Chapter 17

Approaches for Removal of PAHs in Soils: Bioaugmentation, Biostimulation and Bioattenuation

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Abstract

Polycyclic aromatic hydrocarbons (PAHs)-contaminated soils have been a concern during last decades; consequently, physicochemical and biological technologies have emerged and evolved with the aim of remediating them. Particularly, biological technologies are considered promising since they are low cost, safe and environmentally friendly. However, their results so far have been diverse and scattered. This chapter includes a review of the current status on bioaugmentation, biostimulation and bioattenuation techniques, which have been applied in PAHs-contaminated agricultural soils during the last decades. Successes and failures in PAHs remediation applied at microcosm and field levels are exhibited. Furthermore, the effects of microbial inoculum, the soil organic matter and the particle size of the aggregates on the PAHs' availability and on the subsequent microbial biodegradation are reviewed. Finally, agricultural management systems are considered in the prediction of the behaviour and the end-point of some contaminants, as well as in the success of applying a biological technique.

Keywords: bioattenuation, biostimulation, bioaugmentation, PAHs, soil

1. Introduction

Oil-based fuels are currently the major source of energy for industry and daily life. However, leaching and spills that occur during exploration, production, refining, transport and storage cause pollution problems. Polycyclic aromatic hydrocarbons (PAHs) are organic molecules that have two or more fused aromatic rings arranged in a linear, angular or cluster array. PAHs are found ubiquitously in the environment and are present in oil-based fuels. Currently, PAHs had become increasingly important because they are considered as emerging contaminants by their high risk to humans and the environment. According to the US Environmental Protection Agency (EPA), they are toxic, mutagenic and carcinogenic, and are priority to be eliminated from the environment. There are several biological alternatives to eliminate PAHs and this chapter focuses on developments in bioaugmentation, biostimulation and bioattenuation for the removal of PAHs.

2. Remediation and biodegradation technologies

Methods to remove PAHs have been classified as physicochemical, chemical and biological, and are briefly described in **Table 1**. Among biological techniques, bioremediation is considered a viable technology, environmental friendly and inexpensive that uses the metabolic diversity of some microorganisms to degrade and decrease the concentration of toxic compounds.

Technology	Purpos
Solvent extraction	Treatment with two or more solvents, either alone or within mixtures to extract PAHs.
Chemical oxidation	Different types of oxidants such as Fenton's reagent, ozone, potassium permanganate, hydrogen peroxide are used to oxidize PAHs.
Photocatalist degradation	Photocatalytic oxidation-reaction is used to destroy PAHs in presence of UV light.
Electrokinetic remediation	It is applied mainly to treat soils with low permeability and contaminated with heavy metals. In addition, co-contaminated soils with organic pollutants can also be treated.
Thermal technology	PAHs can be either destroyed or volatilized by the use of high temperatures.
Phytoremediation	Plants are commonly used to extract and sequester heavy metals from contaminated soil. However, PAHs removal can also occur through synergistic interaction in the rhizosphere (plant and microorganism).
Biological remediation	Mineralization or biotransformation of toxic organic compounds either by specialized microorganisms or by their enzymes.

Table 1. Technologies suitable to remove PAHs from contaminated soils.

Biological removal or (biodegradation) is a process carried out by aerobic organisms mainly indigenous microorganisms and commonly it reaches the mineralization of toxic compounds to inorganic forms (CO_2 and H_2O). However, anaerobically PAHs biodegradation under denitrifying and sulphate-reducing conditions has been well recognized [1]. The aerobic biodegradation mechanism of PAHs begins with the initial oxidation step, either where two atoms of oxygen are incorporated into the aromatic ring to form *cis*-dihydrodiol or where monooxygenases enzymes are involved in the first initial oxidation to form *trans*-dihydrodiols. Otherwise, bioremediation can be conducted in two ways: (1) ex situ that is held off the contaminated site and requires excavation and site conditioning, and (2) in situ where the soil decontamination is performed without removing it from the area [2].

Three technological processes are well recognized for in situ bioremediation: (1) bioattenuation, which depends on the natural degradation processes to dissipate contaminants through biotransformation; (2) biostimulation, involving the addition of nutrients, water, electron donors or acceptors that stimulates microbial growth; and (3) bioaugmentation, which requires the inoculation of indigenous, allochthonous or genetically modified microorganisms with specific capabilities to degrade or biotransform the contaminant of concern. Bioaugmentation can follow two strategies: (1) isolation of microorganisms able to remove the contaminants from contaminated soils, culturing them in the laboratory and returning them to the original site (reinoculation of indigenous bacteria), or (2) inoculation of microorganisms obtained from different contaminated sites with proven abilities to degrade the contaminants of concern [2].

3. Bioaugmentation, biostimulation or bioattenuation on PAHs removal

In the past decades, prominent microorganisms have been obtained and isolated, as consortia or individual strains, able to grow using aromatic compounds as the only carbon and energy source. These microorganisms have been used for PAHs' degradation in soil by bioaugmentation, as it is mentioned in the following section.

3.1. Bioattenuation

It relies on natural processes to dissipate contaminants through biological transformation, during which the indigenous microbial populations degrade recalcitrants or xenobiotics compounds based on their metabolic processes. Bioattenuation includes a variety of chemical, physical and biological processes that reduce the mass, toxicity, volume or concentration of contaminants. These processes include aerobic and anaerobic biodegradation, sorption, volatilization, and chemical or biological stabilization, transformation of contaminants. The time is not a limiting factor and usually is applied on sites with low concentration of contaminants, where no other remedial techniques are applicable.

In order to reveal that bioattenuation occurs in remote areas consistently and continuously, deep-sea sediments of Artic Ocean were collected in the summer of 2010; the PAHs compositions were examined and the 16 EPA-priority PAHs were from 2.0 to 41.6 ng g^{-1} dry weight, among them, phenanthrene was relatively abundant in all sediments. The 16S rRNA gene of the total environmental DNA revealed potential degraders. Meanwhile, 40 PAH-degrading bacteria were isolated though enrichment culture, of which *Cycloclasticus* and *Pseudomonas* showed the best degradation capability under low temperatures. Based on the 16S rDNA library and isolation of strains, the author suggested that bacteria of *Cycloclasticus, Pseudomonas, Pseudoalteromonas, Halomonas, Marinomonas* and *Dietzia* play the most important role in PAH mineralization in situ [3].

In terrestrial environments, where the biodegradation of a mixture of PAHs (fluorene, phenanthrene and pyrene) in mangrove sediments chronically exposed to industrial discharge, livestock and household waste and wastewater was revealed, the bioattenuation favoured the removal of fluorene and phenanthrene up to 99% while pyrene removal (98%) was only improved by adding salt medium as a nutrient supplement [4]. Besides, the bioattenuation was effective in the removal of total petroleum hydrocarbons (TPHs) and high molecular weight PAH residuals after applying a pilot-scale biopile remediation treatment, by properly enhancing their catabolic capacities with the addition of lignocellulosic substrate as a biostimulant [5].

3.2. Biostimulation

Biostimulation is the addition of nutrients to a contaminated site in order to encourage the growth of naturally occurring chemical-degrading microorganisms. Generally, inorganic additions of macro (as N, P, K) or micronutrients (as Mg, S, Fe, Cl, Zn, Mn, Cu, Na) are important to recover depleted soils by agricultural management systems or contaminated with PAHs, in order to improve the degradation activity of native or foreign microorganisms. Thus, the type and concentration of nutrient can play an important role in biodegradation of PAHs. Particularly, the effect of biostimulation on phenanthrene removal from contaminated soil via adding macro and/or micronutrients revealed that the optimal phenanthrene reduction resulted when a high level of macronutrient in the range of 67–87% and low level of micronutrient in the range of 12–32% were used with the nitrogen as the dominant macronutrient [6]. Other strategies had been implemented by the use of stable organic supplements such as compost, sewage sludge, manure, vermicompost, etc., as biostimulant nutrients to activate the catabolic potentials of microorganisms. The success of applying stable organic residuals may be a promissory technology due to the high content of essential nutrients and the harbouring of large quantities of diverse microorganisms that accelerates the biodegradation of some contaminants in soil. The biostimulation with compost achieves an improved removal of PAHs in an artificially contaminated agricultural soil [7]. The dissipation of phenanthrene, anthracene and benzo(a)pyrene in a spiked agricultural soil amended with manure and vermicompost resulted in a transient effect in the removal of PAHs during the first 30 days [8]. Furthermore, it was observed that the inorganic nutrients or biosolid amendment have a similar effect on the degradation of phenanthrene and anthracene in an artificially contaminated agricultural soil. Polyacrylamide, a flocculant used in wastewater treatment, was added in two different artificially contaminated soils, and the concentrations of phenanthrene and anthracene were removed rapidly in both soils (agricultural soil and alkaline-saline soil) [9].

3.3. Bioaugmentation

It is defined as a technique for improvement of the removal capacity of contaminated areas by the introduction of specific competent strains or consortia of microorganisms to the contaminated site, thus favouring the biodegradation process. In this way, a bacterial mixed culture was added to a PAHs (pyrene and benzo[a]pyrene)-contaminated soil, and after the treatment, the mineralization rate of pyrene was about 36% (after 150 days), and benzo[a]pyrene 5% (after 70 days) [10]. Similar results were observed with *Scopulariopsis brevicaulis* PZ-4 that was able to remove phenanthrene (60%), fluoranthene (62%), pyrene (64%) and benzo[a]pyrene (82%)

in liquid medium after 30 days of incubation; while, in a PAH-contaminated soil, PZ-4 removed 77% of total PAHs and the highest removal of PAHs occurred for phenanthrene (89%) and benzo[a]pyrene (75%) after incubation for 28 days [11]. On the other hand, organic pollutant-contaminated soils are often co-contaminated with heavy metals, and the success of applying a bioaugmentation treatment has been tested by some authors; for example, a bacterial consortium composed by 12 indigenous strains with different catabolic capacities (resistant to heavy metals, producer of surfactants and degraders of hydrocarbons) was added in a soil spiked with diesel oil and heavy metals (Pb and Zn) obtaining the total removal of diesel oil [12]. Consequently, the authors concluded that the entire indigenous community was pushed towards an effective bioremediation by the addition of the microbial consortium.

3.4. Combinations and improvements in biodegradation techniques

Some reports showed that the addition of microorganisms (bioaugmentation) or nutrients (biostimulation) either individually or combined have negligible effects on the removal of PAHs at field or microcosm level. In this manner, the effect of applying bacterial or fungus consortium to artificially contaminated forest soil with a mixture of PAHs reported that bioaugmentation did not improve the removal of naphthalene, phenanthrene, anthracene and pyrene as compared to bioattenuation [13]. However, successful approaches were achieved when nutrients and microorganisms were added simultaneously [14] or successively during the treatment [15]. Therefore, some modifications have been made in bioremediation techniques to improve the removal efficiency of PAHs. A strategy is the use of carriers and the results obtained are promising. Biocarriers have particular characteristics that allow microbial survival by providing a temporary nutrition medium and a protective niche. Immobilization of cells also avoids protozoan grazing and promotes a slow release of cells from the biocarriers, prolonging their degrading activity. Encapsulated *Pseudomonas aeruginosa* strains effectively removed PAHs only in the soil bioaugmented with nutrients, moisture and oxygen supplies [16]. Another modification to bioremediation technique is the dose of the inoculum. It has been seen that the use of several doses of the inoculum improves the removal of contaminants in comparison with a single dose. Thus, the inoculation of two doses in different times of a specialized bacterial consortium, able to degrade alkanes and PAHs, improved the overall removal of TPHs above 30% [15]. The repeated inoculation of *Arthrobacter* sp. to an artificially contaminated soil improved the removal of phenanthrene as compared to one dosage [17].

The addition of compounds with similar characteristics to the contaminants can stimulate indigenous microorganisms of the soils suggested that the ability of indigenous microorganisms to remove a particular contaminant could be enhanced by the presence of other contaminants or by the repeated exposure to the contaminant of concern, which favours the selection of specific microorganisms with desired specific metabolic capabilities. Additionally, the effect of adding various types of chlorophenols at different concentrations on the indigenous population from a calcareous agricultural soil without a previous history of exposure to such contaminants helped microorganisms to survive and stay alive during the treatment even in the presence of a more toxic compound [18]. On the other hand, knowledge of the physicochemical properties of soil is important to establish and design the best strategy bioremediation. The response of indigenous microorganisms in an artificially contaminated agricultural soil was studied, and it was faster during the removal of phenanthrene than fluoranthene. This difference was attributed to the physicochemical properties of both contaminants and the specific metabolic capacity showed by the microorganisms at the onset of the experiment [19]. PAHs-contaminated soil has a negative impact on the stability of an ecosystem, therefore the physicochemical properties of a contaminated soil and its associated microbial community should be considered to ensure the success of bioremediation. The knowledge of these parameters will avoid conflicting reactions between the different techniques of bioremediation. Therefore, it is necessary to conduct assays of the combinations of techniques at laboratory level to determine the synergistic effects and to achieve improvements in the PAHs degradation in the soil.

4. Limiting factors for a successful biological remediation

Bioremediation is influenced by abiotic factors such as temperature, humidity, pH, aeration, nutrient content, redox potential and soil type; however, interaction of biotic factors such as competition, predation and biological factors also play a major role in the success of this technique [20]. Some studies have shown that the microorganisms added for degrading contaminants at laboratory level were not able to mineralize, survive or compete with the native microorganisms when they were introduced into foreign environments, probably due to susceptibility to toxins or predators in the environment, due to the preferential use of easily assimilated organic compounds or due to slow motion throughout the inner porous soil that harbours the contaminant [21]. To facilitate the adaptation of microorganisms added to a soil, the following criteria must be considered: contaminant-availability for microorganisms; microbial activity; survival of microorganisms in the foreign environment; and environmental conditions such as nutrient availability, water content and pore size of the aggregates [20]. On the other hand, when a population is introduced into a foreign site, it tends to decrease with time due to the abiotic and biotic factors mentioned above, and thus the treatment can be adjusted either by adding more specialized microorganisms or by using immobilized bacteria [22]. The introduction of a microorganism in an environment is complex and its permanence may be only temporarily, depending on the ability of the microorganism to adapt to environmental conditions. The strategy to isolate indigenous microorganisms and incorporate them into the environmental is a viable alternative; however, this technique does not always produce the expected results, suggesting that the above factors play an important role.

5. Organic matter content and particle size: sorption or sequestering; how could they affect the bioavailability?

Soil is composed of organic and inorganic components separated by pores containing water or air. The interactions between hydrocarbons and mineral surfaces (clay, silt and sand) are only significant when the organic matter content is <0–1%. Thus, organic matter is very important in the fate and behaviour of organic contaminants in soil. The soil organic matter can be divided into two types: soft carbon (rubbery), which is defined as expanded and flexible structures with humic and fulvic acids as component with reversible sorption, and hard carbon (glassy), defined as rigid and condensed structures with humin, kerogen and pyrogenic carbon as commonly identified components, which are involved in irreversible sequestration [23]. Therefore, the organic matter content can directly affect the bioavailability of contaminants to microorganisms by sorption or sequestration mechanisms, and thus the success of bioremediation technologies can be hindered. The effect of organic matter on the degradation of PAH was studied in [24], and it was found that microbial activity was influenced by the amount of organic matter in the soil by either nutrient limitation or PAHs sequestration. In addition, microbial activities developed in humic acid were much higher than those developed in humin (aged organic matter), demonstrating that humin is able to sequester organic contaminant in a stronger way. In another study, it was demonstrated that a high content of organic carbon in the soil produces a low degradation rate of PAHs by indigenous microorganisms [25], indicating The sequestration of PAHs by organic carbon is the major mechanism for the accumulation of PAH in soils. On the other hand, it has been proposed that humic acids promote degradation of aromatic compounds by changing pore size and the structure of the soil [26]. It has been well known that the mineral complexes also affect the bioavailability of some contaminants because they could be involved in sorption phenomena (adsorption and desorption). Different bioremediation techniques were applied to a clay soil artificially contaminated with diesel oil and the removal rate of PAH was depending on adsorption and desorption phenomena [27]. Additionally, the soil organic matter presents different sorption properties due to its biochemical contents, which include substances such as polysaccharides, lipids, lignin, proteins, humic substances, kerogen and black carbon.

The particle size of the aggregates, the shape and the interconnections amongst the pores of a soil are physical factors that determine the microbial colonization, since they have effect on air diffusion and water infiltration. The association of soil organic matter with secondary minerals, such as clay and amorphous oxides, form complex organomineral aggregates which participates in the soil structure. Furthermore, it has been observed that PAHs distribution in soil depends mainly on the hydrophobicity of the PAH and their affinity towards microcompartments of the aggregates [28]. It is known that as time goes on in a contaminated soil, the contaminants diffuse into hydrophobic areas (ageing), reducing the bioavailability to the microorganisms and thus slowing down their removal. Some authors suggest that biodegradation and removal of contaminants become difficult with ageing of soil; moreover, the rate of desorption of PAH decreases, persisting even in the presence of indigenous microorganism degraders [29]. Bioavailability of anthracene in freshly and aged spiked agricultural soil were studied by its removal efficiency. The 72% of anthracene was removed in freshly spiked soil, while only 34% was degraded in aged soil [30]. However, in experiments conducted in [31], the lack of response of microorganisms to some contaminants is not related to a limited bioavailability, but rather is related to microbial factors, such as lack of co-metabolic substrates or insufficient numbers of hydrocarbon-degrading populations. Besides, it was found that biostimulation with inorganic nutrients and terminal electron acceptors did not improve the removal of PAHs in freshly spiked soil with phenanthrene or pyrene [32]. Moreover, total biodegradation extent was evident in ageing but not in freshly spiked soil, which was considered to be the result of the adaptation of indigenous bacteria *P. aeruginosa* by entering a stationary phase during the time of ageing (200 days) and by the subsequent production of surfactants. On the other hand, it was suggested that ageing of the soil is not the main parameter influencing PAH-availability level, but the complexity of the organic constituents (i.e. coal tar, pitch, soot or coke) influence overall PAH availability in soil [33]. In addition, some bioremediation studies have evidenced the importance of the physicochemical parameters of organic contaminants on the availability to microorganisms, which have effect on the biodegradation rate [27]. Soil properties and the indigenous microbial population affect the level of biodegradation; therefore, a detailed study on soil properties such as physicochemical and biological parameters must be performed to select the bioremediation technique.

6. How does the impact of the agricultural management system have an effect on the response of microbial population to contaminants?

The different responses of indigenous microorganisms to the PAHs degradation in agricultural contaminated soils are attributed mainly to the deficiency in nitrogen and phosphorous availability. As discussed above, organic matter plays a key role in the bioavailabilty of organic contaminants; however, the organic matter in the soil is also the primary source of essential nutrients such as nitrogen, phosphorous and sulphur [34], and it is often a carbon source easier to assimilate than the contaminant. Therefore, a good understanding of soil management systems can help to infer how soil microorganisms behave when facing to a contaminant. By studying the effects of soil management systems (no till and conventional tillage with sequenced or rotation cropping) on the soil microbial community, it was found that an untilled soil and appropriate crop rotation systems favoured richness and diversity of the microbial community. Changes in microbial communities have also been observed in soils with different agricultural management systems, having a considerable impact on the biological activity of the soil [35]. Furthermore, it has been observed that variations in the microbial communities associated with soils are influenced by the type of land use and by time [36]. The leguminous crops contribute to enhance the organic matter levels resulting in small changes in bacterial populations [37]. Besides, the reducing tillage with retention of crop residues improves and preserves the diversity of bacterial communities [35]. On the other hand, soil enzymes are involved in the cycling of nutrients and they can react rapidly to make changes in soil derived from contamination or by the use of different management systems [38]. The activity of six soil enzymes (β-1-4-glucosidase, L-leucine-aminopeptidase, β-1-4-N-acetylglucosaminidase, phenol oxidase, phosphatase and peroxidase) was correlated with the chemistry of soil organic matter in sites with different broad land use (agriculture soil, pine forest, hardwood forest and pasture). They found that biological process and soil texture correlate well with the chemistry of soil organic matter, suggesting that interactions between microbial communities and soil organic matter influence the soil carbon dynamics [39]. However, soil enzymes have been used as disturbance and quality indicators of contaminated ecosystems [40]. Besides, the soil nutrient status, microbial biomass nitrogen and enzyme activities in five different land-use patterns (nature forest, park, farmland, street garden and roadside tree) were compared, and it was found that soil quality and fertility were affected by urban land-use patterns. Nutrients were scarce in urban soil and restricted the soil microbial biomass and enzyme activities (urease, protease and nitrate reductase) [38]. Soil enzymes are usually present in moderate or high levels in agricultural soils and they can be correlated to the bacterial diversity found in contaminated vs agricultural soils [41]. Dehydrogenase activity is a more sensitive parameter than urease activity to evaluate the combined toxic effect of metals and PAHs in soils, and these activities are dependent on the enzymatic concentrations [42]. However, enzymatic activity of dehydrogenase and fluorescein diacetate hydrolase has been found, by some authors, in PAH-contaminated soils, and it has been attributed to the gradual adaptation of microorganisms to contaminants and their utilization as a sole carbon and energy sources [43].

Otherwise, soils can be exposed to physical, chemical or biological degradation having an effect on the diversity of microbial communities. From the foregoing, a good agricultural management system may positively change the microbial diversity and improve the nutrient quality in soil as well as the metabolic variety of the microorganisms, leading to a favourable response in the removal of some contaminants. The response of microbial communities in an agricultural land used to grow wheat and sunflower was studied after the addition of diesel fuel. Despite the majority volatilization of aliphatic hydrocarbons, the soil microbial population was able to entirely remove the aliphatic hydrocarbons, and only 1% of the initial contaminant load in the soil remained after 400 days of monitoring. In addition, soil quality indicators (dehydrogenase activity and soil microbial biomass) decreased their values in the first 18 days; however, they recovered their original levels and then exceeded them, reaching a maximum value at the end of the study [44]. Agricultural management system impacts on the response of microbial population to contaminants by producing changes in the biological activity of the soil accelerating or delaying the biodegradation process, which should be considered as the relevant factor in the remediation at field level.

7. Perspectives and conclusions

The sorption phenomena, sequestering mechanisms, content and quality of organic matter and nutrient availability have a direct role in biodegradation success, together with the microbial metabolism and the biological interactions between the populations, which also play a major role. Many authors have reported some bioattenuation failures in contrast to biostimulation or bioaugmentation, thereby a proper creation of the environmental conditions may be sufficient to remove PAHs as discussed earlier. Therefore, the variation in biodegradation results obtained by several authors can be attributed to complex-multiplex interactions between biological inter- or intra-relationships, soil constituents, the physicochemical properties of contaminants and the environmental conditions. They may stall or diminish the biological activity given by allochthonous or indigenous microbial. A proper understanding of the selection of indigenous or allochthonous microbial consortia, agricultural management systems, the quantity and quality of nutrients and the diversity of microbial communities in the contaminated soils must be envisaged and studied in detail, in order to increase our understanding about the complex physicochemical-biological interactions between the microbial community and its environment. The addition of organic residuals combined with a specialized microbial consortium has the potential to enhance the degradation of such contaminants and may become a promising technology in the near future. However, a combined election of different bioremediation technologies may raise the costs and may become too expensive to use.

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References

[1] Lu XY, Zhang T, Fang HHP. Bacteria-mediated PAH degradation in soil and sediment. Applied Microbiology and Biotechnology. 2011;89(5):1357–1371. DOI: 10.1007/s00253-010-3072-7

[2] Fernández-Luqueño F, Valenzuela-Encinas C, Marsch R, Martínez-Suárez C, Vázquez-Núñez E, Dedooven L. Microbial communities to mitigate contamination of PAHs in soil-possibilities and challenges: a review. Environmental Science and Pollution Research. 2011;18(1):12–30. DOI: 10.1007/s11356-010-0371-6

[3] Dong C, Bai X, Sheng H, Jiao L, Zhou H, Shao Z. Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean. Biogeosciences. 2015;12(7):2163–2177. DOI: 10.5194/bg-12-2163-2015

[4] Yu KSH, Wong AHY, Yau KWY, Wong YS, Tam NFY. Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments. Marine Pollution Bulletin. 2005; 51(8-12):1071–1077. DOI: 10.1016/j.marpolbul.2005.06.006

[5] Lladó S, Covino S, Solanas AM, Viñas M, Petruccioli M, D'annibale A. Comparative assessment of bioremediation approaches to highly recalcitrant PAH degradation in a real industrial contaminated soil. Journal of Hazardous Materials. 2013;248–249:407–414. DOI: 10.1016/j.jhazmat.2013.01.020

[6] Kalantary RR, Mohseni-Bandpi A, Esrafili A, Nasseri S, Ashmagh FR, Jorfi S, Ja'fari M. Effectiveness of biostimulation through nutrient content on the bioremediation of phenanthrene contaminated soil. Journal of Environmental Health Science and Engineering. 2014;12(1):143. DOI: 10.1186/s40201-014-0143-1

[7] Sayara T, Borràs E, Caminal G, Sarrà M, Sánchez A. Bioremediation of PAHs-contaminated soil through composting: influence of bioaugmentation and biostimulation on contaminant biodegradation. International Bioderioration and Biodegradation. 2011; 65(6):859–865. DOI: 10.1016/j.ibiod.2011.05.006

[8] Álvarez-Bernal D, García-Díaz EL, Contreras-Ramos SM, Dendooven L. Dissipation of polycyclic aromatic hydrocarbons from soil added with manure or vermicompost. Chemosphere. 2006;65(9):1642–1651. DOI: 10.1016/j.chemosphere.2006.02.028

[9] Fernandez-Luqueño F, Thalasso F, Luna-Guido ML, Ceballos-Ramírez JM, Ordoñez-Ruiz IM, Dendooven L. Flocculant in wastewater affects dynamics of inorganic N and accelerates removal of phenanthrene and anthracene in soil. Journal of Environmental Management. 2009;90(8):2813–2818. DOI: 10.1016/j.jenvman.2009.03.010

[10] Derz K, Schmidt B, Schwiening S, Schuphan I. Comparison of microbial pyrene and benzo[a]pyrene mineralization in liquid medium, soil slurry, and soil. Journal of Environmental Science and Health B. 2006;41(5):471–484. DOI:10.1080/03601230600701619

[11] Mao J, Guan W. Fungal degradation of polycyclic aromatic hydrocarbons (PAHs) by *Scopulariopsis brevicaulis* and its application in bioremediation of PAH-contaminated soil. Acta Agriculturae Scandinavica Section B: Soil and Plant Science. 2016;66(5): 399–405. DOI: 10.1080/09064710.2015.1137629.

[12] Sprocati AR, Alisi C, Tasso F, Marconi P, Sciullo A, Pinto V, Chiavarini S, Ubaldi C, Cremisini C. Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. Process Biochemistry. 2011;47(11):1649–1655. DOI: 10.1016/j.procbio.2011.10.001

[13] Silva IS, Santos EC, Menezes CR, Faria AF, Franciscon E, Grossman M, Durrant LR. Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia. Bioresource Technology. 2009;100(20):4669–4675. DOI: 10.1016/j.biortech.2009.03.079

[14] Sun GD, Xu Y, Jin JH, Zhong ZP, Liu Y, Luo M, Liu ZP (2012). PAHs-contaminated soil by indigenous microorganisms and bioaugmentation by a PAHs-degrading and bioemulsifier-producing strain. Journal of Hazardous Materials. 233-234:72–78. DOI: 10.1016/j.jhazmat.2012.06.060

[15] Tahhan RA, Ammari TG, Goussous SJ, Al-Shdaifat HI. Enhancing the biodegradation of total petroleum hydrocarbons in oily sludge by a modified bioaugmentation strategy. International Biodeterioration and Biodegradation. 2011;65(1):130–134. DOI: 10.1016/j.ibiod.2010.09.007

[16] Karamalidis AK, Evangelou AC, Karabika E, Koukkou AI, Drainas C, Voudrias EA. Laboratory scale bioremediation of petroleum-contaminated soil by indigenous microorganisms and added *Pseudomonas aeruginosa* strain Spet. Bioresource Technology. 2010;101(16):6545–6552. DOI: 10.1016/j.biortech.2010.03.055.

[17] Schwartz E, Scow KM. Repeated inoculation as a strategy for the remediation of low concentrations of phenanthrene in soil. Biodegradation. 2001;12(3):201–207. DOI: 10.1023/A:1013136524377

[18] Caliz J, Vila X, Martí E, Sierra J, Nordgren J, Lindgren P-E, Bañeras Ll, Montserrat G. The microbiota of an uncontaminated calcareous soil faces up chlorophenols: evidences of resistant strains with potential for bioremediation. Chemosphere. 2011; 83(2): 104–116. DOI: 10.1016/j.chemosphere.2011.01.016

[19] Muckian LM, Grant RJ, Clipson NJW, Doyle EM. Bacterial community dynamics during bioremediation of phenanthrene and fluoranthene amended soil. International Biodeterioration and Biodegradation 2009;63(1):52–56. DOI: 10.1016/j.ibiod.2008.04.005

[20] Lebeau T. Bioaugmentation for in situ soil remediation: how to ensure the success of such a process. In: Singh A, Parmar N, Kuhad RC, editors. Bioaugmentation, Biostimulation and Biocontrol, Soil Biology, Vol. 28, Springer-Verlag Berlin Heidelberg, Germany; 2011. p. 129–186. DOI: 10.1007/978-3-642-19769-7_7

[21] Zafra G, Cortés-Espinosa DV. Biodegradation of polycyclic aromatic hydrocarbons by Trichoderma species: a mini review. Environmental Science and Pollution Research. 2015;22(24):19426–19433. DOI: 10.1007/s11356-015-5602-4

[22] Tyagi M, da Fonseca MMR, de Carvalho CCCR. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation. 2011;22(2):231–241. DOI: 10.1007/s10532-010-9394-4

[23] Chen M, Xu P, Zeng G, Yang C, Huang D, Zhan, J. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnology Advances. 2015;33(6):745–755. DOI: 10.1016/j.biotechadv.2015.05.003

[24] Yang Y, Zhang N, Xue M, Lu ST, Tao S. Effects of soil organic matter on the development of the microbial polycyclic aromatic hydrocarbons (PAHs) degradation potentials. Environmental Pollution. 2011;159(2):591–595. DOI: 10.1016/j.envpol.2010.10.003

[25] Yang Y, Tao S, Zhang N, Zhang DY, Li XQ. The effect of soil organic matter on fate of polycyclic aromatic hydrocarbons in soil: a microcosm study. Environmental Pollution. 2010;158(5):1768–1774.

[26] Tang J, Lu X, Sun Q, Zhu W. Aging effect of petroleum hydrocarbons in soil under different attenuation conditions. Agriculture Ecosystems and Environment. 2012;149:109–117. DOI: 10.1016/j.agee.2011.12.020

[27] Chagas-Spinelli ACO, Kato MT, Lima ES, Gavazza S. Bioremediation of a tropical clay soil contaminated with diesel oil. Journal of Environmental Management. 2012;113:510–516. DOI: 10.1016/j.jenvman.2012.05.027

[28] Styrishave B, Björklund E, Johnsen A, Halling-Sorensen B. The spatial heterogeneity of polycyclic aromatic hydrocarbons in soil depends on their physico-chemical properties. Water Air Soil Pollution. 2012;223(3):969–977. DOI: 10.1007/s11270-011-0916-4

[29] Allard AS, Remberger M, Neilson AH. The negative impact of aging on the loss of PAH components in a creosote-contaminated soil. International Biodeterioration and Biodegradation. 2000;46(1):43–49. DOI: 10.1016/S0964-8305(00)00050-0

[30] Delgado-Balbuena L, Romero-Tepal EM, Luna-Guido ML, Marsch R, Dendooven L. Removal of anthracene from recently contaminated and aged soils. Water Air Soil Pollution. 2013;224(2):1420. DOI: 10.1007/s11270-012-1420-1

[31] Huesemann MH, Hausmann TS, Fortman TJ. Does bioavailability limit biodegradation? A comparison of hydrocarbon biodegradation and desorption rates in aged soils. Biodegradation. 2004;15(4):261–274. DOI: 10.1023/B:BIOD.0000042996.03551.f4

[32] Hwang S, Cutright TJ. Biodegradability of aged pyrene and phenanthrene in a natural soil. Chemosphere. 2002;47(9):891–899. DOI: 10.1016/S0045-6535(02)00016-4

[33] Cébron A, Faure P, Lorgeoux C, Ouvrard S, Leyval C. Experimental increase in availability of a PAH complex organic contamination from an aged contaminated soil: consequences on biodegradation. Environmental Pollution. 2013;177:98–105. DOI: 10.1016/j.envpol.2013.01.043

[34] Horwath W. Carbon cycling and formation of soil organic matter. In: Eldor AP, editor. Soil Microbiology, Ecology, and Biochemistry. 3rd ed. Academic press, USA; 2007. p. 303–339.

[35] Ceja-Navarro JA, Rivera-Orduña FN, Patiño-Zúñiga L, Vila-Sanjurjo A, Crossa J, Govaerts B, Dendooven L. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Applied Environmental Microbiology. 2010;76(11):3685–3691. DOI: 10.1128/AEM.02726-09

[36] Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N. Temporal variability in soil microbial communities across land-use types. ISME J. 2013;7(8):1641–1650. DOI: 10.1038/ismej.2013.50

[37] Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied and Environmental Microbiology. 2003;69(3):1800–1809. DOI: 10.1128/AEM.69.3.1800-1809.2003

[38] Zhao D, Li F, Wang R. The effects of different urban land use patterns on soil microbial biomass nitrogen and enzyme activities in urban area of Beijing, China. Acta Ecologica Sinica. 2012;32(3):144–149. DOI: 10.1016/j.chnaes.2012.04.005

[39] Grandy AS, Strickland MS, Lauber CL, Bradford MA, Fierer N. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. Geoderma. 2009;150(3–4):278–286. DOI: 10.1016/j.geoderma.2009.02.007

[40] Zhan X, Wu W, Zhou L, Liang J, Jiang T. Interactive effect of dissolved organic matter and phenanthrene on soil enzymatic activities. Journal of Environmental Science. 2010; 22(4):607–614.

[41] Andreoni V, Cavalca L, Rao MA, Nocerino G, Bernasconi S, Dell'Amico E, Colombo M, Gianfreda L. Bacterial communities and enzyme activities of PAHs contaminated soils. Chemosphere. 2004;57(5):401–412.

[42] Shen G, Lu Y, Zhou Q, Hong J. Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. Chemosphere. 2005;61(8):1175–1182. DOI: 10.1016/j.chemosphere.2005.02.074

[43] Teng Y, Luo Y, Sun M, Liu Z, Li Z, Christie P. Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbons from an aged contaminated soil. Bioresource Technology. 2010;101(10): 3437–3443. DOI: 10.1016/j.biortech.2009.12.088

[44] Serrano A, Gallego M, González JL, Tejada M. Natural attenuation of diesel aliphatic hydrocarbons in contaminated agricultural soil. Environmental Pollution. 2008;151(3): 494–502. DOI: 10.1016/j.envpol.2007.04.015