contaminants. In this review however, the pollutants of interest are PHCs sourced from crude-oil or refined petroleum by-products [11]. Thus, researches related to soil HC-contamination & its bio-remediation is the focus. In addition, plant–microbe interactions related to bio-degradation of PHCs could offer a robust understanding of the requisite tools for developing on-site biological remediation plans for mitigating risks in PHCs-contaminated soil.

Terrestrial (soil) pollution can be restored by developing new, science-based technique, including a new emerging method, i.e., bio-remediation. Bio-remediation is an environmentally- friendly & efficient technique, where live microbe(s) and its products or other biological agents (such as plants) can be utilized for the remediation of eco-contamination [14].

The fate of hydrocarbon/organic contaminants in soil-plant environment is determined by significant processes driven by plant-microbe interactions, [15, 16], competence of the microbial activity & microbe-degradation or bio-transformation of petroleum hydrocarbons in soil. In field studies, in depth understanding of the fate of a hydrocarbon/organic-pollutant in oil would aid in determining if the contaminant will persist in the environment or not, enhance the success of any remediation strategy & assist in developing a high-throughput risk mitigation approach.

This part of the review focuses on role of plants & microbes in bio-degradation of PHCs-contaminated soil, resulting from increased researches on bio-remediation & field trials. Also, the enzymatic activities of hydrocarbon degrading microorganisms will be discussed. Emphasis is placed on Phyto-remediation, a valuable method that depends on plants to eliminate/decontaminate soil contaminants. Studies that recorded success on the use of trees in the restoration of PHCs-contaminated soils are also cited.

2. Biological remediation

Biological remediation involves elimination, attenuation or transformation of pollutants or contaminants by using biological agents/processes. It is frequently used in moderately PHC-contaminated soils. It is a better remediation tool in soil contamination when compared to physico/chemical remediation. It also offers a cost-effective option, a potentially low-technology, low risk of secondary pollution, & aesthetic value (by phytoremediation) [16–19]. For the past years, the application of bio-degradation and/ or bio-remediation as a remediation/clean-up strategy has become the strategy of choice for remediating PHC-contaminated soil, for the following reasons that; it is cost-effective, sustainable & can enhance natural bio-degradation processes by optimizing limiting factors [9, 20].

Four types of biological strategies useable in soil remediation; (i) micro-organisms (such as bacteria or fungi) to degrade organo-pollutants (also referred to as microbial remediation), (ii) fast growing plants with large biomass, or plant & associated rhizospheric microbial population assisted remediation also known as phyto/rhizo-remediation, (iii) animals in soil (such as worms) to concentrate or stabilize contaminants that cannot be broken down by biological processes, in their body or in the soil; (iv) the combined utilization of the entire strategies aforementioned or the combination of physico/chemical & biological methods. Nevertheless, the focus here is on the use of plants, also known as phyto-remediation [16, 19].

Phyto-remediation is a known bio-remediation technique that uses the degradation potential of microbes & plants to clean up or decrease soil pollutant concentration to permissible risk-levels of site owners and/or regulatory bodies [16, 21–24].

2.1 Phytoremediation

Plants have the capacity to adjust to and modify diverse environmental conditions to certain levels [25]. Phyto-remediation (phyto—Greek for plants) is a universal word that refers to diverse methods that employ plants in the clean-up of environmental (water & soil) contaminants [16, 26, 27]. It is the use of living green plants for on-site remediation. It also involves taking advantage of the symbiotic interaction between plant-based processes & their associated microbial communities to eliminate, transform, &/or mineralize soil inorganic & organic contaminants, as well as pollutants in wastewater, surface water, ground water & sludges [28–30]. In specific words, phytoremediation is a term used to describe a battery of technologies that utilize plants to reduce, remove, degrade or immobilize environmental toxicants/pollutants with the sole purpose of eco-restoration (restoring a site to a condition useable for private or public applications) [16, 31] as described in (**Figure 1** and **Table 1**). Phytoremediation has been widely employed to eco-restoration of soil contaminants such as landfill leachates, crude-oil, metals, pesticides, solvents, explosives, etc. [42, 43]. Phylogenetic diversity of PHC-degraders is huge & numerous recurrent groups reported by most phyto-remediation studies are; *Stenotrophomonas, Sphingomonas, Rhodococcus, Acinetobacter, Alcaligenes, Arthrobacter, Burkholderia, Flavobacterium, Mycobacterium, Micrococcus, Nocardioides, Pseudomonas,* & *Ralstonia* species [10, 44–46].

Individual mechanisms described above are known to have major impact on concentration, environmental outcome & behavior, toxicity, bio-availability & bio-accessibility of PHCs in contaminated soil. Field studies have shown that, certain plants have the ability to eliminate/breakdown xenobiotic organic compounds from the environment by enhancing accumulation & transformation [16, 47], extracellular transformation [48, 49], & metabolic activities of microbes around rhizosphere of the plant [16, 40]. Strategies for phyto-remediation of foreign organics are grouped into two groups; direct phyto-remediation (*in planta*) and phyto-remediation (*ex planta*) [16, 24, 50, 51]. The phyto-remediation *ex planta* depends on a synergy association between substances secreted by roots (root exudates) & metabolic activities of autochthonous rhizosphere related micro-organisms [32, 52]. Plants with the capacity for PHCs phytoremediation have been reported in a number of studies as shown in **Table 2**.

[24, 54], stated that an efficient plant for phyto-remediation should have these traits; (i) capacity for tolerance, build-up, or breakdown of pollutants in their above-ground areas, (ii) tolerate the volume of pollutants built-up, (iii) grow fast & produce high cell mass, (iv) fibrous root systems, & (v) be easy to harvest. The fibrous root systems are advantageous over taproot systems because they offer a larger surface area for colonization of microbial populations & also enhance the interaction between autochthonous rhizospheric-associated microbial communities & the xenobiotic compounds [16, 55].

Aside *in vitro* trials, success reports have been recorded with phyto-remediation field trials aimed at remediating PHC–contaminated soil over 2 decades ago. [56]

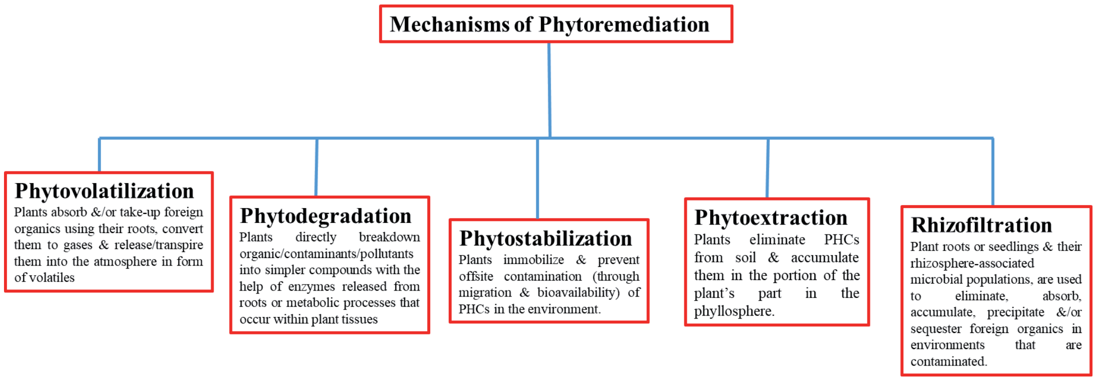

Figure 1.
Mechanisms involved in phytoremediation.

Position	Mechanisms	Description	Pollutants and media	Objectives of phyto-remediation	References
Plant shoots	Phyto-accumulation; Phyto-sequestration; Phyto-extraction	Plants eliminate PHCs from soil & accumulate them in the portion of the plant's part in the phyllosphere	Metals, PHCs & other inorganic toxicants present in sediment, surface water & soil	Phyto-remediation is achieved by removing plants that have accumulated the pollutant	[32, 33]
	Phyto-transformation or Phytodegradation	Plants directly breakdown organic contaminants/pollutants into simpler compounds with the help of enzymes released from roots or metabolic processes that occur within plant tissues, which consequently sustains growth of plant	PHCs, mobile organics such as herbicides in surface water, sediment & soil	Phyto-remediation by complete mineralization	[32, 34]
	Phyto-hydraulics	Plants are used to increase evapo-transpiration, thus, putting the movement of contaminant, water & soil under control	Inorganics & Organics in surface & groundwater	Plants contain the pollutant by controlling water movement	[35, 36]
	Phyto-volatilization	Plants absorb &/or take-up foreign organics using their roots, convert them to gases & release/transpire them into the atmosphere in form of volatiles	Volatile organics & in-organics such as mercury & selenium in soil surface & surface water	Biological remediation by plant removal	[37, 38]
Rhizosphere	Rhizo-filtration	Plants (aquatic/terrestrial), their roots or seedlings (rhizo-filtration or blasto-filtration), & their rhizosphere-associated microbial populations, are used to eliminate, absorb, accumulate, precipitate &/or sequester foreign organics in environments that are contaminated	Inorganics such as heavy metals & Organics in surface water	Containment	[39]
	Phyto-immobilization or Phyto-stabilization	Plants immobilize & prevent offsite contamination (through migration & bioavailability) of PHCs in the environment	Inorganics & organics in water & soil	Containment	[39]
	Phyto-stimulaton or Rhizo-degradation	Plant-assisted bio-remediation which basically depends on degradation of contaminants via metabolic activity of microbes (fungi, yeast, or bacteria) in soil	Hydro-phobic organics such as Polychlorinated biphenyls, PAHs, & other PHCs in water & soil	Remediation by mineralization	[40, 41]

Table 1.
Mechanisms involved in phyto-remediation.

S/N	Botanical name	Common name
1.	*Agropyron smithii*	Western wheat grass
2.	*Andropogon geradi*	Big bluestem
3.	*Bassia scoparia L.*	Burning bush or ragweed
4.	*Biden*	Beggar ticks
5.	*Bouteloua gracilis*	Blue grama
6.	*Cynodon dactylon*	Bermuda grass
7.	*Glycine max*	Soybean
8.	*Lolium perenne L.*	Ryegrass
9.	*Medicago sativa L.*	Alfalfa
10.	*Oryza sativa or Oryza glaberrima*	Rice
11.	*Zea mays L.*	Maize
12.	*Sorghum bicolor*	Sorghum
13.	*Boutelova curtipendula*	Side oats grama
14.	*Sorghastrum nutans*	Indian grass
15.	*Fetusca rubra var. arctared*	Arctared red fescue
16.	*Daucas carota*	Carrot
17.	*Sorghum vulgare L.*	Sudan grass

Adapted from [29, 53].

Table 2.
Some plants with phyto-remediation ability for the clean-up of petroleum hydrocarbon–contaminated soil.

stated that trees & grasses are usually employed for phyto-remediation purposes, with trees classically selected for the bio-remediation of Benzene, Toluene, Ethybenzene, Xylene, whereas grasses are often employed for the bio-remediation of PHC–contaminated soil.

[16, 57] Study on plants with the potential for enhancing bio-remediation of PHC-contaminated soil, obtained results that indicated that the growth of *Glycine max* impacted on soil organic matter contents, moisture & pH of PHC–contaminated soils significantly, with levels of significance; ($P < 0.001$, $P < 0.01$ & $P < 0.05$).

Biodegradation of PHCs was improved in soils spiked with 25 g/L crude-oil & cultivated with *Glycine max* & the soils became more suited for growth of plants as weeds were observed to also grow from the soil. Findings from the study revealed the farming of certain crops like *G. max*, could be proficient in ecorestoration & alleviate risks of PHC–contaminated soil [57]. Phyto-remediation is an inexpensive plant–based remediation strategy for decontaminating PHCs-contaminated soils particularly in the tropics with low finances [58, 59].

Ecological rehabilitation (restoration of contaminated or degraded soils), with the cultivation of *Vetiveria zizanioide* has been reported to increase biomass greatly & consequently improve phyto-remediation of an oil shale mined land contaminated with heavy metals [42, 60]. *Vetiveria zizanioide* (a hydrophyte & xerophytes), tolerates varying abiotic stresses significantly & has been employed in times past in the rehabilitation of coal, gold mines & mining overburdens [61, 62]. In the study by [62], it was discovered that Goose grass (*Eleusine indica*) improved phyto-remediation greatly in soils polluted with PAHs & Total Petroleum Hydrocarbons (TPH).

Comparatively, grasses exhibit traits of fast growth, strong resistance & large biomass in contaminated environment, efficient stabilization & rehabilitation of contaminated lands in sub-tropics & tropics, than trees & shrubs [63, 64]. The capacity of regular tropical grasses, such as elephant grass (*Pennisetum purpureum*), to increase the degradation of a crude-oil contaminated soil has been documented [64]. Field trials have shown that phyto-remediation can be used on soils with moderate PHC contamination or after the use of other biological remediation strategies, in order to alleviate risks linked with PHC-residues in soil further [16, 65]. In a way to improve this bio-remediation strategy, the competence of the plant for phyto-remediation could be significantly enhanced using genetic engineering technologies [24, 66].

Phytoremediation is promising for the onsite treatment of PHC-polluted soils. Treating on site could be challenging to regulate than off-site treatments, for instance, *ex situ* treatment of soil impacted with wastes from refinery. In spite of this, remediation actions on site are largely employed in recent times since they are inexpensive and prevent disruption of contaminated soils. Success of biological degradation is affected by several environmental parameters; contaminants composition, concentration & bio-availability, soil nutrients, oxygen, moisture content, pH, & profile of contaminated area [2, 67, 68]. Understanding ways of controlling these parameters/factors in order to optimize biological activities that will result in bioremediation is paramount.

2.2 Choice of species in phyto-remediation

2.2.1 Plant choice

Several criteria should be considered before choosing plants for phytoremediation. A plant species for phytoremediation should have roots that can spread throughout the whole contaminated site. The principle for plant selection ought to follow the needs of the use, contaminant type & their ability to grow & increase on contaminated soil [16, 18]. Indigenous plants are preferable, to prevent introducing invasive species. E.g. *Hibiscus Cannabinus* (Kenaf) & *Vetivera zizanioides* (Vertiver) which are indigenous plants have been reported to be very proficient in crude-oil pollution remediation in Nigeria [42, 69].

Herbaceous plants, deciduous trees & conifers are renowned plant types [65], based on the environmental conditions & the polluting compound. Peas, clover, reed canary grass & alfalfa (legumes), ryegrass, sunflowers & wheatgrass (grasses) and *Thespesia populnea*, *Populus* sp., *Salix* sp., *Scaevola serica*, *Prosopis pallida* & *Cordia subcordata* (trees) have been reported to display tolerance to PHC-contamination [16, 18, 42, 65]. Tolerance refers to the potential of a plant to grow in hydrocarbon-contaminated soil, however, it does not really suggest the healthiness of the plant [65].

i. **Herbaceous plants in phyto-remediation:** Grasses usually cultivated with trees are largely employed as the main remediation species in hydrocarbon-polluted soils, because they make available great fine roots in topsoil. Grasses are successful as binders & transformers of PHCs like PAHs & BTEX because of the extensive fine root biomass that contains vast microbial community than other species of similar capacity [16, 18, 70].

ii. **Legume—rhizobium symbiosis in phytoremediation:** Nutrients (nitrogen & phosphorus) are especially limiting in contaminated soils. Also, competition for nutrients amongst soil organisms makes nutrients a limiting factor for bio-remediation [71, 72]. When soil moisture content & temperature is low, nitrogen insufficiency is worsened owing to poor mobility of nutrients, limited microbial & enzymes activity [71]. Adequate fertilization & frequent tillage were suggested by [73, 74] as helpful measures in ensuring breakdown of PHC in comparison to un-treated soil. In their research, initial concentrations of PHC were eliminated by 70–81% through bio-remediation in fertilized soils as against 56% elimination without fertilization in natural attenuated soil.

Though, disproportionate application of nitrogen-containing fertilizers could lead to environmental pollution/problems. In order to prevent this, nitrogen-fixing plants like legumes, can be used in place of them [16, 75]. Rhizobia have the capacity to infiltrate roots of legumes, forming symbiotic relationships & nodules, which have the ability to fix gaseous nitrogen into plant in the form of ammonia [24, 76]. *Anabaena,* Blue-green algae, *Azotobacter*, *Azospirillum*, *Rhizobium*, *Actinomycete*s & *Frankia* are generally used Nitrogen-fixers in soils [77, 78].

iii. **Trees as phytoremediation tool:** Trees & their hybrids are extensively employed in clean-up of PHC-impacted soils. CLUIN phyto-remediation databank records that a great percentage of phyto-remediation successful studies were performed using trees (**Table 3**). Plant hybrids that grow fast with desired characteristics like resistance to harsh soil & climatic conditions, resistance to pests & diseases; have been chosen as potential phyto-remediation choice species [16, 80]. For example, hybrid trees from willows & poplars have generally & successfully employed in phyto-remediation of soils polluted with organic & in-organic compounds. But, precautions are advised, to evade risks of utilizing genetically engineered or modified breeds [29, 42, 80].

2.3 Rhizoremediation

Although some studies have endorsed the use of plants only, for effective biological remediation of PHC-impacted soils, [81, 82], using plants associated with PHC–eating microbes &/or plant growth–promoting bacteria (PGPB) for the clean-up or degradation of HC-impacted soil has an edge because it reduces the risks of reverse transformation &/or HC-residues [16, 83–85]. The root system of plants which is generally known to offer support & enhance water & nutrient uptake, is a chemical factory that drives several interactions like mutualistic relationships with beneficent autochthonous micro-organisms (e.g. mycorrhizae, endophytes, plant growth-promoting rhizobacteria (PGPR) & rhizobia) below soil surface [16, 24, 86]. The use of phyto-remediation/plant-associated microbes' combination strategy offer better biological clean-up platform than using plant only.

Rhizo-remediation is thus, the use of plants & their interactive relationships with micro-organisms that inhabit the area around the roots (rhizosphere). This combination has the capacity to breakdown foreign organics in the rhizosphere. In this process, root exudates/secretions enhance the survival & metabolic activities of PHC-degrading microbial populations &/or associated rhizo-microbes, resulting in complete breakdown of organics in hydrocarbon-inundated soils [16].

Rhizo-remediation is one of the most efficient phyto-remediation tools that take advantage of the most active area being near/around the roots of plants for removal/degradation/clean-up of organic contaminants [24, 50, 87]. Practically, microbial communities associated with the rhizosphere are the major contributors to biodegradation & the green plants employed are seen as biological, solar-driven pump & treatment systems [43, 88, 89].

The success of rhizo-remediation relies upon the proficiency of the rhizo-microbes, indicated by their potential to survive & compete for root exudates in the rhizosphere, in order for them to be maintained in requisite numbers & proficiently colonize the emerging root system [16, 90]. In field studies, effectiveness

Name of project (duration)	Trees & other plants employed	Pollutants (initial concentration in media)	Phyto-remediation mechanisms	Results & findings
Phyto-remediation at a gasoline release site in Georgia (1999–2002)	Native sedge, Cattails rush, White willow, Black willow, Wooly bull rush	Gasoline in soil and ground water (Soil average BTEX: 1400 μg/L; average benzene: 44 μg/L)	Phyto-degradation; Phyto-volatilization; Rhizo-degradation	82% reduction in Soil BTEX concentrations; In the 1st year of the growing season, almost 90% of the trees planted survived, although highest death rates was observed in regions with highest concentration of gasoline. In plant branches & leaves, BTEX & benzoic acid (a product of degradation) were present in low concentrations.
Phyto-remediation at the Edward Sears Property in New Jersey (1995–2004)	Hybrid poplar	Mixture of organics (e.g. 2700 mg/L of Xylenes) in groundwater & soil	Phyto-degradation; Hydraulic Control	During the 1st 3 years of monitoring, approx. all of the contaminants decreased
Phyto-remediation at Oneida Tie Yard Site in Tennessee (1997-)	Hybrid poplar	17,500 ppb of PAHs & 18,500 ppb of Naphthalene in soil	Rhizo-degradation; Phyto-volatilization	Concentrations of PAHs & naphthalene were 6400 ppb & 4900 ppb respectively, at the end of 7 years monitoring
Phyto-remediation at Ashland Inc. in Wisconsin (2000-)	Understory grasses; Hybrid poplar	Diesel in soil, BTEX, gasoline, & other organics in ground water & soil	Rhizo-degradation; Hydraulic Control; Phyto-extraction	Trees tripled in height, & subsurface aeration increased in soil since planting. Roots observed at 10 feet depth during 1st growing season

Source: [79].

Table 3.
Studies that used forest trees in phyto-remediation of PHCs contaminated soils.

& success in the use of rhizoremediation strategy depends mainly on the potential of PHC-degrading microbial populations &/or PGPR to efficiently colonize the rhizosphere [91].

Employing rhizo-remediation in breakdown of PHCs have been proven to be an inexpensive strategy & it can be further improved by employing genetically modified/engineered microbes &/or plants fashioned uniquely for the purpose & optimizing favorable conditions for efficient restoration/clean-up of organic pollutants [24].

The study by [87] evaluated the plant/associated rhizo-microbe degradation of diesel-polluted soil using two varieties of rapeseed & HC–degrading microbes. They found out that the rapeseeds defenses responded in different ways. Research by [92] revealed that the mean of the residual PAHs in mixtures (48%), was considerably less than PAHs in soils that used plants alone (55%) & in soils that did not employ plants (70%).

3. Application of plant growth promoting microbes (PGPM) in phytoremediation

Plant growth-promoting rhizobacteria (PGPR) are a class of beneficial microbes associated with root system (rhizosphere) in plants & on colonization, are known to facilitate plant growth via direct & indirect processes [93, 94] (**Figure 2**). Direct mechanisms include heavy mineral uptake by plants [24, 95], phyto-stimulation (also known as phytohormone production), siderophore production which limits the iron (Fe) activity [24, 96], nitrogen fixation, phosphate solubilization & potassium solubilization; while indirect mechanisms include ISR (induced systemic resistance against plant diseases, also known as "systemic resistance"), phyto-remediation, signal interference [97], antibiotics production, quorum sensing [98], chitinase & glucanase activity, exopolysaccharide production [99]. The PGPM facilitates growth of plants under stress by production of vital enzymes like rhizobitoxine exopolysaccharides, 1-aminocyclopropane-1-carboxylate (ACC)-deaminase & chitinase.

Plant growth–promoting rhizobacteria (PGPR) can enhance growth of plant in polluted soils by diverse processes or can assist in biological remediation of polluted soil [100] by using any or a combination of the mechanisms stated above. They are capable of detoxifying the contaminated soil by sequestering metal ions inside the cell [101], biotransformation/transformation of metals from toxic to less toxic ones [19, 100, 102], adsorption/desorption of metals, etc. Some examples of PGPR, pollutants they target & the processes they employ to enhance growth of plants under stressed conditions (pollution) are displayed in **Table 4**.

It is worthy to note that this approach applies PGPR whose action is affected by climate change. Thus, successful remediation with PGPR is greatly connected with climate, for e.g. heat could impair plant physiology & growth, likely resulting to modifications in the structure, population, or activity of plant-associated microbes. Therefore, microbial populations with beneficial impacts on plant health or growth may be reduced under unfavourable conditions [24, 111]. Therefore, understanding growth patterns of plants & its ambient environment prior to using PGPR is essential, particularly in certain conditions. Identifying a particular PGPR unique to a certain area is thus, essential for achieving improved activity by them & effective enhancement of the bio-remediation of polluted soil under evolving climatic conditions [24, 111].

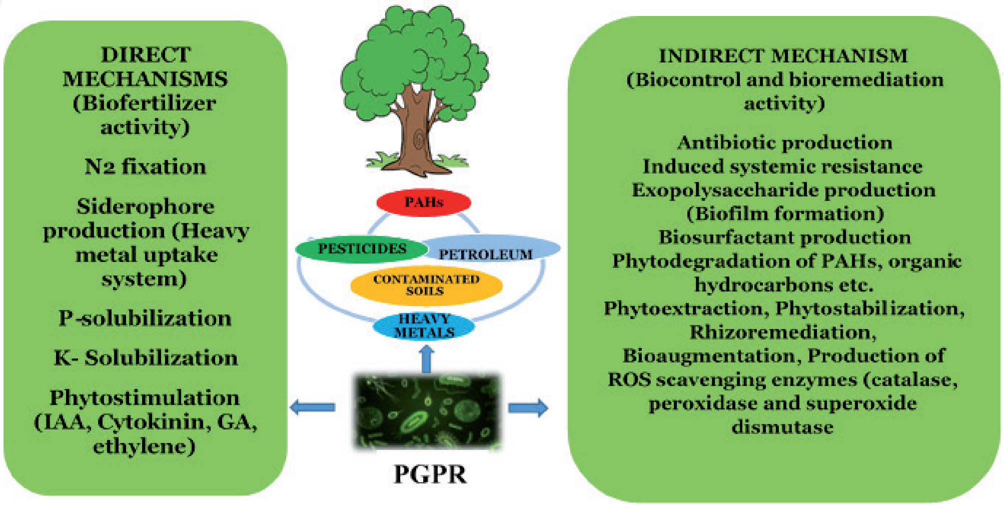

Figure 2.
Mechanisms of action of PGPR in biological remediation of polluted soil.

PGPR	Plants	Target pollutant	Mechanism of action	Reference
Dokdonella, *Luteimonas, Sphingomonas*, *Pseudomonas*, & *Sphingobium* sp.	—	Polycyclic aromatic hydrocarbons (PAHs)	Degradation of phenanthrene, fluorene, & pyrene	[103]
Acinetobacter sp. PDB4	Rice	Anthracene, Pyrene & Benzo(a)pyrene (BaP)	Siderophore production, phosphate solubilization	[104]
Burkholderia sp. XTB-5	*Brassica chinensis, Ipomoea aquatic*	Phenol	Siderophore production, Phosphate solubilization & 1-aminocyclopropane-1-carboxylate (ACC) deaminase synthesis	[105]
Pseudomonas plecoglossicida (JX149549), *P. aeruginosa* (JX100389)	Wheat	Petrol engine oil	Biosurfactant synthesis, petroleum hydrocarbon metabolism, iron sequestration, petroleum hydrocarbons metabolism	[106]
Shinella sp. EIKU6, *Micrococcus* sp. EIKU8, & *Microbacterium* sp. EIKU5	—	Arsenic (As) & Uranium (U)	Oxidation & Resistance, Uranium elimination	[107]
Planctomyces Lysobacter, *Klebsiella* sp. D5A, *Pseudomonas* sp. SB, *Pseudoxanthomonas*	*Testucaarundinacea*	Petroleum hydrocarbons	Production of phyto-hormones & solubilization of minerals; production of biosurfactant; increase root biomass	[108]
Staphylococcus carnosus, Bacillus circulans, & *Enterobacter intermedius*, *Serratia marcescens* BC-3, *Pseudomonas aeruginosa* SLC-2	Maize and Oat	Petroleum hydrocarbons	Siderophore production, Synthesis of Indole acetic acid, 1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity and petroleum degradation	[93, 109]
Pseudomonas fluorescens ATCC 17400	Red clover	Radionuclide cesium	Increase in translocator factor, Resorption of Cesium onto biofilms	[110]

Table 4.
Some PGPR, target pollutant & mechanisms of action.

4. Enzymatic activities of hydrocarbon degrading microbes

High enzymatic potentials present in microbes afford microbial communities the ability to degrade complex hydrocarbons [49, 112]. This petroleum degrading/modifying potential makes them able to breakdown/transform some pollutants like petroleum, & this sums up the significance of enzymes in bio-remediation. The diverse nature of microbial genes adds to the versatility of their metabolic reactions for the transformation of toxicants into less-toxic end-products, subsequently incorporated into natural bio-geochemical cycles [2, 49]. Myriad of micro-organisms (green algae, bacteria, fungi, & cyanobacteria), have PHC-degradative potentials under anaerobic, aerobic, pH, saline & other types of environmental conditions [7, 113]. These enzymatic tools provide these potentials to microbes.

4.1 Aerobic degradation of petroleum & petroleum degrading enzymes

Degradation of petroleum is a gradual process that involves sequential breakdown (metabolism) of its components. The genes that encode the production of petroleum degradation enzyme may be found on plasmid or chromosomal DNA [113, 114]. Biological degradation of hydrocarbons can take place under oxic or anoxic conditions [1, 2].

Under oxic conditions, oxygenases introduce oxygen atoms into hydrocarbons (mono-oxygenases introduce one oxygen atom to a substrate while dioxygenases introduce two). Aerobic breakdown of HCs can be quicker, because of O_2 (oxygen) available as an electron acceptor [115]. Oxidation of saturates (aliphatics) is acetyl-CoA, usually broken down in the Kreb's cycle, with the synthesis of electrons in the electron transport chain (ETC). The ETC is repeated, breaking down HCs further to carbon dioxide (CO_2) [116]. Aromatics like naphthalene & BTEX can also be broken down under oxic conditions. Breakdown of these compounds leads to the first step in catechol synthesis or a similar compound. Once synthesized, catechol could be broken down into precursors in the Kreb's cycle, that are eventually completely mineralized to carbon dioxide (CO_2) [115, 116].

4.1.1 Alkane degradation

In recent times, variation in alkane degradation genes clustering & regulation amongst species has been discovered. The finding is that a species could have multiple genes that encodes for various enzymes performing related functions. alkBFGHJKL operon has been reported as one of the operons that encode the enzymes required for alkanes conversion to acetyl-CoA [2, 117].

The reported alk gene products include; AlkS (positive regulator of the alkBFGHIJKL operon & alkST genes), AlkT (rubredoxin reductase), AlkL (outer membrane protein that maybe involved in uptake), AlkK (acyl-CoA synthetase), AlkJ (alcohol dehydrogenase), AlkH (aldehyde dehydrogenase), AlkF & AlkG (rubreoxins), &. AlkB (alkane hydroxylase). These genes have been identified in several petroleum-metabolizing organisms like *Alcanivorax* sp., *Rhodococcus* sp., *Pseudomonas putida*, *Acinetobacter* sp. & others. [46, 116, 118] reported thirty-six percent (36%) of the hydrocarbon-metabolizing species obtained in their study possessed genes involved in the metabolism of both n-alkanes (alkB) and aromatic hydrocarbons (xylE). [2, 113, 116] indicated the coexistence of multiple-degradative potentials in one microorganism (*Pseudomonas* sp. strain BI7), showing both genetic proof and phenotypic responses. Other microbes with similar potentials include members of the genera *Mycobacterium*, *Rhodococcus* and *Pseudomonas*.

Alkane hydroxylases are a class of enzymes that catalyses the breakdown of alkanes & this class of enzymes are present in many diverse bacterial, fungal & algal species [2, 112, 113, 119]. In addition, [117] projected three classes of alkane-degrading enzyme systems; C17+, C5-C16, & C1-C4, which are for degradation of long chain alkanes (broken down basically by unknown enzymes), pentane to hexadecane (broken down by integral membrane cytochrome P450 enzymes or non-heme iron) & methane to butane (broken down by methane-monooxygenase-like enzymes) respectively. The authors also documented bacterial P450 oxygenase system and di-oxygenase (CYP153, class I), eukaryotic P450 (CYP52, class II), alkane hydroxylases related to AlkB genes, the compositions, cofactors, ranges of substrates, presence of the main groups of alkane hydroxylases (soluble methane mono-oxygenase (sMMO), and particulate methane mono-oxygenase (pMMO). They also added that alkane degrading microbes could have multiple alkane hydroxylases, thus have the capacity to metabolize a variety of substrate ranges.

Over the years, amongst the mostly studied alkane degradation pathways is that explained for *Pseudomonas putida* Gpo1, encoded by the OCT plasmid [1, 113, 120, 121]. Converting alkane to an alcohol by this microbe is initiated by a membrane mono-oxygenase, rubredoxin reductase & soluble rubredoxin [1]. van Hamme et al. [1] developed a model for alkane catabolism in Gram-negative bacteria, describing the position & roles of the ALK-gene products. A class of iron-containing enzymes in bacteria called catechol di-oxygenase is an example of those involved in aerobic catabolism of aromatics. They have the ability to hasten the addition of oxygen (O_2) atoms to 1,2-dihydroxybenzene (catechol) & its derivatives, with subsequent cleavage of the aromatic ring [2, 113, 115, 116]. Catechol di-oxygenases & similar enzymes involved in cleavage of aromatic ring are accountable for the myriad of microbes with aromatic-HC degrading potential [2, 46, 113, 114, 118].

4.1.2 Polycyclic aromatic hydrocarbon (PAHs) degradation

In recent times, majority of reports on PAH-degrading genes arise from studies on naphthalene-degrading plasmids like NAH7 from *Pseudomonas putida* strain G7. Naphthalene dioxygenase is now a known versatile enzyme, with the capacity to mediate the catalysis of a broad array of reactions. Genomic & bio-chemical data have proved that enzyme system for naphthalene degradation is also capable of mineralizing other aromatics like anthracene & phenanthrene. A number of other bacteria with PAH-degrading potential have been obtained & characterized. In addition, more genomic tools to study microbial populations have been invented, there by affording researchers the opportunity to realize diversities of PAH metabolic genes [2, 113, 122].

Novel gene sequence & orders have been reported in many species including, *Pseudomonas* sp. strain U2; nagAaGHAbAcAB, phnFECAcAB, *Norcardiodes* sp. strain KP7; phABC *Burkholderia* sp. strain RP007; etc. The ability of several species to degrade a wide range of aromatics is attributed to presence of multiple oxygenases, presence of multiple metabolic pathways or genes, & relaxed initial enzyme specificity for PAHs. The presence of alkane & aromatic hydrocarbon-degrading genes within single species is common [2, 112, 113].

4.2 Anaerobic degradation

Anaerobic degradation is as important as the aerobic degradation process in bioremediation, even though HC-degradation under aerobic process is faster. This could be attributed to several limiting environmental conditions like insufficient oxygen (typical in aquifers, sludge digesters, mangroves) [3, 7]. Anaerobes like sulphate-reducing bacteria catalyse anaerobic degradation using diverse terminal electron acceptors (TEAs) [1, 2, 123]. Usually, anaerobic degradation involves the conversion of aromatics to benzoyl-CoA (target of the benzoyl-CoA reductase (BCR)) action [2, 113, 124]. Environmental conditions determine the TEAs that could be used in the degradation. Fe (III), sulphate & nitrate are examples of TEAs that could be used [2, 72, 113, 115].

Studies have reported that HCs such as toluene, alkylbenzenes (m, o, & p-xylene & trimethylbenzenes), benzene, naphthalene & phenanthrene, > C6 n-alkanes, branched alkanes & HC mixtures can be catabolized under anaerobic conditions. These reactions may take place under denitrifying, sulphate-reducing & Fe (III)-reducing conditions, by anoxygenic photosynthetic bacteria. Sulphate-reducing

Desulfococcus oleovorans Hxd3 is the only currently known anaerobic bacterium that degrades n-alkanes independently of anaerobic generation of oxygen species [7, 125].

In recent times, electron acceptors shown to be used during anoxic (anaerobic) degradation of HCs include soil humic acids, manganese oxides, etc. Also, the number of pure cultures shown to catabolize different HCs with various electron acceptors has risen. Examples are members of Proteobacteria group, which have helped in explaining the basic genomic & biochemical processes mediating anoxic breakdown of HCs [2, 46, 125].

The diversity & unique characteristics of anaerobic HC-utilizing bacteria are areas that require more studies. More focus is required on isolating & characterizing enzymes mediating anoxic degradation of HCs especially from non-cultivable organisms (using metagenomics) [2, 7, 113].

Bio-catalysis is creating novel paths geared towards improvement & development of processes & products that will cut down on industrial costs, generation of secondary pollutants (toxic sub-products) & subsequently, the adverse effects on the environment. Enzymatic bio-remediation and creation of new clean energy contributes to reduce harm caused by fossil fuel [13, 113, 126]. Enzyme-mediated remediation can be easier compared to using intact microbes. Some advantages, including the enzymatic potential, can be increased in laboratory conditions [113, 127]. Using enzymes alone does not result to production of toxic by-products [113, 128] and competition from intact cell is not needed [113, 126]. [113, 127] stated the major areas to be taken into consideration during enzyme-mediated bio-remediation, range from selection of organisms that has contaminant-degrading potentials, identifying the gene encoding the specific enzyme, to enzyme production.

An example of enzymatic bio-remediation is de-toxification of aromatics (PAHs) & this is can be successful by the application of laccases. Laccases are enzymes that speed up the breakdown (oxidation) of anilines, polyphenols & phenols, with the production of water as the end-product. [113, 129, 130]. The major benefit of enzyme-mediated bio-remediation of partially soluble pollutants or hydrophobics (PAHs) is the fact that it can take place in the presence of solvents organic in nature [131]. However, the drawback is that the relevant enzymes could be denatured, inhibited or un-stable in organic solvents. [2, 131] in their study expressed laccase from *Myceliophthora thermophila* (MtL) in *Saccharomyces cerevisiae*, using directed evolution & extensively improved laccase expression. Years ago, [7, 113, 132] reported success in first field trial with an enzyme-based product, based on the enzyme TrzN, confirming that the enzyme-mediated bio-remediation can effectively clean-up herbicides-contaminated aquatic systems, but only few field studies with enzyme-mediated bio-remediation are available currently. [7, 133] stated that more than 1000 aromatics-degrading enzymes have been documented.

In 2011 the U.S. EPA outlined 20 bio-remediation agents & one pure enzyme additive alone known as “Petroleum Spill Eater II”. The manufacturer described the product as a “bioremediation agent (biological enzyme additive (previously listed as a nutrient additive)),” with a 5-year duration [113, 134]. The manufacturer reported 33.6% decrease in aromatics & 36.9% decrease in alkanes after 7days & 89.6 % & 89.8% decrease of the same compounds respectively, 28 days post Petroleum Spill Eater II application, indicating maximum reductions over a short duration.

Although enzyme-mediated bio-remediation is beneficial, there are requirements & challenges which restrict its application to few classes [132]. Generally, these challenges are associated with enzyme stability & high costs.

Genomic techniques are thus extensively being explored, in order to offer enzyme products that can compete favorably as bio-remediation products. Genomic techniques make detection of genes that encode HC-degrading enzymes in environmental samples or micro-organisms possible, thereby acting as high-throughput technique for bio-prospecting studies. In addition, gene-engineering can significantly enhance cost-effective enzyme production [7, 113, 126]. Brzeszcz and Kaszycki [2, 126] reported that new studies using omics (proteomics, protein engineering & metagenomics) are successfully adding to cost-effectiveness, increased cost-benefit ratios & reduction in chemical application. Using genomic techniques for bio-catalysis (bio-degradation) uses can also assist in tackling the challenge of employing genetically modified organisms (GMO) in the environment [113, 126]; for example, recruiting modified microbes into the environment will not be necessary, if modified enzyme is produced in the laboratory.

Author details

Ajuzieogu Chinyere Augusta[1], Ehis-Eriakha Chioma Bertha[2]*
and Akemu Stephen Eromosele[2]

1 Department of Microbiology, Renaissance University, Enugu State, Nigeria

2 Department of Microbiology, Edo State University Uzairue, Edo State, Nigeria

*Address all correspondence to: bertha_chioma@yahoo.com

References

[1] van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. Microbiology and Molecular Biology Reviews. 2003;**67**(4):503-549

[2] Brzeszcz J, Kaszycki P. Aerobic bacteria degrading both n-alkanes & aromatic hydrocarbons: An undervalued strategy for metabolic diversity & flexibility. Biodegradation. 2018;**29**:359-407

[3] Santos HF, Carmo FL, Paes JE, Rosado AS, Peixoto RS. Bioremediation of mangroves impacted by petroleum. Water, Air, and Soil Pollution. 2011;**216**(1-4):329-350

[4] Speight JG. Refining heavy oil and extra-heavy oil. In: Delmon B, Yates JT, Centi G, editors. Heavy and Extra-Heavy Oil Upgrading Technologies. Vol. 164. New York: Elsevier; 2013. pp. 1-14

[5] Kilbane JJ. Microbial biocatalyst developments to upgrade fossil fuels. Current Opinion in Biotechnology. 2006;**17**:305-314

[6] Santos HF, Cury JC, do Carmo FL, et al. Mangrove bacterial diversity and the impact of petroleum contamination revealed by pyrosequencing: Bacterial proxies for petroleum pollution. PLoS One. 2011;**6**(3):e16943

[7] Ismail WA, van Hamme JD, Kilbane JJ, Gu J-D. Editorial: Petroleum microbial biotechnology: Challenges and prospects. Frontier in Microbiology. 2017;**8**:833

[8] Ehrlich HL. Geomicrobiology. New York: Marcel Dekker; 1995

[9] Epps AV. Phytoremediation of petroleum hydrocarbons. U.S. Environmental Protection Agency. 2006. Available from: http://clu-in.org/download/studentpapers/A_Van_EppsFinal.pdf [Accessed: December 2, 2011]

[10] Khan S, Afzal M, Iqbal S, Khan QM. Plant–bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere. 2013;**90**(4):1317-1332

[11] Environment Agency (EA). Soil quality indicators. IPSS Meeting, Leeds. 2006

[12] Pinedo J, Ibáñez R, Lijzen JP, Irabien A. Assessment of soil pollution based on total petroleum hydrocarbons and individual oil substances. Journal of Environmental Management. 2013;**130**:72-79

[13] Rodríguez-Eugenio N, McLaughlin M, Pennock D. Soil Pollution: A Hidden Reality. Rome: FAO; 2018

[14] Ojuederie OB, Babalola OO. Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. International Journal of Environmental Resource and Public Health. 2017;**14**(12):1504

[15] Karthikeyan R, Kulakow PA. Soil plant microbe interactions in phytoremediation. In: Tsao DT, editor. Phytoremediation. Berlin, Heidelberg: Springer Berlin Heidelberg; 2003. pp. 52-74

[16] Ite AE, Ibok UJ. Role of plants and microbes in bioremediation of petroleum hydrocarbons contaminated soils. International Journal of Environmental Bioremediation and Biodgradation. 2019;**7**(1):1-19.s

[17] Obuekwe CO, Al-Muttawa EM. Self-immobilized bacterial cultures with potential for application as ready-to-use seeds for petroleum bioremediation. Biotechnology Letters. 2001;**23**: 1025-1032

[18] Kamath R, Rentz JA, Schnoor JL, Alvarez PJJ. Phytoremediation of Hydrocarbon-contaminated Soils: Principles and Applications. Iowa: Department of Civil and Environmental Engineering, Seamans Center, University of Iowa; 2007

[19] Shukla KP et al. Bioremediation: Developments, current practices and perspectives. Genetic Engineering and Biotechnology Journal. 2010;**2010**:GEBJ-3

[20] Coulon F, Brassington KJ, Bazin R, Linnet PE, Thomas KA, Mitchell TR, et al. Effect of fertilizer formulation and bioaugmentation on biodegradation and leaching of crude oils and refined products in soils. Environmental Technology. 2012;**33**(16):1879-1893

[21] Burken JG, Schnoor JL. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environmental Science & Technology. 1998;**32**(21):3379-3385

[22] Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, et al. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Applied and Environmental Microbiology. 2001;**67**(6):2469-2475

[23] Lynch JM, Moffat AJ. Bioremediation—Prospects for the future application of innovative applied biological research. Annals of Applied Biology. 2005;**146**(2):217-221

[24] Chitara MJ, Chauhan S, Singh RP. Bioremediation of polluted soil by using plant growth-promoting rhizobacteria. In: Panpatte DG, Jhala YK, editors. Microbial Rejuvenation of Polluted Environment, Microorganisms for Sustainability. Springer Nature Singapore PVT Ltd; 2021. pp. 202-226

[25] Susarla S et al. Phytoremediation: An ecological solution to organic chemical contamination. Ecological Engineering. 2002;**18**(5):647-658

[26] U. S. Environmental Protection Agency (USEPA). A citizen's guide to phytoremediation. EPA-542-F-98-011. Washington DC: Office of Solid Waste and Emergency Response; 1998. 6 pp

[27] U.S. Department of Agriculture (USDA). Conservation buffers work—Economically and environmentally. Program Aid 1615. Washington, D.C.; 2000. 4 pp

[28] Ayotamuno J, Kogbara R, Agoro O. Biostimulation supplemented with phytoremediation in the reclamation of a petroleum contaminated soil. World Journal of Microbiology and Biotechnology. 2009;**25**(9):1567-1572

[29] Kumar R, Das A, Lal S. Petroleum hydrocarbon stress management in soil using microorganisms and their products. In: Chandra R, editor. Environmental Waste Management. Boca Raton: CRC Press; 2016. pp. 525-550

[30] Dickinson N. Phytoremediation. In: Murray BG, Murphy DJ, editors. Encyclopedia of Applied Plant Sciences. 2nd ed. Oxford: Academic Press; 2017. pp. 327-331

[31] Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS. Phytoremediation and hyperaccumulator plants. In: Molecular Biology of Metal Homeostasis and Detoxification. Berlin: Springer; 2006. pp. 299-340

[32] Greipsson S. Phytoremediation. Nature Education Knowledge. 2011;**3**(10):7

[33] Jiao H, Luo J, Zhang Y, Xu S, Bai Z, Huang Z. Bioremediation of petroleum hydrocarbon contaminated soil by Rhodobacter sphaeroides biofertilizer and plants. Pakistan Journal of

Pharmaceutical Science. 2015;**28**(5 Suppl):1881-1886

[34] Al-Baldawi IA, Abdullah SRS, Anuar N, Suja F, Mushrifah I. Phytodegradation of total petroleum hydrocarbon (TPH) in diesel-contaminated water using Scirpus grossus. Ecological Engineering. 2015;**74**:463-473

[35] Hong MS, Farmayan WF, Dortch IJ, Chiang CY, McMillan SK, Schnoor JL. Phytoremediation of MTBE from a Groundwater Plume. Environmental Science & Technology. 2001;**35**(6):1231-1239

[36] U. S. Environmental Protection Agency (USEPA). Phytotechnologies for Site Cleanup. EPA 542-F-10-009. 2010

[37] Shiri M, Rabhi M, Abdelly C, Amrani AE. The halophytic model plant Thellungiella salsuginea exhibited increased tolerance to phenanthrene-induced stress in comparison with the glycophitic one Arabidopsis thaliana: Application for phytoremediation. Ecological Engineering. 2015;**74**:125-134

[38] Limmer M, Burken J. Phytovolatilization of organic contaminants. Environmental Science & Technology. 2016;**50**(13):6632-6643

[39] Masu S, Albulescu M, Balasescu L-C. Assessment on phytoremediation of crude oil polluted soils with Achillea millefolium and total petroleum hydrocarbons removal efficiency. Review Chimie. 2014;**65**:1103-1107

[40] Banks MK, Lee E, Schwab AP. Evaluation of dissipation mechanisms for Benzo[a]pyrene in the Rhizosphere of Tall Fescue. Journal of Environmental Quality. 1999;**28**(1):294-298

[41] Siciliano SD, Germida JJ. Enhanced phytoremediation of chlorobenzoates in rhizosphere soil. Soil Biology and Biochemistry. 1999;**31**(2):299-305

[42] Federal Remediation Technologies Roundtable (FRTR). Remediation Technologies Screening Matrix and Reference Guide, Version 4.0. 4.3 Phytoremediation (In situ Soil Remediation Technology). 2012. Available from: http://www.frtr.gov/matrix2/section4/4-3.html [Accessed: January 18, 2012]

[43] Pokethitiyook P. Phytoremediation of petroleum-contaminated soil in association with soil bacteria. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L, editors. Phytoremediation: Management of Environmental Contaminants. Vol. 5. Cham: Springer International Publishing; 2017. pp. 77-99

[44] Marihal AK, Jagadeesh KS. Plant–microbe interaction: A potential tool for enhanced bioremediation. In: Arora NK, editor. Plant Microbe Symbiosis: Fundamentals and Advances. New Delhi: Springer India; 2013. pp. 395-410

[45] Marmiroli N, McCutcheon SC. Making phytoremediation a successful technology. In: McCutcheon SC, Schnoor JL, editors. Phytoremediation: Transformation and Control of Contaminants. John Wiley & Sons, Inc; 2004. pp. 85-119

[46] Andreolli M, Lampis S, Poli M, Gullner G, Biro B, Vallini G. Endophytic Burkholderia fungorum DBT1 can improve phytoremediation efficiency of polycyclic aromatic hydrocarbons. Chemosphere. 2013;**92**:688-694

[47] Schneider K, Oltmanns J, Radenberg T, Schneider T, Pauly-Mundegar D. Uptake of nitroaromatic compounds in plants. Environmental Science and Pollution Research. 1996;**3**(3):135-138

[48] Siciliano SD, Goldie H, Germida JJ. Enzymatic activity in root exudates of Dahurian Wild Rye (Elymus dauricus) that degrades 2-chlorobenzoic acid. Journal of Agricultural and Food Chemistry. 1998;**46**(1):5-7

[49] Alexander M. Biodegradation and Bioremediation. San Diego: Academic Press; 1994

[50] Anderson TA, Guthrie EA, Walton BT. Bioremediation in the rhizosphere. Environmental Science & Technology. 1993;**27**(13):2630-2636

[51] Salt DE, Smith RD, Raskin I. Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology. 1998;**49**(1):643-668

[52] Phillips LA, Germida JJ, Farrell RE, Greer CW. Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biology and Biochemistry. 2008;**40**(12):3054-3064

[53] Moubasher HA, Hegazy AK, Mohamed NH, Moustafa YM, Kabiel HF, Hamad AA. Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. International Biodeterioration & Biodegradation. 2015;**98**(Supplement C):113-120

[54] Eapen S, D'Souza SF. Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnology Advances. 2005;**23**(2): 97-114

[55] Schwab AP, Banks MK. Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In: Anderson TA, Coats JR, editors. Bioremediation through Rhizosphere Technology. Washington, DC (USA): American Chemical Society; 1994. pp. 132-141

[56] Cook RL, Hesterberg D. Comparison of trees and grasses for rhizoremediation of petroleum hydrocarbons. International Journal of Phytoremediation. 2013;**15**(9):844-860

[57] Njoku KL, Akinola MO, Oboh BO. Phytoremediation of crude oil contaminated soil: The effect of growth of Glycine max on the physico-chemistry and crude oil contents of soil. Nature and Science. 2009;**7**(10):79-87

[58] Merkl N, Schultze-Kraft R, Infante C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water, Air, and Soil Pollution. 2005;**165**(1):195-209

[59] Collins CD. Implementing phytoremediation of petroleum hydrocarbons. In: Willey N, editor. Phytoremediation: Methods and Reviews. Totowa, NJ: Humana Press; 2007. pp. 99-108

[60] Xia HP. Ecological rehabilitation and phytoremediation with four grasses in oil shale mined land. Chemosphere. 2004;**54**(3):345-353

[61] Khan AG. Role of Vetiver Grass and Arbuscular Mycorrhizal fungi in improving crops against abiotic stresses. In: Ashraf M, Ozturk M, Athar HR, editors. Salinity and Water Stress: Improving Crop Efficiency. Dordrecht: Springer Netherlands; 2009. pp. 111-116

[62] Lu M, Zhang Z, Sun S, Wei X, Wang Q, Su Y. The use of Goosegrass (Eleusine indica) to remediate soil contaminated with petroleum. Water, Air, & Soil Pollution. 2010;**209**(1): 181-189

[63] Ayotamuno JM, Kogbara RB, Egwuenum PN. Comparison of corn and elephant grass in the phytoremediation of a petroleum-hydrocarbon-contaminated agricultural soil in Port Harcourt, Nigeria. Journal of Food, Agriculture & Environment. 2006;**4**(3,4):1-12

[64] Meeinkuirt W, Kruatrachue M, Tanhan P, Chaiyarat R, Pokethitiyook P. Phytostabilization potential of Pb mine tailings by two grass species, Thysanolaena maxima and Vetiveria zizanioides. Water, Air, & Soil Pollution. 2013;**224**(10):1750

[65] Frick CM, Germida JJ, Farrell RE. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Technical Seminar on Chemical Spills. 1999;**16**:105a-124a

[66] Cherian S, Oliveira MM. Transgenic plants in phytoremediation: Recent advances and new possibilities. Environmental Science & Technology. 2005;**39**(24):9377-9390

[67] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for total petroleum hydrocarbons (PHC). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999. Available from: http://www.atsdr.cdc.gov/ToxProfiles/tp123.pdf [Accessed: March 20, 2012]

[68] Margesin R, Schinner F. Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology. 2001;**56**:650-663

[69] MERCK. “Indigenous Plants to the Rescue.” Science in Africa. 2002. Available from: http://www.scienceinafrica.co.za/2002/february/oil.htm [Accessed: January 15, 2012]

[70] Chiapusio G et al. Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (Lolium perenne L. and Trifolium pratense L.) in three spiked soils. Plant Soil. 2007;**294**:103-112

[71] Wenzel WW. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil. 2009;**321**:385-408

[72] Bian X-Y, Mbadinga SM, Liu Y-F, Yang S-Z, Liu J-F, Ye R-Q, et al. Insights into the anaerobic biodegradation pathway of n-alkanes in oil reservoirs by detection of signature metabolites. Scientific Reports. 2015;**5**:9801

[73] Chaineau CH, Yepremian C, Vidalie JF, Ducreux J, Ballerini D. Bioremediation of a crude oil-polluted soil: Biodegradation, leaching and toxicity assessment. Water, Air, and Soil Pollution. 2003;**144**:419-440

[74] Bachmann R, Johnson A, Edyvean R. Biotechnology in the petroleum industry: An overview. International Biodeterioration and Biodegradation. 2014;**86**:225-237

[75] Miller AJ, Cramer MD. Root nitrogen acquisition and assimilation. Plant and Soil. 2004;**274**:1-36

[76] Suominen L, Jussila MM, Mäkeläinen K, Romantschuk M, Lindström K. Evaluation of the Galega-Rhizobium galegae system for the bioremediation of oilcontaminated soil. Environmental Pollution. 2000;**107**: 239-244

[77] Havlin JL, Beaton JD, Tisdale SL, Nelson WL. Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 7th ed. Published by Pearson Education, Inc; 2010

[78] Yadegari M, Rahmani HA, Noormohammadi G, Ayneband A. Plant growth promoting rhizobacteria increase growth, yield and nitrogen fixation in Phaseolus vulgaris. Journal of Plant Nutrition. 2010;**33**(12):1733-1743

[79] CLUIN phytoremediation database. Available from: http://cluin.org/products/phyto/ [Accessed: August 20, 2012]

[80] Interstate Technology & Regulatory Council (ITRC). Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised. Phyto-3. Washington, DC (USA). 2009. Available from: http://www.itrcweb.org/Documents/PHYTO-3.pdf [Accessed: December 29, 2011]

[81] Peng S, Zhou Q, Cai Z, Zhang Z. Phytoremediation of petroleum contaminated soils by Mirabilis jalapa L. in a greenhouse plot experiment.

Journal of Hazardous Materials. 2009;**168**(2):1490-1496

[82] Zhang Z, Rengel Z, Chang H, Meney K, Pantelic L, Tomanovic R. Phytoremediation potential of Juncus subsecundus in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). Geoderma. 2012;**175**:1-8

[83] Afzal M, Yousaf S, Reichenauer TG, Kuffner M, Sessitsch A. Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel. Journal of Hazardous Materials. 2011;**186**(2):1568-1575

[84] Gkorezis P, Daghio M, Franzetti A, Van Hamme JD, Sillen W, Vangronsveld J. The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: An environmental perspective. Frontiers in Microbiology. 2016;**7**:1836

[85] Spada V, Iavazzo P, Sciarrillo R, Guarino C. Successful integrated bioremediation system of hydrocarbon-contaminated soil at a former oil refinery using autochthonous bacteria and rhizo-microbiota. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L, editors. Phytoremediation: Management of Environmental Contaminants. Vol. Volume 5. Cham: Springer International Publishing; 2017. pp. 53-76

[86] Tewari S, Arora NK. Transactions among microorganisms and plant in the composite rhizosphere habitat. In: Arora NK, editor. Plant Microbe Symbiosis: Fundamentals and Advances. New Delhi: Springer India; 2013. pp. 1-50

[87] Wojtera-Kwiczor J, Żukowska W, Graj W, Małecka A, Piechalak A, Ciszewska L, et al. Rhizoremediation of diesel-contaminated soil with two rapeseed varieties and petroleum degraders reveals different responses of the plant defense mechanisms. International Journal of Phytoremediation. 2014;**16**(7-8):770-789

[88] Erickson LE. An overview of research on the beneficial effects of vegetation in contaminated soil. Annals of the New York Academy of Sciences. 1997;**829**(1):30-35

[89] Singh RP, Dhania G, Sharma A, Jaiwal PK. Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Singh SN, Tripathi RD, editors. Environmental Bioremediation Technologies. Berlin, Heidelberg: Springer Berlin Heidelberg; 2007. pp. 223-258

[90] Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ. Rhizoremediation: A beneficial plant-microbe interaction. Molecular Plant-Microbe Interactions. 2004;**17**(1):6-15

[91] Lugtenberg BJJ, Kravchenko LV, Simons M. Tomato seed and root exudate sugars: Composition, utilization by Pseudomonas biocontrol strains and role in rhizosphere colonization. Environmental Microbiology. 1999;**1**(5):439-446

[92] Meng L, Qiao M, Arp HPH. Phytoremediation efficiency of a PAH-contaminated industrial soil using ryegrass, white clover, and celery as mono- and mixed cultures. Journal of Soils and Sediments. 2011;**11**(3):482-490

[93] Ajuzieogu CA, Ibiene AA, Stanley HO. Laboratory study on influence of plant growth promoting rhizobacteria (PGPR) on growth response and tolerance of Zea mays to petroleum hydrocarbon. African Journal of Biotechnology. 2015;**14**(43):2949-2956

[94] Asad SA. Soil–PCB–PGPR interactions in changing climate scenarios. In: Xenobiotics in the Soil

Environment. Cham: Springer; 2017. pp. 281-298

[95] Ma Y, Prasad MN, Rajkumar M, Freitas H. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances. 2011;**29**: 248-258

[96] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012;**28**(4):1327-1350

[97] Cassán F, Vanderleyden J, Spaepen S. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus Azospirillum. Journal of Plant Growth Regulators. 2014;**33**(2):440-459

[98] Podile AR, Vukanti RV, Sravani A, Kalam S, Dutta S, Durgeshwar P, et al. Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proceedings of the National Academic Science. 2014;**80**(2):407-413

[99] Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology Advances. 2014;**32**:429-448

[100] Patel PR, Shaikh SS, Sayyed RZ. Dynamism of PGPR in bioremediation and plant growth promotion in heavy metal contaminated soil. Indian Journal of Experimental Biology. 2016;**54**:286

[101] Antony R, Sujith PP, Sheryl OF, Pankaj V, Khedekar VD, Loka Bharathi PA. Cobalt immobilization by manganese oxidizing bacteria from Indian Ridge System. Current Microbiology. 2011;**62**:840-849

[102] Cheung KH, Gu JD. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. International Biodeterioration and Biodegradation. 2007;**59**:8-15

[103] Bacosa HP, Inoue C. Polycyclicaro matichydrocarbons(PAHs) biodegradationpotentialand diversity of microbial consortia enriched from tsunami sediments in Miyagi, Japan. Journal of Hazardous Mater. 2015;**283**:689-697

[104] Kotoky R, Das S, Singha LP, Pandey P, Singha KM. Biodegradation of Benzo (a) pyrene by biofilm forming and plant growth promoting Acinetobacter sp. strain PDB4. Environmental Technology Innovation. 2017;**8**:256-268

[105] Chen W, Li J, Sun X, Min J, Hu X. High efficiency degradation of alkanes and crude oil by a salt-tolerant bacterium Dietzia species CN-3. International Biodeterioration and Biodegradation. 2017;**118**:110-118. DOI: 10.1016/j.ibiod.2017.01.029

[106] Gangola S, Kumar R, Sharma A, Singh H. Bioremediation of petrol engine oil polluted soil using microbial consortium and wheat crop. Journal of Pure Applied Microbiology. 2017;**11**(3):1583-1588

[107] Bhakat K, Chakraborty A, Islam E. Characterization of arsenic oxidation and uranium bioremediation potential of arsenic resistant bacteria isolated from uranium ore. Environment Science and Pollutant Research. 2019;**26**(13):12907-12919

[108] Hou J, Liu W, Wang B, Wang Q, Luo Y, Franks AE. PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. Chemosphere. 2015;**138**:592-598

[109] Liu JL, Xie BM, Shi XH, Ma JM, Guo CH. Effects of two plant

growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase on oat growth in petroleum-contaminated soil. International Journal of Environmental Science and Technology. 2015;**12**(12):3887-3894

[110] Hazotte A, Péron O, Gaudin P, Abdelouas A, Lebeau T. Effect of Pseudomonas fluorescens and pyoverdine on the phytoextraction of cesium by red clover in soil pots and hydroponics. Environment Science and Pollutant Research. 2018;**25**(21): 20680-20690

[111] Compant S, Clément C, Sessitsch A. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology and Biochemistry. 2010;**42**(5):669-678

[112] Gao X, Gao W, Cui Z, Han B, Yang P, Sun C, et al. Biodiversity and degradation potential of oil-degrading bacteria isolated from deep-sea sediments of South MidAtlantic Ridge. Marine Pollution Bulletin. 2015;**97**:373-380

[113] Peixoto RS, Vermelho AB, Rosado AS. Petroleum-degrading enzymes: Bioremediation and new prospects. Enzyme Research. 2011;**34**:1-8

[114] Broderick JB. Catechol dioxygenases. Essays in Biochemistry. 1999;**34**(11):173-189

[115] Cao B, Nagarajan K, Loh KC. Biodegradation of aromatic compounds: Current status and opportunities for biomolecular approaches. Applied Microbiology and Biotechnology. 2009;**85**(2):207-228

[116] Madigan MT, Martinko JM, Dunlap PV, Clark DP. Brock Biology of Microorganisms. 12th ed. Benjamin Cummings; 2010

[117] Whyte LG, Bourbonniere L, Greer CW. Biodegradation of petroleum hydrocarbons by psychotrophic Pseudomonas strains possessing both alkane (alk) and napthalene (nah) catabolic pathways. Applied Environmental Microbiology. 1997;**63**:3719-3723

[118] Steliga T, Jakubowicz P, Kapusta P. Changes in toxicity during in situ bioremediation of weathered drill wastes contaminated with petroleum hydrocarbons. Bioresources Technology. 2012;**125**:1-10

[119] Stosky JB, Greer CW, Atlas RM. Frequency of genes in aromatic and aliphatic hydrocarbon biodegradation pathways within bacterial populations from Alaskan sediments. Canadian Journal of Microbiology. 1994;**40**: 981-985

[120] van Beilen JB, Funhoff EG. Alkane hydroxylases involved in microbial alkane degradation. Applied Microbiology and Biotechnology. 2007;**74**(1):13-21

[121] van Beilen JB, Wubbolts MG, Witholt B. Genetics of alkane oxidation by Pseudomonas oleovorans. Biodegradation. 1994;**5**(3-4):161-174

[122] Kweon O, KimSJ BJ, Kim SK, Kim BS, Baek DH, Park SI, et al. Comperative functional pan-genome analyses to build connections between genomic dynamics and phenotypic evolution in polycyclic aromatic hydrocarbon metabolism in the genus Mycobacterium. BMC Evolutionary Biology. 2015;**15**:21

[123] Zhou J, Bian X-Y, Mbadinga SM, Yang S-Z, Yang J-F, Gu J-D, et al. Synthesis and characterization of anaerobic degradation biomarkers of n-alkanes via hydroxylation/carboxylation pathways. European Journal of Mass Spectroscopy. 2016;**22**:31-37. DOI: 10.1255/ejms.1402

[124] van Beilen JB, Panke S, Lucchini S, Franchini AG, Rothlisberger M, Witholt B. Analysis of Pseudomonas putida alkane-degradation gene clusters and flanking insertion sequences: Evolution and regulation of the alk genes. Microbiology. 2001;**147**(6):1621-1630

[125] Rabus R, Boll M, Heider J, Meckenstock RU, Buckel W, Einsle O, et al. Anaerobic microbial degradation of hydrocarbons: From enzymatic reactions to the environment. Journal of Molecular Microbiology and Biotechnology. 2016;**26**:5-28

[126] Alcalde M, Ferrer M, Plou FJ, Ballesteros A. Environmental biocatalysis: From remediation with enzymes to novel green processes. Trends in Biotechnology. 2006;**24**(6):281-287

[127] Sutherland TD, Horne I, Weir KM, et al. Enzymatic bioremediation: From enzyme discovery to applications. Clinical and Experimental Pharmacology and Physiology. 2004;**31**(11):817-821

[128] Setti L, Lanzarini G, Pifferi PG. Whole cell biocatalysis for an petroleum desulfurization process. Fuel Processing Technology. 1997;**52**(1-3):145-153

[129] Alcalde M, Bulter T, Zumarraga M, et al. Screening mutant libraries of fungal laccases in the presence of organic solvents. Journal of Biomolecular Screening. 2005;**10**(6):624-631

[130] Smith M, Thurston F, Wood DA. Fungal laccases: Role in delignification and possible industrial applications. In: Messerschmidt A, editor. Multi-Copper Oxi-Dases. Singapore: World Scientific Publishing; 1997. pp. 201-224

[131] Bulter T, Alcalde M, Sieber V, Meinhold P, Schlachtbauer C, Arnold FH. Functional expression of a fungal laccase in Saccharomyces cerevisiae by direct edevolution. Applied and Environmental Microbiology. 2003;**69**(2):987-995

[132] Scott C, Lewis SE, Milla R, et al. A free-enzyme catalyst for the bioremediation of environmental atrazine contamination. Journal of Environmental Management. 2010;**91**(10):2075-2078

[133] Whiteley CG, Lee DJ. Enzyme technology and biological remediation. Enzyme and Microbial Technology. 2006;**38**(3-4):291-316

[134] U. S. Environmental Protection Agency (USEPA). Oil: Crude and Petroleum Products. 2011. Available from: http://www.eia.gov/energyexplained/index.cfm?page=oil_home [Accessed: December 24, 2011]