Chapter 9
Bioremediation: A Viable Approach for Degradation of Petroleum Hydrocarbon

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9.1 Background

Soils contaminated with diesel or benzine are much harder to treat than more volatile petroleum hydrocarbons (PHCs), e.g., specific contaminants that are components, such as BTEX (benzene, ethylbenzene, toluene and xylene), n–hexane, jet fuels, fuel oils, and mineral-based crankcase oil, gasoline. The harmful effects of PHCs on the environment can be diverse: some aromatic and polyaromatic compounds have shown carcinogenic effect, being toxic or mutagenic, benzene for example; having carcinogenic effect even upon absorption through the skin; inhalation of vapors can lead to nausea, acute toxic reactions, liver disease, or teratogenic effects; some compounds can affect the taste and smell, so that their presence in surface and groundwater, even in small quantities, makes them no longer suitable for consumption; volatile compounds can form flammable or explosive mixtures with oxygen from air (Waters et al. 2017).

Figure 9.1 shows some structures of some PHCs (aliphatic hydrocarbons, cyclic and polycyclic aromatic hydrocarbons from PHCs).

Most of the chemicals in the category of harmful pollutants are nonpolar organic compounds; as a result they are hydrophobic, with extremely low solubility in water; they are lipophilic, which causes the accumulation in the fatty tissues of organisms. Due to the phenomenon of bioaccumulation and bio-amplification, the concentration of harmful pollutants can be much higher in the tissues of organisms at the end of the trophic chains (species of fish, mammals, birds, and humans) than the concentration in the environment (even 70,000 times higher).

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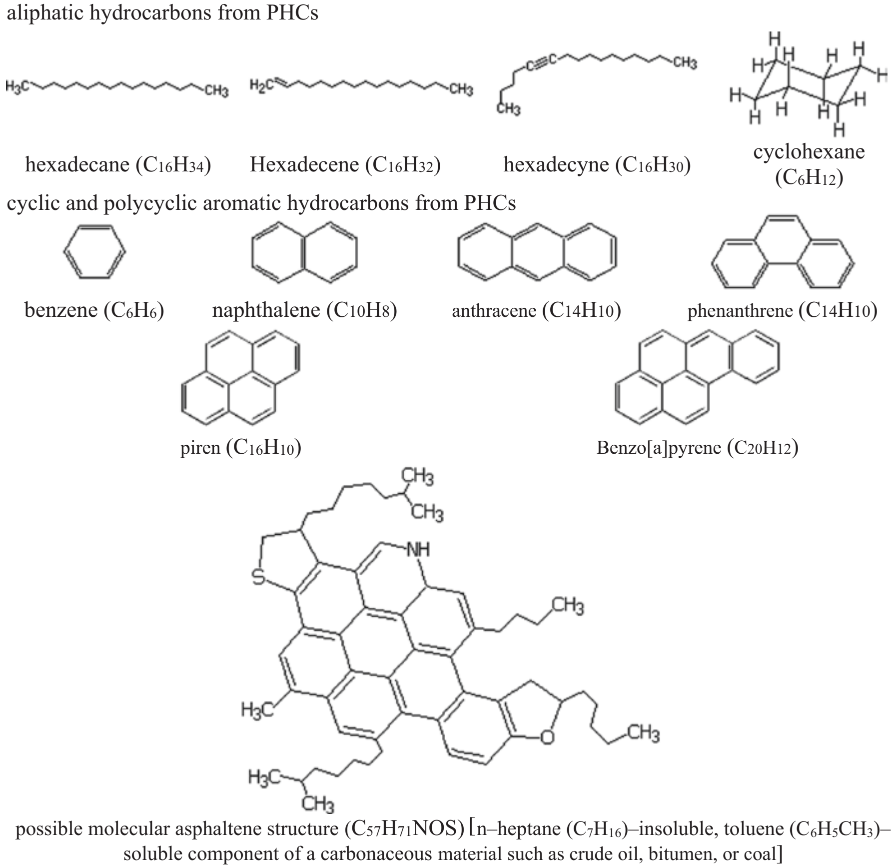

Fig. 9.1 The chemical structure of some PHCs representatives

In the literature, most studies address the assessment of artificially contaminated soils with individual pollutants. The in situ washing technology of the soil, in which the oxidant is introduced in the washing solution (chemical oxidation in situ), coupled with the electrokinetic process (EKOSF), was evaluated. EKOSF was made using different oxidants, sodium hypochlorite (NaOCI), permanganate ($KMnO_4$), and sodium persulfate ($Na_2S_2O_8$), which were transported from the electrode chambers into the solid matrix sections under the action of the electric field. Initially, the soil was characterized and several ex situ experiments were performed to determine the relationship between the pollutants present in the soil and the key process parameters and to evaluate the efficiency of each oxidant in the PHCs degradation process present in the soil. Sensitive soils include all soils in residential and recreational areas, soils used for agricultural purposes and soils in undeveloped areas. Less-sensitive soils include all soils for commercial and industrial use and land areas that will have such use in the near future. The toxicity of oil and petroleum products (PHCs) falls into two categories: immediate and long term. The immediate one is caused by the saturated hydrocarbons which in high concentrations cause the death of organisms. Aromatic hydrocarbons are the most toxic, and olefinic hydrocarbons have intermediate toxicity between saturated and aromatic ones.

Long-term toxicity is caused by the interference between hydrocarbons and soil components dissolved in water, interference with numerous chemical messengers, with the role of nutrition and reproduction of many aquatic organisms, leading to ecological imbalances (Errington et al. 2018a, b). The profile distribution of the pollutant is dependent on the amount of pollutant, the characteristics of the pollutant, the terrain configuration, the soil characteristics, and the dwell time. In soils with poorly permeable or impermeable horizons, an area of pollutant concentration appears above them, because of its heterogeneous polydisperse body properties, the soil acts as a chromatographic column. The components of the oil are retained especially in the upper horizons, and the film water that accompanies the oil in various proportions, with higher density and less viscous, penetrates faster in the lower horizons. Volatile PHCs fractions containing 6–7 carbon atoms in the molecule are volatilized, and nonvolatile hydrocarbons tend to concentrate and solidify. With the passage of time, the process of redistributing the oil components on the soil profile is accentuated, with a tar and asphalt retention taking place.

Oil stains lead to an imbalance of the C:N ratio in all spots with oil stains, as fresh oil is essentially a mixture of C and H. This causes a deficiency of N in soils soaked in oil, which slows the growth of bacteria and use of carbon sources. In addition, deficiencies of N and different nutrients such as phosphorus can limit the growth rate in soils soaked in oil. In addition, high concentrations of biodegradable organic products in the upper horizon of agricultural soils lower oxygen reserves in the soil and reduce the rate of oxygen diffusion into deeper layers.

PHCs pollutants tend to persist in the soil until remedial measures, involving the application of nutrients, are used, because O and N are limiting factors for all types of oil degradation. The contradiction between the tendency of concentration and bioaccessibility for a soil contaminant can be explained by the stronger, almost irreversible sorption by Fe and Al oxides or silicate clays, or by chemical complexation with organic matter. In addition, the contaminant molecules can become physically isolated in three ways.

First, the contaminant molecules can be trapped in the soil nanopores, the ends of pore spaces from 1 to 100 nm in diameter. These pores, inside the humus and the clay colloids, are large enough to house the contaminant molecules, but too small for the entry of bacteria or even extracellular enzymes that would otherwise be capable of attacking the contaminant. Second, the contaminant molecules can diffuse into the pores; they can be absorbed into the solid structure of the humus particles, where, again, they are no longer exposed to living cells or their enzymes. Third, the contaminant molecules may become buried, or occluded, beneath the precipitated mineral layer, and isolated again from biological interactions. The PHCs pollutant molecules trapped in either of these mechanisms can offer a reduced risk of environmental mobility or biological toxicity even if their total soil concentration remains high. Due to this aging process, some researchers believe that environmental cleanliness standards need to be based more on the measured bioaccessibility of the contaminant than on their total concentration.

The term remediation covers: measures to eliminate or reduce pollution (decontamination measures) and measures to prevent or reduce the spread of pollutants in a safe manner, without removing pollutants (encapsulation measures).

Among the methods of treating soils contaminated with PHCs are:

– Physical immobilization: sealing, treatment barriers, hydraulic locking, and stabilization and inerting
– Biological methods: natural attenuation, phytoremediation (hydraulic control, phytoextraction or phytoaccumulation, phytodegradation or phytotransformation, rhizodegradation, phytostabilization, phytovolatilization and phytostimulation), bioremediation, and alternative systems of bioremediation (bioreactor, land-based treatment, composting, and bio-oxidation systems in soil piles)
– Chemical methods: photo-oxidation, dehalogenation, chemical extraction, oxidation, reduction, dechlorination, precipitation, and microbial fuel cell
– Physicochemical methods: excavation, pumping, washing, flotation, biosparging, venting, electrokinetic extraction, electroacoustic decontamination, washing and applying electromagnetic waves, and passive absorption on polymers
– Thermal methods: incineration, pyrolysis, thermal desorption, vitrification and microwave treatment

The treatment techniques that meet the requirements are selected and then evaluated on the basis of nine mandatory criteria: complete protection of human health and the environment; compliance with the relevant applicable or relevant requirements; long-term and permanent efficiency; reducing toxicity, mobility, or volume; short-term efficiency; implementability; cost (Errington et al. 2018a, b); state acceptance; and community acceptance.

9.2 Treatments Can Be Performed In Situ or Ex Situ

The main advantage of in situ treatment is that it allows the soil to be treated without being excavated and transported. In situ treatments generally require longer periods of time and are less certain about the uniformity of the treatment due to the variability of soil characteristics under unverified conditions.

In situ bioremediation refers to the biological treatment of contaminated soil without excavation prior to treatment. In situ technologies can be cheaper, create less dust, and result in lower losses of contaminants in the environment compared to ex situ technologies. It is also possible to treat larger volumes of soil at a given time. In situ techniques can be slower, harder to drive, and are more efficient in places with permeable soils (sandy or coated).

Ex situ treatments (including application on agricultural land, composting, soil-based bioremediation, and sludge bioremediation) using soil work, continuous digging, or mixing of sludge, to apply oxygen and nutrients, and is performance in the training bed or reactor.

The researches that have been developed worldwide have had the major objective to develop simple, economically efficient, rapid methods of decontamination, which will ensure by applying them in situ blocking the migration of pollutants underground, destroying the pollutant, and restoring the natural environment.

Bioremediation technology includes biostimulation (stimulation of viable native microbial populations), bioaugmentation (artificial introduction of viable microbial populations), bioaccumulation (in living cells), biosorption (in dead microbial biomass), phytoremediation (with plants), and rjizo-remediation (through interaction between plants and microorganisms).

Rhizo-remediation, which is the most evolved process of bioremediation, involves the removal of specific contaminants from the residual products from the contaminated sites through the mutual interaction of the plant roots and the suitable microbial flora.

Bioremediation efficiency is often a function of the microbial population and how they can be enriched and maintained in the environment. Strategies for cheap in situ bioremediation of oil-contaminated soils include stimulation of indigenous MOs by stimulation with nutrients (biostimulation) and/or by inoculation with enriched microbial mixture cultures in soil (bioaugmentation).

Biodegradation has proven to be suitable for: PHCs such as diesel, light liquid fuel, gasoline, lamp oil, mineral oils, benzene, toluene, xylene, etc. wastes from oil production, sludge, and oil residues; organic products and residues from the basic chemical industry (alcohols, acetone, phenols, aldehydes, and other solvents); and complex compounds such as polycyclic aromatic hydrocarbons and pesticides.

During the biodegradation treatment, we have to keep track of physical factors (characteristics of the polluted environment: permeability, porosity, granulometry, structure, texture, etc., the presence of O_2, humidity, temperature, pH, adherence of the pollutant to soil particles or sediments), chemical factors (nature and the concentration of the pollutant, the degree of toxicity of the pollutant, the solubility of the pollutant, the bioaccessibility of the pollutant, the presence or absence of a co-substrate, the salinity of the environment, the presence of accessible nutrients, the organic content in the environment, the biodegradability of the pollutant), and the biological factors (the density of indigenous MOs capable of degrading). Bioremediation is the creation and maintenance of a favorable environment for MOs, some indigenous others nonindigenous, to use soil contaminants as a carbon source. The most important factors influencing the biodegradation process are:

- Addition of surfactants, carriers
- Quantity and quality of the contaminant
- The biodegradation capacity of the indigenous microflora
- The characteristics of the polluted soil (pH, humidity, texture, structure, content in nutrients, aeration, temperature, biological activity, etc.)
- The presence of nutrients essential for the growth of MOs and especially the ratio between P and N
- The type of MOs presents in the polluted environment and so on

Bioremediation of oil-contaminated soils is an efficient, safe, economically feasible technique, environmentally friendly, a versatile or complementary alternative to physicochemical treatments although the bioaccessibility of hydrophobic organic compounds for MOs can be a limiting factor during biodegradation processes.

Bioremediation can be organized on the spot, the residues are permanently eliminated, it is very economically efficient, nondestructive or with a minimum of destruction, relatively uncomplicated in implementation, it requires nonspecialized equipment, the risks associated with long-term liability related to the remains of the contamination are eliminated, it eliminates transportation costs and liability for debris removal, can be coupled with other treatment techniques, and can be extremely effective in removing recalcitrant PHCs (new synthetic compounds that are slowly biodegradable or nonbiodegradable are known as recalcitrant compounds) (Fuentes et al. 2014). Bioremediation techniques are used to treat contaminated soils, contaminated waters, sludge, and aquifer materials. The choice of the most suitable processes for a specific place depends on the physical–chemical environment, the desired final results, and the economic conditions.

Disadvantages of bioremediation include the following: design criteria for high-efficiency remediation are site specific and may require extensive monitoring, some chemicals cannot be bioremediated, contaminant toxicity, the level of scientific knowledge, potential production of unknown by-products, and perceived as a technology with no contingencies.

In order to improve the low-moderate soils polluted with oil, the following improvement works are required: removals of excess oil; amendments, scarification, deep plowing, organic ameliorative fertilization, mineral fertilization with NPK (biostimulation), with the increase of the nitrogen weight in order to reduce the C:N ratio; o treatments with selected MOs (bioaugmentation); o preparation of the germinating bed and sowing of plants with large seeds (maize, peas, etc.) (Sturla Lompré et al. 2018).

9.2.1 Methods to Stimulate Bioremediation

Biostimulation is a method of stimulating the activity of indigenous populations of MOs for remediation of contaminated soil. The term “enhanced bioremediation” encompasses a large number of technologies that differ with respect to their inputs. These technologies may involve the addition of electron acceptors or electron donors to stimulate the natural development of microbial populations (biostimulation) or may introduce specific MOs to improve the biodegradation of target compounds (biostimulation). The efficiency of biodegradation of PHCs in soils is limited by many factors (e.g., types of MOs, nutrients, oxygen, and contaminant concentration). Therefore, rates of pollutant degradation can be improved by adding nutrients, oxygen, and primary substrates to contaminated systems. These can increase the populations of indigenous MOs and thus improve the efficiency of pollutant biodegradation (Liu et al. 2019).

Among the methods of stimulation of bioremediation are: the use of inoculum of MOs, the use of fertilizers, the application of absorbents, the amendment, the works of the soil, the use of plants, and stimulation with surfactants. Experiments were organized in the reactor where the efficiency of the addition of nutrients, oxygenated water, and molasses from sugar cane were pursued; washing the soil with a biodegradable surfactant (Simple Green SG); and pretreatment of soil by Felton-type oxidation for bioremediation of oil-contaminated soils.

The native MOs dominant in oil-contaminated soils after each treatment process were determined by polymerase chain reaction, gel electrophoresis gradient determination, and nucleotide sequence analysis. The results show that approximately 32–56% of the total PHCs were removed (the initial concentration was 5000 mg/kg) from the reactor by adding nutrients and molasses from the sugar cane (1000 mg/L), with 9% from PHCs removed from the control reactor maintained under untreated conditions, after 120 days of incubation.

The addition of sugar cane molasses produced the growth of the population of MOs and therefore the rate of PHCs degradation improved. The results also show that about 61% of the removed PHCs was observed in the reactor in which H_2O_2 (100 mg/L) and nutrients were added after 120 days of incubation. This indicates that the addition of the reduced concentrations of H_2O_2 (100 mg/L) will result in the desorption of PHCs from the soil particles and the increase of dissolved oxygen and thus the efficiency of the bioremediation in the reactor (Hou et al. 2019).

9.2.2 Removal of Excess Petroleum Hydrocarbons

It is necessary that the first intervention in the event of accidental oil pollution consists in the fastest removal of hydrocarbons from the surface of the soil to reduce the depth of penetration into the soil. Collection gutters for pollutant and small pits to concentrate the pollutant will be executed so that it can be collected with the help of suction systems in a tank and transported to a treatment station (Zhu et al. 2005).

9.2.3 Application of Absorbents

The use of absorbents has several main objectives: the absorption of oil and its retention on the surface of the soil in order to be collected and sent to a recovery and treatment station; the retention of oil in order to prevent the penetration of the soil profile and in this way make the remediation process difficult; preventing the formation of an impermeable film on the surface of the soil; stimulating the multiplication of MOs involved in bioremediation; regulating the aerohydric regime of the soil; source of nutrients and water. Some absorbers are made from Sphagnum peat and have an absorption capacity of 10–14 times its weight. Such an absorbent (Nature Sorb) absorbs and encapsulates, almost instantly, the PHCs in the plant cells of the dehydrated peat muscle. In parallel, a biochemical process of breaking the chemical bonds of hydrocarbons begins by the enzymes produced by the bacterial flora existing in soil and water. In the presence of oxygen, a minimum temperature of 10–12 °C, and humidity, the biodegradation process normally proceeds (Paulauskiene 2018).

Biogenic elements such as nitrogen, phosphorus, and potassium stimulate this process. Other experts recommend to apply immediately after pollution, absorbents in the form of carpets, barriers, rollers, pillows, linings, gloves (metasorb, sorbx, sorbs 2), which preferentially absorb oil and other hydrocarbons but do not absorb water-based liquids.

After soaking with oil, these absorbent materials are taken to an oil extraction and treatment plant. This reduces the risk of deeper soil pollution, which would make it difficult to remedy. The use of absorber (AbsE) has been tested to stimulate biodegradation by promoting the development of bacteria and increasing the rate of biodegradability of PHCs. AbsE is composed of 10 naturals cellulose fibers mixed with nutrients to activate MOs. AbsE is obtained from plant fibers and cellulose waste, all treated and additivated. The studies were carried out on a cambic chernozem with vegetation polluted with 5% and 10% of crude oil, inoculated and not inoculated with selected bacteria and treated with various doses of AbsE.

The bacteria selected were: *Pseudomonas*, *Mycobacterium*, *Arthrobacter globiformis*, and *Bacilus megaterium*. The best results in the bioremediation process of the oil-contaminated soil were obtained in the variants that received the maximum absorbent dose (30 t/ha in the case of 10% oil pollution and 15 t/ha in the case of 5% oil pollution) accompanied by the application of bacterial inoculum. The bacterial inoculum led to a 5% reduction in the total hydrocarbon concentration, so in the polluted variant with 5% oil, the decrease was 12% in the case of treatment with 7.5 t/ha AbsE, and in the treated versions with 7.5 t/ha AbsE with inoculum, decrease in total hydrocarbon concentration was 17% after 90 days from pollution. In the case of 10% oil pollution, the level of the oil residue after 90 days decreased by 20% in the case of treatment with 15 t/ha AbsE and by 25% in the case of treatment with 15 t/ha AbsE plus the bacterial inoculum (Ram et al. 2019).

The research carried out on a soil (with vegetation) contaminated with 3% crude oil in which peat and zeba were absorbed in different doses, basic fertilization with $N_{200}P_{200}K_{200}$ and liquid fertilizers based on humic substances extracted from KH lignite (fertilizer containing potassium humate with microelements), fertilizer 1 (potassium humate in NPK and magnesium matrix, with nitrogen in amide form), fertilizer 2 (fertilizer containing potassium bytes in an NPK matrix with microelements and 4% monosaccharides, where nitrogen is in amide form), and fertilizer 3 (fertilizer containing potassium peroxide in an NPK matrix with microelements and 8% monosaccharides, in which nitrogen is in amide form) led to best results in the bioremediation process on a nonabsorbent basis were obtained in the variants fertilized with fertilizer 1 (potassium humate in NPK and magnesium matrix, with nitrogen in the form amide) where PHCs values decreased by 47% within 45 days of treatment; the process of bioremediation against the use of peat as an absorber at a dose of 16 t/ha was carried out with the highest intensity in the variants in which fertilizers of the type 2 (fertilizer containing potassium in a micro-element NPK matrix with microelements were applied and 8% monosaccharides in which nitrogen is in amide form) and bacterial inoculum, the PHCs values being reduced by 50% after 45 days after treatment; against the background of using the peat as an absorbent, of the fertilizers best behaved fertilizer; against the application of 16 kg/ha absorbent the most effective variant of bioremediation was shown to be the one in which it was fertilized and bacterial inoculum was applied, where the content in PHCs was reduced by 57% after 45 days after treatment; against the application of at a dose of 32 kg/ha the best results in the bioremediation process are obtained by applying the fertilizer at a dose of 11,650 l/ha + 64 kg/ha glucose, in which the PHCs level decreases by 58% after 45 days from the application of the treatment and 60 days from the oil pollution.

Under the conditions of nonuse of an absorbent, the greatest number of bacteria was determined in the variants where mineral fertilizers were applied in the dose of $N_{200}P_{200}K_{200}$ together with the bacterial inoculum.

The application of the bacterial inoculum together with the fertilizer (fertilizer containing potassium humate in an NPK matrix with microelements and 4% monosaccharides, in which the nitrogen is in amide form) in the bioremediation process led to the increase of the soil content in mobile potassium (Karppinen et al. 2017). Some researchers recommend the application of superabsorbent polymer in the amount of 1000 kg/ha to absorb the residual oil that remains after collecting the oil layer/film, in order to prevent its infiltration into the soil profile and subject them to the degradation process. Polymers are catenary-type combinations with large molecular mass, which respect a common formation principle which consists in repeating, along the macromolecular chain, a minimal group of atoms called a structural unit. These units are identical or similar in composition to the monomers from which the polymers were (or could have been) synthesized. The number of structural units included in a macromolecular chain represents the degree of polymerization of the polymer. Polymers can be water soluble or water insoluble.

Polyelectrolytes are polymers whose monomer units possess ionized or ionizable groups. In aqueous solution, the polyelectrolytes dissociate into polyvalent macroions (polyions) and a large number of small ions of opposite-counterions (Cartmill et al. 2014).

Depending on the nature of the ionizable groups, the polyelectrolytes can be classified into:

- Polyacids or anionic polyelectrolytes, which possess as a functional group the carboxylic group – COOH, the sulfonic group – SO_3H, or the phosphoric group – PO_3H
- Cationic polybases or polyelectrolytes, having as functional group the amine group – NH_2, the imine group> NH, the quaternary ammonium group – NH_4, etc.
- Amphoteric polyampholytes or polyelectrolytes, which have both acidic groups (carboxylic, sulfonic, phosphoric, etc.) and basic groups (amines, ammonium, etc.) on the macromolecular chain

Polyelectrolytes are useful as soil conditioning agents. Improvement of the physical, chemical, and biological characteristics of the soil creates conditions for the intensification of the bioremediation processes (Wrede et al. 2012). Amendment with activated carbon resulted in a dramatic reduction in PHCs concentration in the aqueous solution and the bioaccessibility of pollutants for diverse estuarine biota such as *Macona balthica* mollusk, *Hinia reticulata* gastropod, and *Nereis diversicolor* polychaete. Potential obstacles to the successful application of this technique include dirt from active coal by dissolving organic matter in sediments and slowing the transfer of pollutant mass to active coal under field conditions. Biodegradation and stabilization can become competitive processes when carbonaceous material is added, by reducing the free concentrations in the solution and reducing the accessibility of pollutants to degrading MOs. If the pollutants are biodegradable, the addition of sorbent materials may prevent them from decomposing, but if they are not rapidly biodegradated, sorbent amendment may be an effective alternative method for reducing environmental risk (Zhen et al. 2019).

Bioremediation and amendment with activated carbon (AC) were compared as remediation strategies for sediments in the Tyne River containing 16.4 ± 7.3 μg/g PHCs and approximately 5% carbon particles from the total sediment weight. Unamended, amended with nutrients (biostimulated), and amended with nutrients and *Pseudomonas putida* (bioaugmentation) inside the sediments failed to show a significant decrease in the total concentrations of PHCs in the sediment after a period of 1 month. The passive polyethylene samples were incorporated for 21 days in this sediment for the purpose of measuring the accessible portion of PHCs and the accumulation of passive polyethylene 4.70 ± 0.25, 12.43 ± 1.78, and 23.49 ± 2.73 μg PHCs s/g PE from unaltered, biostimulated, and biodocumented material. The higher uptake of PHCs by the samples with activated polyethylene under biostimulation and bioaugmented conditions coincides with the slowing down of the target phenanthrene in the free sediment filtrate from these experiments compared to the unamended variant filtrate (Varjani and Upasani 2019).

Analysis of the microbial community revealed changes in the bacterial community followed directly by the addition of nutrients, but the addition of the *P. putida* community failed to establish itself. Addition of 2% activated carbon to the weight of the dry sediment reduces the PHCs uptake of PE (passive polyethylene) samples to 0.28 ± 0.01 μg PHCs /g PE, more than 90% reduction compared to unamended variant (Zhang et al. 2013).

9.2.4 Amending

For the normal development of the physicochemical processes involved in the degradation of the oil and the stimulation of the activity of MOs, especially of bacteria, it is recommended that the corresponding amendments be applied on acid and basic soils. To improve the reaction of acid soils, it is recommended to apply limestone-based amendments, the dose being determined according to the formula:

$$\mathrm{DCA}(\mathrm{t/ha}) = \mathrm{SBi}\left(\mathbf{100}/\boldsymbol{Vi} - \mathbf{1}\right)1.5\left(\mathbf{100}/\mathbf{NPA}\right)$$

DCA – dose of calcareous amendments;
SBi – the sum of the initial exchange bases;
Vi – the degree of saturation in bases calculated with hydrolytic acidity;
NPA – the neutralization power of the amendment in $CaCO_3$.

This formula is used in the elaboration of agrochemical recommendations and aims to bring pH values in the range 6.5–7.3, optimal for plant growth and for the normal development of the physicochemical processes involved in oil degradation and the activity of MOs, especially bacteria. In the case of saline and alkaline soils, the dose of the amendment is calculated taking into account the content of the soil in exchangeable Na, the alkalinity given by Na carbonates, the cation exchange capacity, and the acidification value of the amendment. The sodium in the complex is removed until a balanced Na/Ca ratio is achieved according to the needs of the cultivated plants. This ratio corresponds to the time when the soil contains only 5% exchangeable Na of T. Practically, it has been shown that a satisfactory improvement is obtained even when the soil contains up to 10–12% exchangeable Na of T (Liu et al. 2018). The dose of amendment is expressed in $CaSO_4 \cdot 2H_2O$ (gypsum). The following relationships can be used for calculation:

$$\text{Dose of } CaSO_4 \cdot 2H_2O(\mathrm{t/ha}) = 0.086\left(\mathrm{b} - \mathrm{T}^{\mathrm{C}}/100 - \mathrm{d}\right) + \mathrm{H} \cdot \mathrm{Wv}$$

in which:

b – soil content in exchangeable Na (me/100 g soil)
T – soil cation exchange capacity (me/100 g soil)
c – the percentage of exchangeable Na that can remain in the soil (5–12%)
d – alkalinity due to carbonate and bicarbonate of Na (me/100 g soil)
H – the depth of the plowing layer that improves (cm)
Wv – the volumetric weight of the soil (t/m3) having the values: 1.3–1.7 in the alluvial soils; 1.3–1.5 on sandy soils; 1.5–1.75 on heavy soils; 1.55–1.65 in swamp; 0.086 – coefficient of transformation of the milliequivalents of convertible Na into g $CaSO_4 \cdot 2H_2O$

$$\text{Dose of } CaSO_4 \cdot 2H_2O(\mathrm{t/ha}) = \mathrm{a}\left(\mathrm{b} - \mathrm{T}^{\mathrm{C}}/100 - \mathrm{d}\right)$$

in which: “a” is the acidification value of the amendment, the size of which varies as follows: 1.00 for gypsum; 1.25 for phosphogips; 0.38 for concentrated sulfuric acid; 0.18 in elemental sulphur; 0.58 at $CaCO_3$. In this formula, the amendment dose is calculated for a 100 mm thick soil layer (Kai et al. 2018).

9.2.5 Soil Works

The homogenization of the soil profile is a work that aims to create optimal conditions under the relationship of soil–water relations and water circulation. This is done by successive plows, at different depths, realizing the most intense mixing of soil horizons, diminishing and combating the negative influences of pollutants on the profile. There is also a horizontal dilution of the pollutant and the provision of 14 aerobic conditions in the polluted area to stimulate the bioremediation process (Yao et al. 2018). It has been recommended to scrape the soils with high porosity, with low aeration porosity, in order to improve the aerohydrous conditions in the soil. The scarification will be carried out at a depth of 60 cm, and the distance between the active organs will be 1 m. The optimum time for execution is when the soil has humidity of 60–80% of the active humidity range. The work is performed perpendicular to the drying channel or the natural emissary. On certain polluted soils and with salt water, it is also recommended to perform mole drainage. Fertilization and soil work increased the diesel losses in the soil by 55% compared to the initial level (soil polluted with 5% oil over a depth of 15 cm) compared to 24% in the unfertilized and untreated control.

The accessibility of the terminal acceptors of suitable electrons (e.g., oxygen, nitrates, iron, manganese, sulfates) more than the single nutrients will be a factor that determines the expanding of biodegradation. Soil works provided oxygen and nitrogen fertilization provided nitrates. Fertilization, works and bioaugmentation increased the percentage of biodegradation up to 89% and registered an increase of 34% compared to bioremediation only with fertilizers and works and by 58% compared to the untreated control (Khudur et al. 2019).

9.2.6 Stimulation with Surfactants

Surfactants, surface active agents, are synthetic organic chemicals having a polar (hydrophilic) and a nonpolar (hydrophobic) portion. They are usually classified as anionic, cationic, amphoteric, and nonionic. Surfactants are used in the formulation of synthetic detergents for personal or household cleaning, as dispersants and emulsifiers, for different industrial uses, and as an ingredient or additives with potential multiscope in food, mining, oil, and textiles. Some nonionic surfactants, such as alcohol ethoxylate and ethoxylated alkylamino, are used as pesticide adjuvants (Li et al. 2018). Increased use of these compounds leads to increased dispersal in the environment. For this reason, they are seen as a source of pollution and carefully controlled. Using surfactants to improve the restoration of highly polluted soils is a forerunner of high-efficiency remediation technologies.

The surfactant improves soil washing, a process that generates high toxicity effluents, usually including potentially hazardous chemicals extracted from contaminated sites. In particular, where surfactants are present, processes such as bioremediation, ultrasonic irradiation, advanced oxidation, activated carbon, and the biological membrane reactor are applied (Liu et al. 2016a, b).

There are two types of surfactants: synthetic and biosurfactants. The application of surfactants or emulsifying agents can cause a decrease in interphase tension and an increase in the solubility of hydrocarbons (Liu et al. 2016a, b). The sorption of surfactants by soil particles depends on the type of surfactant, the properties of the soil, and the amount and structure of the clay in the soil. Biosurfactants (surfactants with properties to increase microbial activity) have characteristics superior to synthetic surfactants such as absence of toxicity, biodegradability, and efficacy at extreme values of temperature, pH, and salinity. In addition, biosurfactants can be produced from cheap raw materials. Adding surfactants enhances the efficiency of processes in sludge bioreactors by increasing the rate of mass transfer of contaminants into the aqueous phase, thus increasing the contact of contaminants with the soil microbial population and improving their biodegradation. There are, however, cases when the application of biosurfactants is not effective because the culture of MOs produces sufficient biosurfactants (Li et al. 2016).

Research has shown that after 14 days after soil pollution with 5% crude oil, PHCs values in the improved variants begin to decrease compared to the untreated pollutant variant, where decontamination in the soil was achieved through natural attenuation processes. After 21 days, it becomes obvious that when the soil was conditioned with the higher dose of AbsE, i.e., 0.5% (100 g/vessel), and inoculated with selected bacteria, the rate of disappearance of the pollutant from the soil was much higher, compared to the other experimental variants, the results having a high degree of statistical assurance. In the case of variants in which the soil was polluted with 10% oil, a relatively slow rate of oil degradation was found in the interval between 30 and 300 days. The significance of this long period of slowing down of the processes of biodegradation of PHCs, in the case of the excessively polluted soil with oil, may be the difficulty of adapting the biodegradating MOs, both indigenous and those introduced by adding bacterial inoculum, after the excessive pollution.

Relatively few works related to the simultaneous treatment of hydrocarbons and surfactants include wastewater from soil washing. With all these technological approaches, flocculation–coagulation processes are suitable methods for treating wastewater generated in the field, on site, without requiring expensive equipment and in a small space, with high efficiency at a very low price. Flocculation–coagulation was used to treat municipal and industrial wastewater, with medium and high load of organic material. The amount of wastewater generated in the process can be highly variable, but a proportion of 3–5 l of water per kg of washed soil is produced. This is of course dependent on many factors such as the initial oil hydrocarbon concentration, the type and amount of surfactants used, the soil texture, the energy supplied to the washing system, etc.

At the end of the washing process, the generated wastewater must be deposited in a drainage system, or discharged into the natural watercourses, so it is compulsory to treat the contaminated stream up to the levels required by the legislative framework (Li et al. 2019). Applying surfactants in contaminated soils can potentially reduce interphase tension, increase the solubility and bioaccessibility of

PHCs, and thus facilitate their biodegradation. The addition of synthetic surfactants in the environment contaminated with PHCs has been studied as a way in which their inhibitory effects of biodegradation have been recognized, especially at concentrations above the critical micellar concentrations. The potential benefit of biodegradable surfactants includes their unusual structural diversity, which can lead to unique properties, economically efficient production, and their biodegradability (Cheng et al. 2019).

9.2.7 Use of Plants

The combined effect of plant growth and different microbial strains on improving the bioremediation of soil contaminated with PHCs was studied. Raygras (*Lolium perenne*) was planted and microbial strains were used as both simple and mixed agents. The results show that combining the raygras with the mixture of *Bacillus subtilis*, *Sphingobacterium multivolume*, *Acinetobacter radioresystems*, *Rhodococcus erythropolis*, and *Pseudomonas fluorescens* microbial strains gave the best results with a 58% degradation rate after 162 days.

Fractional analysis of PHCs gives indications that the degradation of saturated hydrocarbons appears to be more intense at the combination of MOs and raygras; the degradation of polar fractions is more intense through phytoremediation, and the aromatic fractions have been further degraded by microbiological remediation. Higher polyphenol oxidase activity occurs in all applied treatments (compared to control), but lower dehydrogenase activity was found in microbiological remediation processes.

The number of degrading MOs was higher in the rhizosphere of the alfalfa (*Medicago sativa* L.) and the alpine meadow-grass (*Poa alpina* L.), than in the mass of the soil where they grew in the soil amended with organic contaminants. The number of pyrene-degrading MOs was significantly higher in the thick rhizosphere (*Cynodon dactilon* cv. Guymon) compared to soil mass. The number of total hydrocarbons degrading MOs was lower in the soil without vegetation compared to the soils with vegetation (Teng et al. 2011). It has been reported that the number of PHCs-degrading MOs per gram of dry soil was 100 times higher in treatment with vegetation than in treatment without vegetation. The number of MOs that degrade hydrocarbons, alkanes, and PHCs per gram of soil was significantly higher in the rhizosphere of the Sudan grass (*Sorgum sudanenese*) compared to the soil outside the rhizosphere in the six doses of N used. Sudan grass grew for 7 weeks in soil with a PHCs level of 16.6 g PHCs/kg soil (Kirkpatrick et al. 2006). Nitrogen was added based on PHCs C:N total ratios (PHCs–C:TN) ranging from 44: 1 to 11: 1. The presence of Sudan grass led to significantly more total hydrocarbon and alkane degrading MOs per vessel when grown in the fine soil with an 11: 1 ratio (inorganic nitrogen) PHCs–C:TN compared to higher ratios PHCs–C:TN (carbon from total PHCs: nitrogen from total nitrogen added). The soil outside the rhizosphere in the Sudan grass vegetation vessels did not contain a number of contaminants degrading

MOs significantly different per gram of soil than in the plant less vessel. Increased doses of N stimulated the growth of the roots of Sudan grass which led to the increase of the potential for degradation in the contaminated soil due to the influence of the rhizosphere (Hou et al. 2019).

The results demonstrate the importance of the rhizosphere in increasing the potential for contaminant biodegradation. For efficient phytoremediation, additional doses of nitrogen will be sufficient to optimize plant growth and maximize the number and activity of contaminant-degrading MOs. A positive correlation between the reduction of PHCs and n-hexadecane and the number of degrading MOs has been reported by other studies (Chaturvedi et al. 2019).

9.2.8 Use of Microorganism Inoculum

The experiments of inoculation with specific MOs aim to intensify certain biological processes in the soil, being shown that enrichment of the polluted soil with these selected MOs intensifies the initiation and the development of biodegradation. For this reason, bioremediation technologies have been developed which involve: knowledge of ways to optimize the conditions of biodegradation, behavior and effects of chemicals introduced into the soil on the ecosystem, and selection of MOs with superior degradative abilities (Meyer et al. 2012). MOs that are capable of degrading xenobiotic substances are generally present in polluted environments, but natural biodegradation occurs at very low rates. The application of bacterial biopreparations is the main technological link in the process of bioremediation of soils contaminated with biodegradable compounds, as is the case of PHCs (Dos Santos and Maranho 2018). Treatment of 10% oil-contaminated brown luvosoil, with an inoculation of bacterial strains of the genera *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Mycobacterium*, *Micrococcus*, *Escherichia*, and *Streptomyces*, selected from oil-polluted soils, led to the following conclusions: significantly increases the total number of bacteria and microfungus in the variants treated with the inoculum compared with the untreated control; during the dry summer, usually July and August, the number of bacteria is reduced, so also the biodegradation activity, which requires the application of irrigation; significantly increased the taxonomic diversity of microorganism species in the variants treated with the inoculum, especially in years 2 and 3 after pollution; in the last experimental year, there was a net differentiation in terms of the number of bacteria in the inoculated versus noninoculated variants (14.4–33.3·10^6/g dry soil compared to 5.3–9·10^6/g dry soil); after 3 years after treatment, the level of pollution became insignificant (Hou et al. 2019). Numerous heterotrophic MOs in soil, both bacteria and fungi, are able to use crude oil as an energy source for cell growth and development. Some MOs, especially bacterial species from the genera *Bacillus*, *Pseudomonas*, and *Acinetobacter*, were tested on a soil contaminated with crude oil from India and found to be most effective in decontamination; it was a species of the genus *Bacillus*, achieving a rate of biodegradability of 59%, followed by species of the genera *Acinetobacter* 37% and

Pseudomonas 35%. Great progress has been made in biodegradation of oil-contaminated soils and in decreasing their level of toxicity, up to the ability to support plant growth. In all cases, biodegradation is effective only within certain pollutant concentrations. If a certain limit of the pollutant concentration in the environment is exceeded, the MOs may remain at the periphery of the contaminated area or may be destroyed due to the high toxicity of the pollutant (Aguelmous et al. 2018). Microbial cultures must have the ability to adapt to environmental conditions and survive in the presence of other MOs. Most studies based on inoculation with selected MOs are carried out by inoculating filamentous fungi in polluted soil, which have the ability to biodegrade polynuclear aromatic hydrocarbons with up to four nuclei.

Inoculation technology with selected MOs is used in particular for the degradation of pure compounds. Various studies have investigated the use of exogenous bacteria or groups of bacteria. This method is called bioaugmentation and involves the selection of microbial strains for their ability to degrade the contaminants and add them to a particular place (Kotoky et al. 2018). Were examined nine highly adapted, commercially available microbial strains. Their results indicated that commercial products do not adapt quickly to the environment in which they were exposed. Therefore, the efficiency of the adapted microbial strains was severely limited. In fact, the conclusion showed that indigenous bacteria have sufficient potential for degradation if they are provided with the proper conditions and nutrients. Eight cultures of commercial bacteria were tested on PHCs brought daily to North Alaska in closed laboratory reactors. It was concluded that indigenous bacterial populations already present in crude oil were the first to be responsible for biodegradation.

They applied *Bacillus pumilus* MVSV3 (*Bp* MVSV3) on two plots of land polluted with PHCs and found an increase in the degree of decontamination from 53% to 76% compared to the method of "land farming." The addition of *Bp* MVSV3 leads to stimulation of microbial activity, evidenced by both increased respiration and the number of bacteria. After 6 months of treatment with *Bp* MVSV3, the value of aromatic hydrocarbons decreased from 37 mg/L to 2 mg/L. Research carried out in the vegetation house showed that by applying a bacterial inoculum consisting of three strains belonging to the genus *Pseudomonas* and two strains belonging to the genus *Arthrobacter*, the content of PHCs in the soil was significantly reduced, their level decreasing from 3% to 1.99% (33%) in only 26 days. The application of bacterial biopreparations has been appreciated as being the main technological link in the bioremediation of hydrocarbon-polluted soil (Hemala et al. 2014). In order to streamline the action of this link, the biopreparations were made using 19 bacterial strains isolated from the polluted soil subjected to decontamination, as well as strains composed of bacterial species isolated from various polluted sites with crude oil, subjected to selection and laboratory tested for biodegradability of crude oil.

Bacterial polyculture mainly contained representatives of the genera: *Pseudomonas* sp., *Escherichia*, *Micrococcus*, *Bacillus megaterium*, *Bacillus subtilis*, *Arthrobacter* sp., and *Streptomyces*. The inoculum was used to stimulate the biodegradation process by composting and applying it on the oil-contaminated farmland after the oil shell was removed. After 7 months, the rate of disappearance of the pollutant was 17.3% for the untreated control, 36.5% for the treatments with bacterial biopreparations, and 42.3% for the application of microbial biopreparations with $N_{200}P_{100}K_{100}$. In the variants treated with the bacterial inoculum, mineral fertilizers with NPK, and superabsorbent polymers, the disappearance rate of the pollutant was 71.2% (Varma et al. 2017). Contamination of fresh oil reduced the number of actinomycetes, fungi, and MOs that break down cellulose and increased the number of bacteria that are isolated on pepton and starch. Between the quantity of hydrocarbons and bacteria in the genetic horizons of the soil there is a close regression relation. It is often the case that bioaugmentation (inoculation with MOs) of contaminated soils to be applied during bioremediation strategies in an attempt to improve both the rate of contaminant degradation and the final concentrations (Ugochukwu et al. 2013). Both the addition of single strains and of the consortium of MOs in contaminated soils usually produce low added values in terms of degradative ability compared to indigenous soil MOs, which are often only limitations in the degradative capacities of the contaminant due to the suboptimal soil condition (Table 9.1).

The promotion of indigenous MOs is therefore the first target and will lead to the optimization and improvement of both biotic and abiotic conditions; what the rams can improve (Kim and Lee 2012). If the soil is polluted with both PHCs residues and salt water, the bioremediation measures are combined with appropriate desalination measures, i.e., arrangements for washing the salts on the soil profile and capturing the washing water in a drainage system to be purified prior to discharge into the outlet. The optimal environmental conditions required for oil degradation are: soil pH 6.5–8.0, humidity 30–90%, oxygen content 10–40%, temperature 20–30 °C, and nutrient ratio C:N: P = 100: 10: 1. Soils treated with *Acinetobacter* sp. A–3 allowed better germination and growth of bean seedlings, showed a better plant length, weight, and chlorophyll content in leaves. This shows that *Acinetobacter* sp. A–3 was capable of reducing the phytotoxicity of oil by biodegradation. It has been shown that many researchers have put complete mineralization of PHCs on the account of a consortium of MOs rather than a strain.

To assume that only a strain of bacteria is responsible for decomposition would mean ignoring biochemical relationships that are well established in microbiology. Studies have shown cooperation between two strains that were able to degrade pollutants. Other researchers have found that a mixture of bacterial cultures is responsible for the processes of decomposition. It was concluded that a consortium of bacteria is responsible for pollutant degradation. The complete degradation of some contaminants is due to the aerobic and anaerobic microbial communities. In part, this is because soil and groundwater are contaminated with a mixture of compounds more often than with pure substances (Mujumdar et al. 2019). The efficacy of *Bacillus subtilis* DM4 and *Pseudomonas aeruginosa* M and NM strains isolated from oil-contaminated soil samples from North-East India for the bioremediation of fresh oil in soil and stirring vials was compared. These bacterial strains can use fresh PHCs as their sole source of carbon and energy. Bioaugmentation of the PHCs-contaminated pilot with *P. aeruginosa* M and NM in consortium with

Table 9.1 Limitations of aerobic bioremediation and the main effects of the debris that minimize these limitations

No.	Limitations of bioremediation	Creating displacement/shelter channels	Ingestion, digestion, and excretion	Coprolite
1.	Lack of oxygen, anaerobic conditions	*		
2.	Soil heterogeneity	*	*	
3.	Compact soil	*	*	
4.	Improper ratio C:N		*	*
5.	Insufficiency of accessible nutrients		*	*
6.	Reduced accessibility of related residues		*	
7.	Presence, number, and activity of degrading MOs	*	*	*
8.	Contaminant concentration too low to induce catabolic ability			
9.	Toxic levels of contaminants, or presence of contaminants that restrict the induction of catabolic ability			
10.	Temperature			
11.	pH			*
12.	Improper humidity conditions	*		

* Effects

B. *subtilis* strains significantly reduced PHCs levels in treated soil compared to untreated control soil at the end of 120 days of experience. *P. aeruginosa* strains were more effective than strains of *B subtilis* in reducing PHCs content in the environment. The strains of *B. subtilis* DM-04 and *P. aeruginosa* M and NM can be effective in in situ bioremediation. Mixed microbial cultures have degraded Louisiana crude oil both in closed vials and in large basins with seawater.

In field simulation studies, the application of a mixed microbial culture to an oil film produced a measurable decrease in the thickness and quantity of the film on the water surface. The initial rate of removal of oil by MOs was at least twice the rate of evaporation. The highest oil removal rates occurred during the initial period of growth (Wang et al. 2019). This has been attributed to the use of n-paraffins less than C15. The number of cells indicated that sown cells preferentially remain at the oil–water interface during the entire experimental period. Studies in closed vials have shown a clear metabolic preference for the use of hydrocarbons by microbial cultures for saturated paraffins. Oxidation rates of saturated paraffin were directly dependent on incubation temperature. The highest oxidation rates of saturated paraffin appeared during the initial growth periods when n-paraffins lower than C18 were used. The range of use of n-paraffins was between C12 and C30. Aromatic and naphthenic compounds do not appear to be metabolized. Within bioremediation, it is often necessary to improve and maintain moisture, oxygen, and nutrient levels and to ensure their homogeneous distribution over time, especially if it is necessary for bioremediation to be applied to the soil depth, if the soil is compacted, or rich in clay.

Although they are time consuming, intensive labor and expensive methods can be used to help the optimize these variables, there may be cheap inputs to solve this problem (Al-Dhabaan 2019). The earthworms move through the soil, where they make shelters (shelters being species-specific) acting as entry points to, and preferential pathways for, the movement of water and particles and the flow of nutrients and aeration. In addition, the occurrence of coprolites and thus of ingested and digested soil leading to a positive effect on the soil environment, as the mechanical fragmentation of soil particles (Sun et al. 2019) the forces applied to soil particles as they cross the gut remodel and reorganized them. For example, it was noted that the earthworms (*Eisenia fetida*) reduced by digestion the particles of organic matter larger than 2000 μm with values between 97% and 27% (200–2000 μm), while it was observed that the earthworms (*Millsinia anomala*) divided the organic matter from the soil causing a reduction in size by 25–30%. Such research underscores the major role of earthworms in the fragmentation of soil litter in aggregates with smaller sizes. It has been hypothesized that there is potential for earthworms to contribute to the disposal of organic contaminants associated with the soil. In addition, such activities have been shown to increase the size of the soil organic matter surface, which will have the role of exposing the fractionated material to interaction with MOs, leading to an increase in the potential for contaminant degradation.

Fractions of organic matter processed by digestion and excretion have positive effects on soil structure and soil environment. These include increasing soil porosity, increasing oxygenation, water retention, improving soil fertility by accelerating the decomposition of organic matter, and improving nutrient accessibility (Zhou et al. 2019).

Although the process is dependent on the species of earthworms, it is known that earthworms interact with soil MOs (fungi, bacteria, and actinomycetes) in three broad spatial directions (lining the shelters, coprolites, and gut or bowels). For example, mucus and coprolites deposited on the walls of shelters and other sources of organic carbon transferred through shelter systems promote the growth and distribution of MOs in shelters, while mucus, urine, and glucose can lead to microbial biomass growth. This is relevant, because the increase in microbial biomass is related to the increase of microbial catabolic activity, related to the potential increase of bioaccessibility due to the action of the earthworms on the soil through gut (Martinkosky et al. 2017) which may increase the potential for loss of microbial compounds through mineralization.

The higher number of MOs, the diversity and the activity are also known to be related to the passage of MOs through the intestines. The earthworms intestines, as well as the promotion and the stimulation of the dormant flora from the intestines, however, are in very close relation with the passage time through the intestine (Ekperusi and Aigbodion 2015). Importantly, the growth of the microflora is associated with the intestine, which is thus excreted into the environment by the coprolites of the earthworms and by the microbiological adhesion to the skin of the earthworms, while the mechanisms of transit and dispersion are associated with the flow of water which also helps in the subsequent dissipation of MOs. Such actions are clearly relevant to any phytoremediation methodology that wants the spatial incorporation of MOs capable of degrading organic contaminants.

9.3 Application of Fertilizers

Ameliorative fertilization is the action of restoration and appreciable growth of the fertility of the soils that have lost this property due to various causes. It aims, first of all, to raise to an optimum level, from an agrochemical point of view, and in a limited period of time, the content in nutrients and implicitly of the ratio C:N. Mineral fertilization is absolutely necessary in the case of oil pollution, due to the insufficient nitrogen in the soil found in abnormal relationships with the organic carbon brought by hydrocarbons (C:N ratio too high).

It is desirable to apply both nitrogen and phosphorus so that the C/N/P ratio of 300/10/1–100/10/1 is achieved, but without applying in doses greater values than 200 kg/ha N to avoid unnecessary wasting loss and toxicity to MOs. To achieve and maintain the balance of nutrients in the soil, to create conditions for rapid multiplication of MOs, doses of 200 kg/ha N, 100 kg/ha P and 100 kg/ha K were applied, and the results of the bioremediation process were very good on the chernozem. In order to carry out the physicochemical processes involved in oil degradation and stimulate the activity of MOs in good conditions, some researchers recommend: amending the soil with calcium carbonate to bring the pH to values above 6.5; annual application of mineral fertilizers all over the area, in the dose of $N_{200}P_{100}K_{100}$; plow at 15–30 cm depending on the thickness of soil horizon, for breaking the oil film and diluting the pollutant in a larger mass of soil; the annual organic fertilization with 150 t/ha of semi-fermented manure (Chen et al. 2019), by uniform application throughout the polluted surface and its immediate incorporation into the soil.

9.3.1 Application of Bacterial Inoculum

Organic fertilization of hydrocarbon-polluted soils is essential in the bioremediation process. The role of organic fertilizer is multiple:

- It has a high capacity of cation exchange and adsorption, which makes it absorb (fix) a large quantity of crude oil, preventing percolation into the depth and being able to break any oil film installed on the surface of the soil after pollution.
- It is a source of nutrients for plants and soil MOs.
- It is a source of various MOs, which can be involved in oil degradation and stimulate soil biological activity.
- It increases the resistance of plants to the content of salts in the soil.
- It allows better aeration of the soil, so it stimulates the decomposition process, which is more intense under aerobic conditions.
- It improves soil aerohydric regime and all physicochemical parameters.

Contaminated land has priority at organic fertilization. In vegetation vessel experiments, it was concluded that a concentration of 220 N, 110 P, and 110 K mg/kg soil is suitable for growing vetiver (*Vetiveria zizanioides* (L.) Nash) on soil contaminated with 5% PHCs.

In biostimulation methods, additional nutrients for oil hydrocarbon degradation were usually directed to the addition of nitrogen and phosphorus, or other organic or mineral fertilizers. Because carbon is a major constituent of oil, its traditional role in bioremediation research has typically watched as an index to determine the amount of N and P needed to be added to reach the optimal C:N:P ratio. More recently, the role of carbon supplementation in biodegradation of contaminants has been investigated through the use of molasses from sugar cane, surfactants, glucose, urban sludge, or composting (Nwankwegu et al. 2016).

9.3.2 Species of Microorganisms Identified in the Degradation of Certain Hydrocarbons

Bioremediation uses live MOs, primary MOs, to degrade contaminants from the environment into less toxic forms. MOs may be native to the contaminated area or may be isolated elsewhere and brought to the contaminated area. For bioremediation to be effective, MOs must enzymatically attack pollutants and convert them to less toxic products. This process often involves manipulating environmental parameters to allow microbial growth so that degradation occurs at a higher rate. The *Achromobacter* genus has been successfully used in the degradation of carbazole (Farajzadeh and Karbalaei Heidari 2012) and phenanthrene degradation (*Achromobacter xylosoxidans*) (Hou et al. 2018). Within the genus *Bacillus*, the following species are noted: *Bacillus firmus* – strain APIS272 is capable of completely degrading acenaphthylene, anthracene, and benzofluoranthene and reducing concentrations of naphthalene, dibenzoanthracene, and indenopyrene. *Bacillus licheniformis* – strain APIS473 is capable of completely degrading only anthracene, while the rest of the hydrocarbons only reduces the concentration. *Bacillus subtilis* – strain SBS526 completely degrades acenaphthene, anthracene, and benzofluoranthene and reduces the concentration of naphthalene, indenopyrene, and toluene. *Bacillus pumilus* has been shown to be the most efficient in the process of biodegradation of hydrocarbons, with a degradation of 86.94%. The genus *Brevibacterium* isolated from Nigerian soils degraded 40% of the hydrocarbons in 12 days. *Mycobacterium* species that have the ability to biodegrade hydrocarbons are *M. lacticola, M. luteum, M. phlei, M. rubrum*, and the last three species listed degrade gasoline, petroleum, and paraffin (Cheung and Kinkle 2001). Within the genus *Pseudomonas* we notice: *Pseudomonas alkaligenes* – strain DAFS311 which completely degrades naphthalene, benzofluoranthene, and indenopyrene and reduces the concentrations of anthracene, benzoanthracene, and benzoperylene, and *Pseudomonas putida*, which has the ability to degrade organic solvents such as toluene or naphthalene.

In the case of fungi, results were obtained regarding the biodegradation capacity of hydrocarbons, some strains of the genera *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Fusarium*. We were able to identify the genus *Penicillium*, which had the ability to degrade 90% phenanthrene and 75% acenaphthylene and fluoren (Kozlovsky et al. 2020).

Studies have shown the ability of *Cladosporium resinae* species to degrade aliphatic hydrocarbons. *Rhizopus* genus includes filamentous fungi, found in soil, fruits, vegetables, and old bread. The genus has been isolated and studied from the point of view of the biodegradation capacity of hydrocarbons, obtaining satisfactory results (Balaji et al. 2014). Biodegradation occurs in several stages and is not the result of a single specific organism; usually it acts synergistically with several strains of MOs. In this regard, the synergistic phenomenon between *Nocardia* and *Pseudomonas* is described, which in combination are capable of degrading cyclohexane: *Nocardia* by the use of cyclohexane produces intermediate compounds, which are taken over by *Pseudomonas*. *Pseudomonas* produces the growth factors (mainly biotin) needed for the growth of *Nocardia* bacteria. Research was carried out on the development of a consortium of bacteria and fungi.

From the experiments it has been observed that *Penicillium* and *Rhodococcus* have been shown to be very effective in degrading polycyclic aromatic hydrocarbons, while *Rhodococcus* with *Aspergillus terreus* did not have synergistic relationships (Yi et al. 2011).

Bioremediation has been defined as "the activity of adding materials to the contaminated environment to produce an acceleration of the natural biodegradation process." This technology is based on the premise that a high percentage of the oil components is readily biodegradable in nature. The success of the bioremediation depends on the ability to establish and maintain the conditions that favor the increase of speed of biodegradation of crude oil in the contaminated environment. Biodegradation is a particularly important mechanism for removing nonvolatile components of crude oil from the environment. This is a relatively slow process and may take months or years for MOs to degrade a significant fraction of the oil spilled in soil. Factors to be considered when deciding on the type of soil remediation include: the type of oil; the total concentration of PHCs in the soil; salinity; the existence of a microbial population capable of degrading pollutants; soil type; the age of pollution; pH; temperature; humidity; nutrients; the presence of oxygen; microelements (Akbari and Ghoshal 2015).

Organo-mineral fertilizers, having in their composition natural organic and/or synthetic polymers associated with different mineral salts, which besides providing the deficient elements in the nutrition of plants also have qualities of improvement of some properties of the soil, are relatively new products used in agriculture and remediation of degraded soils. They were created and developed, in particular, as a result of the need for improvement of sandy, luvic, degraded soils, as well as of other soils with low humus content, under the intensive development of agriculture, as well as for the improvement of soils contaminated with products, organic, or heavy metals. There are numerous references in the recent specialized literature on the effects of humic substances, as well as their salts, extracted from different natural sources (lignite, leonardite, oxidized lower carbons, peat, compost, lignosulfonates, polysaccharides, etc.) on plant growth and development as well as modification by their application of the bioavailability of the nutrients in the soil (Sui et al. 2014).

There are numerous studies that describe the positive relationships between the content of humic substances in soil or applied extraradicular and the yield and quality of agricultural production, as follows:

– Plant metabolism is improved due to the high availability of various minerals present in humic molecules. When suitable humic substances are present in the soil, the need for NPK fertilizer to be applied is reduced. The application of liquid or solid humic products to the soil dramatically improves the efficiency of classical fertilizers and plant metabolism. Also, foliar application of mixtures of liquid humic fertilizers reduces the need for fertilization doses.
– Humic and fulvic acids stimulate the growth and development of plant roots. In the controlled experiments in which these acids were applied, an increase of the roots by 20–50% was observed compared with the roots of the untreated plants.
– Foliar fertilizers containing humic and fulvic acids intensify the metabolism of the plant immediately after application. By this the photosynthesis in the leaves intensifies and the synthesized sugars are quickly transported to the root and released into its rhizosphere. These substances eliminated by the root provide the nutrients to the MOs in the soil that live on its surface. Active MOs synthesize the substances needed for the plant in the growth process.
– Humic and fulvic acids have a direct effect on the plant cell membrane. Thus, humic acid molecules influence the permeability of the cell membrane resulting in the intensification of electronic transport and the exchange of minerals required in specific metabolic processes.
– Humic substances increase the production of adenosine triphosphate (ATP) in plant cells.
– Humic and fulvic acids play the role of growth hormones by inhibiting the Indole-3-Acetic Acid (IAA) oxidase enzyme.
– Carbon-containing compounds from humic substances are an important source of energy and mineral elements for soil organisms that ensure microbiological activity (algae, ferments, bacteria, fungi, nematodes, mycorrizae, actinomycetes, protozoa, and small animals).
– Humus has the role of improving the soil's ability to retain water. Humic and fulvic acids present in the soil bind to the clay molecules and form stable organic-clay complexes that retain an amount of water of approx. 4–7 times their weight. This water retained in the soil can be released to the roots of plants during periods of drought. At the same time, the rate of evaporation of water and soil temperature is stabilized by humic substances.
– Humic substances buffer the pH of the soil and are key components of a friable (noncompact) soil structure, which allows gas to enter and infiltrate water (Koshlaf and Ball 2017).

Among the current techniques for remediation of contaminated soils, the techniques in which the treatment is performed locally, without excavation, either by extracting the pollutant or by degrading or fixing it in the soil should be highlighted, these being known as in situ techniques. This group also includes electroremediation or electrokinetic remediation. Soil electrokinetic remediation is a developing technology, consisting of the movement and entrainment of contaminants, mainly by electromigration of ionic species and by electroosmosis. It is a relatively safe method and easy to implement, which can be applied to different types of soils and a wide variety of pollutants that are organic, inorganic, or with a mixed composition. Despite the proven efficacy of this technique, there are some limitations in particular due to PHCs properties. These compounds are hydrophobic, have low water solubility, and are resistant to degradation. On the other hand, advanced oxidation processes, which are based on the reactivity of species with high oxidation potential, appear as an efficient alternative for degradation of such pollutants. Of these, the Fenton process is a very interesting option, as we can deduct from the large number of manuscripts dealing with this topic. However, there are some limitations that need to be addressed. One of the main problems of the homogeneous electro-Fenton process is the generation of sludge, rich in iron, at the end of the process, and the need to complete this reagent throughout the remediation process, which can be an environmental problem and may entail additional costs. However, as has already been investigated and demonstrated in a number of studies, this problem can be avoided by using heterogeneous catalysts (Huguenot et al. 2015). Electrochemical oxidation of organic compounds during electro-Fenton treatment is strongly influenced by the nature of the electrode material. Many researchers have already investigated the effect and properties of different types of anodes. Since the generation of the main oxidant (H_2O_2) occurs by reducing the oxygen at the cathode, it is important to evaluate the electrode materials that favor this reaction. In addition, another major drawback of the electro-Fenton process is the high energy consumption. Thus, in recent years, by combining different technologies, the energy consumption has been reduced and an efficient alternative has been ensured, minimizing or even eliminating some disadvantages of the individually applied remediation processes, by coupling them (Ouriache et al. 2019). Although the electro-Fenton oxidation process has been considered one of the most efficient in eliminating different pollutants, given that it is not always possible to apply this treatment and considering that it may be more expensive compared to other technologies, it is necessary an in-depth study aimed at improving the process and identifying those technologies that can lead to an increase in the efficiency of the decontamination process, which are economically feasible and do not present the risk of secondary pollution.

9.4 Technological Recommendations

Digging holes for the rapid collection of PHCs spread on the ground and transporting them to a treatment and recovery station. Application of 16 t/ha peat to absorb the remaining oil and limit its penetration into the soil, to aerate the soil and to stimulate the development of hydrocarbon degrading MOs. Collecting the peat soaked with oil and transmitting it to a treatment station.

Loosening by plowing the soil to a depth of 25 cm to ensure aeration of the soil. If the quantity of oil is large, the work will be repeated every 2–3 months. The following ventilation work can be done using the harrows. If the soil is acidic or basic, it is recommended to apply amendments and incorporate them into the soil. Scarification of heavily soiled soils, with low aeration porosity, in order to improve aerohydrous conditions in the soil. The scarification will be carried out at a depth of 60 cm, and the distance between the active organs will be 1 m.

The optimum time for execution is when the soil has a humidity of 60–80% of the active humidity range. The work is executed perpendicular to the drying channel or the natural emitter. On certain polluted soils and with salt water, it is also recommended to perform mole drainage. Absorber is applied at a dose of 32 kg/ha to keep water in the soil and thus stimulate the multiplication of degrading MOs. Microbial polyculture selected from the fields contaminated with hydrocarbons is applied. Bacterial polyculture mainly contained representatives of the genera: *Pseudomonas* sp., *Escherichia*, *Micrococcus*, *Bacillus megaterium*, *Bacillus subtilis*, *Arthrobacter* sp., and *Streptomyces*, which have been shown to be involved in the breakdown of hydrocarbons. Fertilization is done with 150 t/ha composted fermented manure. It will be applied as homogeneously as possible on the polluted land.

Garbage ensures not only better aeration of the soil, but also a great diversity of MOs participating in the bioremediation process, breaks the oil film, retains the hydrocarbons on the surface of the soil where the area is more aerated, and most are lost through volatilization, which assures a very large range of macro- and micronutrients accessible to plants, stimulates water retention in the soil, soil structuring, and improves all soil physical parameters. It is fertilized with mineral fertilizers. Very good results were obtained by applying $N_{200}P_{200}K_{200}$. But the recommendations differ with the concrete characteristics of the soil. Very good results provided the liquid fertilizers based on humic acid at a dose of 650 l/ha. Of these, the fertilizer (potassium humate in an NPK matrix with microelements and 8% monosaccharides or 100 g/l glucose applied at a dose of 650 l/ha + 64 kg/ha glucose) against the application of absorber in the dose of 32 kg/ha, in which the PHCs level decreased by 58% 60 days after oil pollution, is considered to be a favorable alternative for bioremediation. The land could be cultivated with maize, *Lolium perenne*, sunflower, alfalfa, barley, oats, *Lolium multiflorum*, mixtures of grasses and legumes, etc.

The analysis of the degradation of the main compounds identified can lead to a better knowledge of the degradation of the different types of hydrocarbons. Stabilizing the pH value (buffer capacity) associated with the addition of a complexing agent has been shown to be necessary in situ treatment, so as to facilitate the availability of metals, avoid precipitation in the soil, and favor the transport of hydrogen peroxide agents. The EK–Fenton process was successfully coupled with selected surfactants and used to achieve in situ decontamination of historically polluted soils.

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