Chapter 3
Phytoremediation of Soils Contaminated by Hydrocarbon

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Abstract It is estimated that more than one-third of the world soils are seriously contaminated due to anthropological activities. Much of this contamination is due to oil industry activities which cause significant changes in the ecosystems due to the processes of exploration, refining, transportation and commercialization of products derived from oil. Plants have become biotechnologies for the recovery of hydrocarbon-contaminated soils given that they can absorb and degrade significant amounts of the pollutants. Most plants live in symbiosis with ectomycorrhizal fungi and/or arbuscular mycorrhizas that can facilitate the remediation of contaminated soils. In addition, rhizosphere microorganisms such as bacteria, fungi and nematodes have the ability to consume hydrocarbons as sources of energy and carbon, thereby playing a very important role in the remediation of contaminated soils. The remediation of areas contaminated with oil hydrocarbons is making it necessary to conduct studies on each contaminant regarding the damages and/or benefits they may be causing in the rhizosphere and in plant physiology.

Keywords Hydrocarbons · Hydrocarbonoclastic bacteria · Mycorrhizae · Phytoremediation · Rhizosphere · Soil microorganisms

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B. R. Shmaefsky (ed.), *Phytoremediation*, Concepts and Strategies in Plant Sciences,
https://doi.org/10.1007/978-3-030-00099-8_3

3.1 Introduction

On a global level, more than one-third of the world soils are seriously contaminated due to anthropological activities (FAO 2011; Abhilash et al. 2013; Weber et al. 2013; Prasad et al. 2016). One of these activities is the oil industry, which has been the direct cause of significant changes in the ecosystems due to the processes of exploration, refining, transportation and commercialization of products derived from oil (Rivera-Cruz et al. 2005). Oil is a very complex mixture which contains mainly hydrocarbons (molecules with carbon and hydrogen atoms) and compounds with heteroatoms such as sulfur (S), nitrogen (N), oxygen (O) and low concentrations of metallic constituents, mainly nickel (Ni), vanadium (V), sodium (Na), calcium (Ca) and copper (Cu) (Namihira-Guerrera 2004; PEMEX 2011; Feijoo-Ruiz 2012).

Contamination by petroleum hydrocarbons (PH) has become a critical environmental problem, affecting the homeostasis of the soil system through the PH contamination generated and causing a negative impact on the safety of ecosystems and human health (Tripathi et al. 2015). However, as the soils become more and more limited by the contamination, the need to recover these affected areas is increasingly evident (Wagner et al. 2016).

In the search to find a solution for the problem of soil pollution from oil spills, approximately three decades ago, a research project was initiated, which has allowed the use of diverse flora as raw material for environmental decontamination (Sangabriel et al. 2006; Ochoa-Gaona et al. 2011; Prasad et al. 2016). One of the first studies dealing with the effect of oil-contaminated soils on plants was Bossert and Bartha (1985), and specifically Radwan et al. (1995) who used the roots of the *Senecio glaucus* L. for remediation processes in soils contaminated by hydrocarbons. On the other hand, Cunningham and Berti (1993) addressed the remediation of contaminated soils from a theoretical revision with plants, while Cunningham and Ow (1996) initiated the study of phytoremediation with an analysis of the promises and perspectives of this biotechnology.

In this way, plants have become biotechnologies for the recovery of contaminated soils given that they can absorb between 10–50% of some contaminants within their organs and tissues on interaction with water. The remediation can be *in situ* (in the contaminated site) or *ex situ* (in a laboratory or nursery); it generates little waste, creates socioeconomic benefits such as wood (in the case of timber species) or as firewood and thus as a source of bioenergy. In addition, remediation helps to improve the physico-chemical parameters (pH, texture, MO, CIC, N, P) and to reduce salinity in soils contaminated by hydrocarbons, thereby increasing nutrient availability, mitigating soil erosion, capturing carbon and increasing biodiversity (Abhilash et al. 2012; Hu et al. 2012; Chan-Quijano 2015; Thijs and Vangronsveld 2015; Tripathi et al. 2016).

The upper terrestrial plants consist of an aerial system and a root system which can represent between 10 and 20% of the total plant material in forest trees, 10–50% in cultivated plants and 50–80% in grassland vegetation. The root system is responsible for anchoring the plants and for proportioning water and nutrients. In doing this, the roots also absorb oxygen in order to produce the necessary energy for the metabolic processes during photosynthesis. In this way, root respiration, along with the microorganisms and fauna of the soil, all contribute significantly to soil respiration and the liberation of CO_2 in the pore spaces; this is important as it decomposes the organic material and at the same time degrades the contaminating agents (Strawn et al. 2015; Blume et al. 2016).

The root system of plants is divided into thick roots (>2 mm in diameter) and fine roots (<2 mm in diameter); the extremities of the fine roots present a large number of root hairs with a thickness of 5–20 μ and a length of up to 1 km; these only remain functional for a few days and then die. Their function is to increase the surface area of absorption of the root which is why they invest the energy to carry out this activity (Blume et al. 2016). These roots are affected in the event of an oil spill; initially, the plants which cannot support the contaminant die, while those that are able to become acclimatized to the affected area begin to suffer a state of stress, resulting in the appearance of chlorosis on the leaves, slow growth and a reduction in root growth and leaf production (Rivera-Cruz 2011; Ochoa-Gaona et al. 2011).

In the soil–root interface, the narrow volume surrounding the roots (a few mm) is known as the rhizosphere; this is defined as the direct interaction between the microorganisms and the root of the plants, or as the compartment of the soil influenced by the plant roots (Atlas and Bartha 1998; Gregory 2006a; Lugtenberg 2015). It is characterized by various processes such as the exudation of organic compounds, root respiration (absorption of O_2 and liberation of CO_2), liberation of protons and other mineral ions and the absorption of water and solutes which modify significantly the properties and the function of the soil and also favor microbial activity, with the aid of the exudation from the root. The main process associated with the rhizosphere is formed mainly by organic acids of low molecular weight which assist in the degradation of the hydrocarbons with metabolic processes (Morel et al. 1999; González-Mendoza 2013).

Given that oil hydrocarbons initially damage the soil and the plants (death of foliage, damage to the root and wood tissues), the aim of this chapter is to explain the degradation of the hydrocarbons with the rhizosphere of plant species which have potential in the remediation of soils contaminated by oil.

3.2 Contaminated Soil and the Rhizosphere

The plants used for the remediation processes of soils contaminated with hydrocarbons must be fast-growing, resistant and competitive with a capacity of tolerance to the contaminants, as well as good liberation of exudates for the proliferation of microorganisms and a greater development of biomass and roots. Once germinated, the plants need soils in optimal conditions in order to continue their development; in other words, the physical, chemical and biological properties must be in excellent health and have good nutritional quality (Morel et al. 1999; Fenner and Thompson 2005; Gregory 2006a). However, many of the aspects of plant root growth reflect an acclimatization and adaptation to demanding environments, showing complex growth patterns and tropism which allow them to explore and exploit a heterogeneous environment full of obstacles such as the contaminants (Pepper et al. 2004; Taiz and Zeiger 2010).

At best, plant roots, in particular those of the trees, can penetrate down to deeper levels of the soil (in comparison with grasses), and in doing so, they achieve a propagation of the microorganisms at different depths, with which they are able to incorporate nutrients, deliver oxygen and improve redox conditions which help in the degradation of oil hydrocarbons (Pérez-Hernández et al. 2016). Moreover, most plants, in particular trees, live in symbiosis with ectomycorrhizal fungi and/or arbuscular mycorrhizas (Bonfante and Desirò 2015).

Blume et al. (2016) mention that both thick and thin roots penetrate the thick pores of the soil (diameter $>10\ \mu m$) and that the numerous root hairs penetrate a large part of the mesopores (2–10 μm), allowing the absorption of water and nutrients. In the range of fine pores ($<0.2\ \mu m$), these substances can only reach the plant roots by means of slow diffusion processes along the gradients of concentration in the soil solution. In contrast, oxygen is administered to the roots mainly through the large, thick pores in the soil (between 6.5 and 9.3 μm). The density of root length frequently reaches various meters by dm^{-3}. However, normally, less than 1% of the total soil volume available is rooted by the plants, up to a maximum of 10–20% even in the A horizons. In this way, the dynamics of the biogeochemical properties of the rhizosphere and their relationship with soil aggregation have morpho-functional mechanisms such as root depth, root-aggregate contact, density and distribution of the roots, size, distribution and form of the pores created by the roots and the soil structure (Gregory 2006a, b; Gregory et al. 2009; Torres-Guerrero et al. 2013). However, almost all the plant processes are directly or indirectly affected by the water supply. More than 90% of the living structures in plant cells (protoplasm) consist of water; this utilization of water varies among plants from 15 to 100% humidity of the soil (moisture content); in addition, water enters the plants through the leaves, stems and mainly through the roots (Aguilera-Contreras and Martínez-Elizondo 1996).

When the oil falls on the ground or in the water, it adheres to the bark of the roots, forming a layer which does not allow water absorption, causing the slow death of the tree (Radwan et al. 1995; Tansel et al. 2015; Feng et al. 2017). In the same way, the hydrocarbons provoke deformations in the calyptra and, as a consequence, induce damage to the apical meristem of the root; they also obstruct the absorbent, root hairs, which do not allow the passage of water and nutrients to the rest of the plant (Gregory 2006a; Taiz and Zeiger 2010; Feng et al. 2017). When an oil spill occurs in the soil, the oil undergoes a process of intemperization; the volatile hydrocarbons begin to evaporate and the aromatic hydrocarbons (nonvolatile) such as benzene, toluene, xylene, napthalene, biphenyls, dimethylphenanthrene, methylcrisine, methylpirene, benzanthracene and benzopyrene remain in the soil and are deposited in the form of asphalt, provoking toxicological damage to the ecosystem (Toledo 1982; Ferrera-Cerrato and Alarcón 2013).

When the oil falls on the soil, it infiltrates vertically. The heavier hydrocarbons, such as fuel oil, penetrate more slowly, while the lighter ones, such as benzene, show a rapid movement in the soil profile; however, this varies depending on the soil group (Toledo 1982). Moreover, the oil also modifies the structure of the soil (ruptures of the aggregates), reducing the exchange of gases with the atmosphere, increasing the content of organic carbon (through oxidation processes) and thereby reducing the cation exchange capacity (by loss of bases), resulting in an acidification of the soil (Elías-Murguía and Martínez 1991; Zavala-Cruz et al. 2002; Weil and Brady 2008; Ferrera-Cerrato and Alarcón 2013).

This acidification is involved in the capture or liberation of ions and in the catalysis of the redox reactions which are seen to be saturated or limited by the concentrations of hydrocarbons, since it essentially transforms carbon (C), nitrogen (N) and sulfur (S) in ions or molecules which are easily absorbed by plants and microorganisms. Similarly, when the value of pH is not optimal in the soil, this gives rise to serious problems for the development of microorganisms and plants, given the elevation of toxicity in the aluminum (Al), iron (Fe) and manganese (Mn) and a deficiency of calcium (Ca), magnesium (Mg) and molybdenum (Mo) (Sposito 2008).

In addition, when the concentrations of hydrocarbons in the soil are greater than 3000 mg kg^{-1} (milligrams over kilograms), the apparent density tends to decrease to 0.6 Mg m^{-3} (megagrams per cubic meter); this can vary the quantity of organic materials found in the area; the organic material will be influenced by biogenic material (decomposition of plant and animal species) and the petrogenic material (hydrocarbons; Martínez and López 2001; Beltrán-Paz and Vela-Correa 2006).

The organic contaminants such as hydrocarbons integrate with the organic material of the soil due to their greater hydrophobicity, allowing the microorganisms to carry out the mineralization of the contaminant (Cang et al. 2013; Tripathi et al. 2015). These contaminants are submitted to different biotic and abiotic interactions, such as adsorption, volatilization, chemical oxidation, photolysis and microbial degradation (making the contaminant less toxic or innocuous, while also helping in the detoxification through biostimulation among the roots and microorganisms; Zhao et al. 2008; Lors et al. 2012; Masakorala et al. 2013).

The degradation of the hydrocarbons that reach at deeper levels of the soil will depend on root development and soil transpiration (Komives and Gullner 2006; Pérez-Hernández et al. 2013, 2016). When the roots reach these depths, metabolic transformation processes occur, mediated by a large variety of enzymes, allowing the contaminants to be assimilated by the plant tissues (Kuiper et al. 2004; Mezzari et al. 2004, 2005). The process of metabolic transformation of the contaminants will depend on the physico-chemical and structural properties of the soil, as well as its relationship with the rhizosphere, given that the hydrocarbons are organic compounds and moderately hydrophobic (characterized with the partition coefficient of octanol–water, log K_{ow}, with values between 1 and 3; Mezzari et al. 2011).

According to Kuiper et al. (2004), the exudates deriving from the plants, such as amino acids and sugars, among others, can help to stimulate the survival and biostimulation of the microorganisms, resulting in a more efficient degradation of the contaminants. In the same way, the root system of the plants helps in the propagation of the microorganisms, which filter down to impermeable layers of soil affected by the oil spill.

In order for the plants to become acclimatized, they must adjust to the conditions of the affected area. Their capacity to achieve this depends on nutrient availability, the physico-chemical properties of the soil, their biomass production and their response to the stress caused by the oil hydrocarbons (Kuppens et al. 2015; Tripathi et al. 2016). In addition, the conditions of temperature, humidity, sunlight, rainfall, wind and water in the soil all help to accommodate the plants so that they can adjust to the area (Mclntosh et al. 2017). Water, for example, plays a vital role in the extraction of nutrients and hydrocarbons, as these elements can be dissolved in water and thus assimilated by the plants during the process of absorption (Licht and Isebrands 2005).

The plant absorbs nutrients and water through the roots in order to develop; therefore, the intimate contact between the surface of the root and the soil is essential. However, this contact is easily broken when the soil is altered, degraded and/or contaminated (Taiz and Zeiger, 2010). In particular, one of these mechanisms of acclimatization of the plants to contaminated soils is that the new roots, which develop after a contamination event, try to reestablish the optimal contact with the soil, which contributes to a greater resistance of the plant to stress (Luo et al. 2016).

The work of the rhizosphere is based on the catabolic potential of the microorganisms which have the capacity to tolerate the hydrocarbons with the support of the exudates from the roots which creates a favorable microenvironment (Ortega-Calvo et al. 2003). The effect of the rhizosphere is carried out between 1–5 mm of the root surface and the soil. Given that the roots exudate organic compounds, the microbial populations increase their activity 5 to 100 times more, in comparison with soils without plants (Atlas and Bartha 1998; Gregory 2006a; Lugtenberg 2015).

Among the exudates released by the plants can be found sugars, fatty acids, amino acids, water, inorganic ions, oxygen, riboflavin, carbon dioxide, bicarbonate ions, protons, electrons, ethylene, mucilage, enzymes, siderophores, allelopathy inducing compounds, as well as root residues which include calyptra cells and cellular contents, to mention a few (Uren 2007; Ferrera-Cerrato and Alarcón 2013). These are liberated through physical and environmental effects such as luminosity, temperature, pH, damage to the root and the water content in the soil (Ferrera-Cerrato 1995). The exudates are generated inside the mitochondria, in the cytosol and in the vacuole of the plant cells, from the tricarboxylic acid cycle (Young et al. 1998; González-Mendoza, 2013).

Similarly, the exudates have an influence on the solubility of essential and nonessential elements through the acidification, chelation, precipitation and oxide reduction processes in the rhizosphere and also through microbial activity, which contributes to root growth and the elimination of oil hydrocarbons thanks to the mutualistic interactions among arbuscular mycorrhizal fungi, microorganisms and plant roots (Strong and Phillips 2001; Zavala-Cruz et al. 2002; Oldroyd 2013; Philippot et al. 2013).

The rhizosphere, therefore, is an interface between the roots of the plants and the soil where the interactions between the microorganisms and invertebrates intervene in the biogeochemical cycle and in many other aspects such as plant growth, tolerance to biotic and abiotic stress, degradation of oil hydrocarbons and in the complex and dynamic ecology for the improvement and functionality of the ecosystem (both natural and contaminated).

3.2.1 The Role of the Microorganisms and the Rhizosphere in the Degradation of Hydrocarbons

The degradation of oil hydrocarbons by microorganisms is widely used, given that it is an efficient and economical method for the detoxification of contaminants while respecting the natural environment. Plant roots are fundamental for stimulating the proliferation of degrading microorganisms within the dynamic region of their rhizosphere; therefore, they are of significant importance in phytoremediation (Radwan et al. 1995; Masakorala et al. 2013). Microorganisms such as bacteria, fungi and nematodes have the ability to consume hydrocarbons as sources of energy and carbon, thereby playing a very important role in the remediation of contaminated soils.

Bacteria are the most active degrading agents of oil hydrocarbons (Hassaine and Bordjiba 2015; Mayz and Manzi 2017). These bacterial groups use naphthalene and phenanthrene or other hydrocarbons catabolically as the only source of carbon and energy, while the compounds which are less soluble in water, such as anthracene, pyrene and fluoranthene are used as growth sources. These bacteria, capable of eliminating the hydrocarbons, are known as hydrocarbonoclasts (Table 3.1; Kube et al. 2013).

There are also native microorganisms of the Gammaproteobacteria class which can metabolize hydrocarbons at extremely low temperatures, for example, the genera which degrade the alkene hydrocarbons such as *Alcanivorax* spp. and *Cycloclasticus* spp.; also the *Pseudoalteromonas* spp., which can decompose the aromatic hydrocarbons (Pham and Anonye 2014).

Then, we have the *Bacillus* sp., *Rhodococcus* sp., *Mycobacterias* sp., *Pseudomonas* sp. and several yeasts such as *Micromycetes* sp. which use simple and complex organic compounds as a source of energy, since their metabolic versatility allows them to convert substrates which are generally nondegradable into easily absorbed metabolites or susceptible to enzyme catalysis (Mackey and Hodgkinson 1996; Rolling et al. 2003; Echeverri-Jaramillo et al. 2010). Besides inhabiting approximately 0.1% of the contaminated sites (Matsumiya et al. 2007), the *Pseudomonas* sp. can attain an efficiency of up to 92.46% in the degradation of 0.1% polycyclic aromatic hydrocarbons *in situ* in the laboratory, which would suggest that this bacteria and its lipopeptides have great potential in the remediation of contaminated soils (Xia et al. 2014). It is also capable of producing surfactant compounds which provide an efficient degradation of hydrocarbons such as phenanthrene (86.65%); this degradation is by the metabolic pathway of the protocatequito (Masakorala et al. 2013).

Table 3.1 Genus of hydrocarbonoclastic bacteria which eliminate hydrocarbons

Genus	Reference
Alcaligenes sp.	Kim et al. (2000)
Alkanibacter sp.	Zhao et al. (2008)
Altererythrobacter sp.	Kim et al. (2000)
Arthobacter sp.	Radwan et al. (1995), Rivera-Cruz (2011), Zhang et al. (2011)
Azospirillum sp.	Rivera-Cruz (2011), Masakorala et al. (2013)
Bacillus sp.	Radwan et al. (1995), Rolling et al. (2003)
Microcella sp.	Zhao et al. (2008), Philippot et al. (2013)
Mycobacterium sp.	Parés and Juárez (2002), Xia et al. (2014)
Nicardioides sp.	Iwabuchu et al. (1998), Ortega-Calvo et al. (2003)
Promicromonospora sp.	Wu et al. (2017)
Pseudomonas sp.	Parés and Juárez (2002), Philippot et al. (2013), Xia et al. (2014)
Rhodococcus sp.	Radwan et al. (1995)
Sphingomonas sp.	Wu et al. (2017)
Tistrella sp.	Xia et al. (2014)
Xanthomonas sp.	Iwabuchu et al. (1998), Xia et al. (2005)

Surfactin, fengycin and liquenisina are recognized as common metabolites produced by *Bacillus* sp. and these form the group of lipopeptides (Radwan et al. 1995; Das and Mukherjee 2007; Mayz and Manzi 2017). This group of bio-surfactants comprises a hydrophobic fatty acid and one molecule of hydrophilic peptide; it contains a low critical concentration of micelles, stable emulsion property, strong surface activity and an excellent foaming property, as well as the presentation of stable physico-chemical properties at different temperatures and pH levels (Das and Mukherjee 2007), which produce degradation of the hydrocarbons, due to the fact that the microorganisms use the *n*-alkanes and the polycyclic aromatic hydrocarbons, such as fluorine, naphthalene, phenanthrene and pyrene, as carbon sources (Van Beilen et al. 2001; Zhang et al. 2011; Xia et al. 2014).

Temperature and pH have an influence on the bio-stimulation of microorganisms which in turn is associated with the capacity of the bacteria for degradation of the polycyclic aromatic hydrocarbons (Masakorala et al. 2013). This involves a complex process of monooxygenase and dioxygenase; in other words, they transfer oxygen atoms to the contaminated substrate (Hayaishi 2005; Sligar et al. 2005; Waterman

2005), thereby achieving a degradation through the pathways of salicylate or protocatechuate decarboxylase; these compounds provoke the oxidative rupture of the aromatic ring by the lactonizing enzyme (Parés and Juárez 2002; Lalucat et al. 2006).

Degradation of the alkane and alkene hydrocarbons involves the assimilation of O_2 molecular alkanes. This assimilation is carried out by bacteria such as the *Pseudomonas* sp. and members of the coryneform group and actinomycetes, in particular those of the genera *Mycobacterium* sp. and *Nocardia* sp. (Parés and Juárez 2002). Rivera-Cruz (2011) reported low population densities of *Azospirillum* sp., *Azotobacter* sp., phosphate solubilizing bacteria and heterotrophic fungi in the rhizosphere of two soils contaminated with total oil hydrocarbons with concentrations of 25,000 ± 345 mg kg^{-1} (Eutric Fluvisol soil) and de 65,890 ± 156 mg kg^{-1} (Mollic Gleysol soil).

The ectomycorrhizal fungi act with the root system to improve the absorbent surface of the plants; they also participate in nutrient recycling and are often more resistant to abiotic stress such as contamination from oil spills (Thijs and Vangronsveld 2015). In addition, with the help of these arbuscular mycorrhizal fungi, billions of bacteria help to absorb minerals and to produce vitamins and plant hormones which are able to degrade organic compounds such as the hydrocarbons (Bonfante and Desirò 2015; Lugtenberg 2015; Thijs and Vangronsveld 2015).

The roots of the plants must be able to tolerate the contaminants and, in conjunction, to develop the architecture of their roots in order to produce a biochemical environment very different from that which can be expressed in uncontaminated soil. Moreover, the roots must have an interrelationship with the different physical, chemical and biological factors of the affected soil in order to generate the acclimatization, growth and development of the plant (Ferrera-Cerrato and Alarcón, 2013). Once the plant is acclimatized, the degradation process of the organic contaminants among the microorganisms and the rhizosphere begins; this is usually beneficial for plant growth because the hydrocarbons become less toxic or innocuous (Chan-Quijano 2015; Thijs and Vangronsveld 2015).

3.3 Degradation of Hydrocarbons Through the Combination of Tree Species and Organic Fertilizers

Research on the key factors and biogeochemical processes that form the microbiota in the rhizosphere is still scarce in tropical areas and even more so in the areas impacted by hydrocarbon contamination. The plant must resist hydric stress, chemical toxicity, mechanical impedance and nutrient deficiency, to mention just a few; evaluations of root development in plant species which must withstand oil hydrocarbons are also scarce, and the same can be said regarding the studies of plant physiology in contaminated environments.

According to Albrecht and Kandji (2003), Alberto-Pardos (2010) and Philippot et al. (2013), the development of the rhizosphere contributes to the conservation of soil and to the mitigation of the effects arising from global environmental change; this is due to the fact that the roots store a significant amount of carbon at a greater depth, making its release more difficult. Moreover, the application of organic fertilizers favors biostimulation of the microorganisms present in the soil, as well as an increment in their diversity; thus, they could represent an alternative in the degradation of hydrocarbons and, at the same time, capture CO_2 in the roots (Adekunle, 2011; Wang et al. 2011).

Velasco-Trejo and Volke-Sepúlveda (2003) mention that the use of organic fertilizers presents important perspectives in the resolution and remediation of soils contaminated with hydrocarbons. Chan-Quijano (2015) reports that, with the combination of organic fertilizer (sheep manure in a dosage of 3.85 g kg^{-1}) and *Tabebuia rosea* (Bertol.) DC. in a soil contaminated with 158,674 mg kg^{-1} of oil hydrocarbons, a degradation of 85% was achieved over a period of one year; in other words, 135,113 mg kg^{-1} of oil hydrocarbons was eliminated.

In this way, the use of fertilizers associated with plant species increases the α diversity, and the activities of the microorganisms in the contaminated soil increase the degradation of oil hydrocarbons (Chan-Quijano 2015; Wu et al. 2017). Moreover, with the addition of nutrients to the soil through organic fertilizers, there is a corresponding increase in the number of microorganisms which degrade oil hydrocarbons, and thus, the rate of contaminant elimination increases (Litchfield 2005). For this reason, biodegradation by bacteria has been taken into consideration as a potentially useful tool in the remediation of soils contaminated by oil hydrocarbons (Yuste et al. 2000).

When the organic fertilizers and the plants are combined (with the aid of the roots), these two elements, in conjunction, can participate significantly in the degradation of contaminants or in the active absorption, in the case of heavy metals. These biotechnologies are less expensive and more environmentally friendly and are also more efficient in the cleansing of contaminated sites (Litchfield 2005; Rivera-Cruz 2011; Ferrera-Cerrato and Alarcón 2013; Chan-Quijano 2015). However, the plant roots occasionally suffer from a negative geotropism; that is to say, a knot is formed due to the concentration of hydrocarbons found in the contaminated soil as a result of a deficiency in oxygen, nutrients and water in the soil; moreover, when knot formation does not occur, development of the root occasionally presents shorter lengths in comparison with plants growing in non-contaminated soil. The formation of a greater number of secondary roots has also been observed in the species growing in contaminated soils (Fig. 3.1).

3.4 Perspectives and Necessary Research

According to Thijs and Vangronsveld (2015), the rhizosphere is a specific subset with the soil and the microorganisms; these organisms are involved in the biodegradation

Fig. 3.1 Negative geotropism (knot formation) in the main root of two tree species **a** *Swietenia macrophylla* and **b** *Tabebuia rosea* developed in soil contaminated with hydrocarbons, **c** and **d** are the same species but growing in uncontaminated soils

processes of the organic contaminants. It is important, therefore, to carry out studies on the rhizosphere in tropical areas and on native species, given the lack of sufficient information. It is also necessary to evaluate the behavior of the physico-chemical parameters and the biogeochemical characteristics between the contaminated soil and the rhizosphere of the plants at different depths, since the electric properties of the contaminated area change with time (Luo et al. 2016).

The use of native tree species in association with their rhizosphere helps the areas affected by oil hydrocarbons and, at the same time, provides certain benefits to the local inhabitants, for example, as timber species, living fences, raw material for craft trades, firewood, among others. At the same time, the people living in the area can foment a biologically based economy for the sustainable development of the impacted areas while also providing bioproducts such as biofuels, biopaints, biolubricants, among others, and also ecosystem services (Ceccon and Miranda 2012; Hu et al. 2012; Ceccon et al. 2015; Prasad 2016; Tripathi et al. 2016; Wagner et al. 2016).

However, in order to work with the rhizosphere in the evaluation, behavior and response of the plant species to be used in the remediation of soils contaminated with hydrocarbons or other contaminants, it is necessary to elaborate a profile of the contaminated soil to determine (1) the current ecological state and the degree of contamination, (2) the level and type of contaminants and (3) the toxicity of the contaminants. In addition, with the support of laboratory work and specialized equipment, we can determine (1) the morphology and physiology of the plants in order to understand the level of stress inflicted by the contaminant (tolerance and resistance to the contamination) and (2) the level of accumulation/acclimatization/adaptation of the plants (Tripathi et al. 2015, 2016).

The procedure described above can provide support in the ecological restoration and remediation of the affected areas through frameworks of ecological and sociocultural value, as well as economic aspects for a sustainable remediation (Fig. 3.2).

In addition, certain guidelines must be established for the remediation of soils contaminated by hydrocarbons (IMP 2010; Chan-Quijano et al. 2015), in order to put into effect strategies of remediation and restoration in the areas contaminated by oil spills with the aid of the rhizosphere provided by certain plant species (Ochoa-Gaona et al. 2011; Qixing et al. 2011) and to implement rehabilitation processes (the oil tends to concentrate in only one part of the altered habitat), the recovery

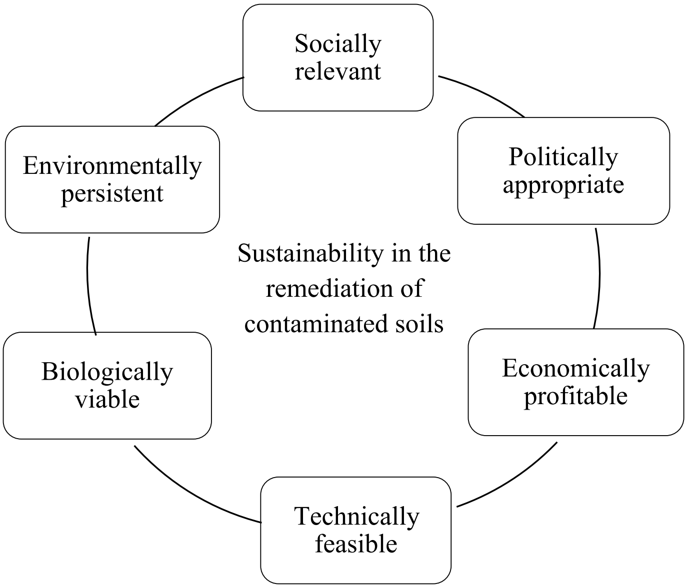

Fig. 3.2 Frameworks of ecological, sociocultural and economic values for a sustainable remediation

(rehabilitation of the gravely perturbed habitat), the recreation (construction) of an alternative, but desirable state in a gravely perturbed site where very little remained to be restored, improvement (ecological improvements) and mitigation or compensation (very often focused on a different system) in order to restore the structure and function of the contaminated ecosystems (Bradshaw 1987; SER 2004; Cooke and Suski 2008; Qixing et al. 2011).

The degradation of oil hydrocarbons requires a metabolic activation exercised by biological activities including mutagenicity or carcinogenicity, mediated through the formation of metabolites such as flavanone, flavone, iso-flavanone, 7-hydroxyflavone and 6-hydroxyflavone, to mention a few (White and Burken 1998; Yan et al 2004; Thijs et al. 2017).

When studying the rhizosphere of plants for the remediation, it is necessary to implement plant physiology as part of the conservation and management of populations and ecosystems. Physiology has been used very little in the field of restoration ecology. It is possible to use physiological metrics, such as gas exchange, transfer of energy, changes in metabolism, stress response, nutritional state and gene expression, among others, in order to understand the biogeochemical, metabolic and enzymatic processes of root function and of the plants in general, growing in contaminated soils, as well as to have a better understanding of the factors influencing their structure (Cooke and Suski 2008).

In relation to the application of genomic tools, including genomic sequencing, expressed sequence tags, transcription profiles and molecular markers, this would be very useful in monitoring activity to determine if the hydrocarbons penetrate the plant and with this information to evaluate the quality of the wood from tree species which are used in the remediation of soils contaminated with oil hydrocarbons; metaproteomics can also be used to evaluate the functional and phylogenetic relationships of the microorganisms in the degradation of oil hydrocarbons in contaminated soils (Merkle and Nairn 2005; Batista et al. 2016).

Plants are autotrophic organisms which are capable of using sunlight and carbon dioxide as sources of energy and carbon. The roots of the plants absorb a wide range of natural and anthropogenic, toxic compounds for which they have developed a number of extraordinary mechanisms of detoxification. Further basic and applied research is required in order to generate sufficient knowledge of the natural mechanisms of detoxification of many contaminants, deriving from the hydrocarbons (Alagic et al. 2015). It is important to mention that each hydrocarbon differs in its chemical composition, and for this reason, the Environmental Protection Agency (EPA) of the USA published a list of 126 priority contaminants which cause the most damage to ecosystems and human health (Yan et al. 2004; EPA 2014). Thus, further studies must be carried out on cytotoxicity in the microorganisms and phytotoxicity in the plant species and the rhizosphere which will be used in the remediation of soils contaminated by hydrocarbons in tropical areas.

3.5 Conclusions

The study of the remediation of areas contaminated with oil hydrocarbons is faced with a challenge to develop innovative and cost-effective solutions for the decontamination of contaminated environments. To achieve this, it is necessary to conduct studies on each contaminant regarding the damages and/or benefits they may be causing in the rhizosphere and in plant physiology.

The public in general should be encouraged to participate in the recovery of contaminated areas with the use of native plant species which provide more viable benefits for the sustainability of the ecosystem and for society. Studies on the rhizosphere must be integral, with the evaluation of soil quality, during and after the site remediation process.

Phylogenetic and physiological responses of the microbial community in the contaminated soils and their relationship with the rhizosphere must be evaluated in order to understand all the possible processes in the behavior of oil hydrocarbons in the soil resource.

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