Bull Environ Contam Toxicol (2008) 81:19–24
DOI 10.1007/s00128-008-9438-1

Evaluation of Plant–Microorganism Synergy for the Remediation of Diesel Fuel Contaminated Soil

Xin Lin · Xiaojun Li · Peijun Li · Fei Li · Lei Zhang · Qixing Zhou

Received: 17 July 2007 / Accepted: 14 April 2008 / Published online: 21 May 2008

Abstract The remediation of diesel fuel contaminated soil over a 2-year period by the plant–microorganism synergy was evaluated. Results indicated that the growth of *Astragalus adsurgens* was affected significantly, when the diesel fuel concentration was higher than 10 g kg^{-1} dry soil. After a 2-year period, the removal of diesel fuel was >67%, and about 58–70% removal of aromatic hydrocarbons was obtained in these treatments. The removal of diesel fuel and its components was 13–30% higher than that of plant alone. These results show that an appropriate plant–microorganism synergy may serve as a low-cost, effective remedial technology for diesel-contaminated soil.

Keywords Diesel fuel · TPHs · AHs · Soil · Removal

Human economic activities have resulted in the contamination of large amounts soil. Agricultural soil is one of the most frequently contaminated soil around the world due to pesticide application and sewage irrigation which introduces many contaminants such as organics (petroleum hydrocarbons, hydroxybenzene, etc.), metals (Cr, Pb, etc.) (Jacks et al. 2000; Liu et al. 2005) and so on. The remediation of agricultural soils which have the direct risk for humans is therefore imperative. Many laboratory and field tests have demonstrated that the biological methods for soil remediation could be a cost-effective and environmental-friendly technology to treat organic contaminants, particularly for petroleum hydrocarbon contaminated soils (Banks et al. 2003; Mathew et al. 2006), although there are many limits on them (insufficient microorganism or plant biomasses, incomplete decomposition, time consuming, etc.) (Alexander 1999).

However, for diesel-fuel contaminated soil, the combination of microbe and plant would be practical and effective, and better than either of them alone. The use of degrading microbes is capable of rapidly metabolizing some available compounds, and some microbes called plant-growth-promoting rhizobacteria can improve the plant tolerance to oily matter and other stresses (Trindade et al. 2005; Betancur-Galvis et al. 2006). At the same time, the plants exudates and sloughed tissue may enhance the degradation of the complex compounds due to increased bioavailability of the contaminants and the interaction among microbes, nutrients and contaminants (Tesar et al. 2002; Banks et al. 2003; Kaimi et al. 2006). Root growth also opens deeper soil to better water infiltration and oxygen diffusion (Singer et al. 2003), disrupts soil aggregates and enhances biodegradation of entrapped hydrophobic contaminants (Banks et al. 2003). It also changes the conditions of the soil including carbon dioxide concentration, pH, osmotic potential, redox potential, oxygen concentration, and moisture content (Anderson et al. 1993), all of which can help microorganism to achieve high biomass. The

X. Lin · P. Li · Q. Zhou
Key Laboratory of Environmental Engineering, Shenyang 110044, People's Republic of China

X. Lin · X. Li (✉) · P. Li
Institute of Applied Ecology, Chinese Academy of Sciences, P.O. Box 417, Wenhua Road 72, Shenyang 110016, People's Republic of China
e-mail: lxj1206@163.com

F. Li
College of Urban and Environmental Sciences, Northeast Normal University, Changchun 130024, China

L. Zhang
College of Resources and Environment, Qingdao Agricultural University, Qingdao 266109, People's Republic of China

present study was conducted with the following objectives: (a) to test the effects of diesel fuel concentration on the growth of *A. adsurgens* in screened growth room under field conditions, and (b) to evaluate the degradation of diesel fuel by plant-microorganism synergy at various concentrations in aged oil-contaminated soil.

Materials and Methods

Soil was obtained from a typical sewage-irrigation area on the outskirts of the city of Shenyang, Liaoning Province, China. The site had been used exclusively for agricultural activities. The soil samples were taken from the upper layer (0–20 cm). Soil samples were air-dried in a dark room, mixed well, sieved through a 3 mm sieve, and stored at 4°C. The soil characteristics were summarized in Table 1.

The diesel fuel containing alkanes 90.01%, aromatics 0.82%, colloid and asphaltene 0.91% and others 8.26% was purchased from Fushun second oil factory, Fushun, Liaoning Province, China.

Erect Milkvetch (*Astragalus adsurgens* Pall) with a strong root system was used for phytoremediation assays. It is a drought- and sandstorm-tolerant legume distributed throughout China, and had been widely cultivated in north of China as highly palatable forage for more than 100 years. The plant seeds were purchased from Shenyang Agricultural University. As nitrogen and phosphorus are the mostly limiting inorganic nutrients for oil-degrading bacteria. The fertilizer autoclaved and dried before used in the experiment was chicken manure with organic content of 39.3%, N 1.82%, P_2O_5 5.69%, K_2O 2.62% and pH 8.02.

The dominant degrading microorganisms used in this study were isolated from aged oil-contaminated soil by Lin et al. (2004). One hundred and fifty millilitre liquid culture medium containing 20% (v/v) potato juice was added into a 500 mL conical flask, and autoclaved at 120°C for 20 min. The degrading microorganisms were then inoculated and shaken on a mechanical shaker (120 rpm), at 25°C for 5–7 days. Rice husk and bran (3:1(w/w)) were mixed and autoclaved at 120°C for 1 h. The microbial inoculants prepared were then inoculated in a proportion 150 ml culture medium per kg in aseptic conditions. Meanwhile, the water content was adjusted using potato juice to 20–25%. The microbial inoculants were then cultured at 25°C for 5–10 days.

One-and-a-half kilogram of unsterilized, air-dried soil was weighed into the Ceramic pots (13 top diameter × 10 bottom diameter × 11 height cm). The diesel fuel in acetone solution (50% (v/v)) was added to the soil bringing the concentrations of 2, 5, 10, 30, 50 g $kg^{-1}$of air-dried soil, respectively. After each addition, the soil was carefully mixed to obtain homogenous. The soil–diesel mixture was allowed to stabilize and evaporate for 14 days in the dark at room temperature before planting in the laboratory. Twenty-five *A. adsurgens* seeds were placed on top of the soil in each pot. Meanwhile, the fertilizer (2% (w/w)), microbial inoculants (0.5% (w/w)) and fungi (0.5% (w/w)) and the water (40% (w/w) of the soil water holding capacity) were added. When the first leaf developed, the seedlings were emeraged, leaving 15 plants in each pot in the first year. Plants were grown for 125 days in a screened growth room (covering with a net in a field) each year, and the plants in the second year were regrowth of the original plants. During the growth period, the plants were watered only as needed. Control treatments consisted of pots without diesel fuel application. All experiments were performed in triplicates.

At harvest, shoots were cut at the soil surface, and height was measured. The fresh above-ground biomass was measured according to Kaimi et al. (2006). After the plants had been harvested each year, three replicate samples were collected from each pot with the upper 2–5 mm of soil discarded, then well mixed, air-dried in the dark at room temperature and stored at 4°C until analysis. The contaminants (diesel fuel and its components) were measured according to Mishra et al. (2001). Soil samples from the experiment were collected after the plants had been harvested each year, air-dried in the dark at room temperature and stored at 4°C until analysis. The air-dried soils (5 g) were extracted by ultra-sonication for 2 h into 20 ml of dichloromethane. After centrifugation, the supernatant was then concentrated under a stream of nitrogen gas to allow the solvent to evaporate completely, and the amount of extracted sludge (diesel fuel) was determined gravimetrically. After gravimetric quantification, the diesel fuel was dissolved in n-pentane and separated into soluble and insoluble fractions. The soluble fraction (TPHs), the amount of which was determined gravimetrically, was loaded on to a silica gel column and eluted with different solvents. After the alkane fraction was eluted with hexane, the aromatic fraction (AHs) was eluted with benzene, and

Table 1 Chemical and physical properties of the study soil

Parameter	pH	K–N (%)	P_2O_5 (%)	K_2O (%)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
Values	5.73	0.137	0.092	1.876	2.49	14.75	57.82	27.43

% (w/w)

was then analyzed by an Agilent 6890(+) gas chromatography equipped with a flame ionization detector. The capillary column used was a DB-5 (30 m × 0.32 mm i.d. × 0.25 μm film thickness. During analysis, the injector and the detector temperature for GC were maintained at 300°C and the oven temperature was programmed to rise from 80 to 240°C in 5°C/min increments and to hold at 240°C for 30 min. Individual compounds present in the aromatic fractions were determined by matching the retention times with authentic standards (Sigma Chemicals) and identified by GC. Extraction efficiency for diesel fuel ranged from 90% to 97%. The detection limits for analyzed diesel fuel, TPHs and AHs were 0.2 mg kg^{-1}, 0.2 mg kg^{-1} and 1–5 μg kg^{-1} respectively.

The difference between treatments was investigated using ANOVA analysis and post hoc Tukey test with SPSS Version 13.0, at 95% confidence.

Results and Discussion

The remaining concentrations of diesel fuel, TPHs and AHs after 2-week ageing were presented in Table 2. The concentrations of diesel fuel and its components (TPHs and AHs) decreased significantly after ageing, with loss of 44–77%, 39–79%, and 4–84% respectively. The decrease of diesel fuel was mainly from TPHs. The decreasing differences of the component concentrations except the control were statistically significant among treatments ($p < 0.05$). The highest loss rate was the No. 3 treatment, followed by No. 4, No. 5, No. 2 and No. 6 treatments.

In an environmental situation, decreases of diesel fuel concentrations in soil would be expected through a number of loss processes, including photo-, chemical- and/or biological-degradation as well as volatilization and/or leaching of contaminants to groundwater. In this study, the diesel fuel added to soil was aged for 2 weeks prior to sow the seeds. This controlled conditions over the course of the incubation meant that volatilization could be the main loss process, and the little loss processes included biological degradation and sorption of the diesel fuel to the soil mineral and/or organic fractions, and /or diffusion into the micro- and nanopores within the solid phases of the soil. Approximately 44–78% of the diesel fuel had been lost in the stability period, presumably by volatilization and sorption in soil. This loss rate was similar to that reported in Dibble and Bartha's study, which showed that in drummed oily waste approximately 50% of TPH would be lost after 6-week weathering mainly due to volatilization (Dibble and Bartha 1979). However, in some studies, this was not taken into account and usually in an overestimation on the degradation of the contaminants (Vinas et al. 2005).

The shoot heights and above-ground biomass of *A. adsurgens* for different treatments in the 2 years were shown in Fig. 1. The significant difference in the shoot heights of the plant was not observed between the 2 years ($p < 0.05$), although the shoot heights were higher in the second year than those of the first year except for the last three treatments. The last two treatments were significantly different from other treatments and there were not the

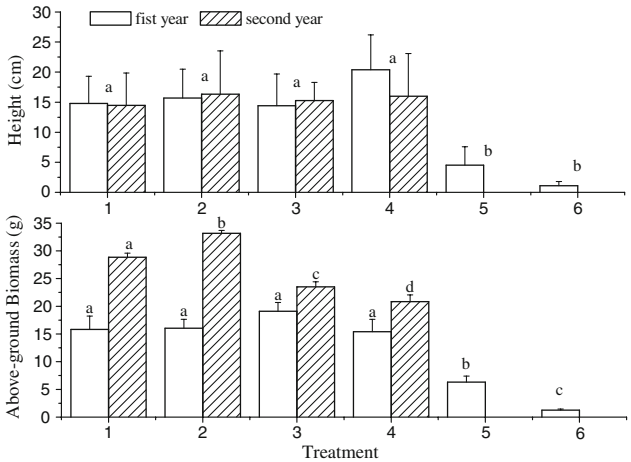

Fig. 1 Shoot heights and above-ground biomass of *Astragalus adsurgens* for designed treatments over 2 years. The error bars are standard errors (n = 3). Labels (**a**)–(**d**) indicate statistically significant differences between treatments in the same situation ($p < 0.05$). The treatments no. were same as Table 1

Table 2 Concentrations of diesel fuel, TPHs and AHs in contaminated soil after 2-week stability

Treatment no.	Concentrations of added diesel fuel (mg kg^{-1})	Initial concentrations for experiment (mg kg^{-1})		
		Diesel fuel	Including	
			TPHs	AHs
1	0	495 ± 35.4	460 ± 8.8	9.6 ± 0.3
2	2000	1270 ± 42.4	1070 ± 15.6	13.7 ± 0.7
3	5000	1600 ± 21.2	1410 ± 132.7	16.2 ± 0.5
4	10000	4450 ± 205.1	4100 ± 121.1	74.9 ± 0.9
5	30000	13805 ± 268.7	12720 ± 236.7	232.3 ± 25.8
6	50000	28540 ± 183.9	27710 ± 205.2	406 ± 7.3

growth of *A. adsurgens* in the second year. The significant difference of above-ground biomass was observed between the 2 years, and the highest combined biomasses were the No. 3 and No. 2 treatments in first and second year respectively. The concentrations higher than 10 g diesel fuel kg^{-1} treatments significantly affected the plant growth in this experiment.

Compared to the first year, the vegetation did not survive in the last two treatments in the second year. This might be due to the toxicity of diesel fuel and its metabolites. So far, most commercial bioremediation trials tended to monitor the success of the treatment by the degree of removal of the parent contaminant and did not consider the possibility of the biological production of more toxic breakdown metabolites (Mendon and Picado 2002; Lundstedt et al. 2003). However, it was important to ensure that the contaminants were suitably detoxified at the end of the treatment process. Some studies have demonstrated that those compounds such as PAH-ketones, quinines and coumarins which are formed during the microbial metabolism, chemical oxidation and phototransformation of PAHs, could be equally toxic at least to human health, when compared with the parent PAHs (Lundstedt et al. 2003). Thus, monitoring the metabolites of bioremediation specifically for toxic dead-end products, and assessing the toxicity of the material both before and after treatment were very important (Bamforth and Singleton 2005).

The removal of diesel fuel, TPHs and AHs by plant–microorganism synergy in this study was shown in Fig. 2. In Fig. 2 the removal of contaminants (D%) in ordinate was given by the formula: D% = 100(Mi-Mj)/Mi, in which Mi was the concentration of contaminants at the beginning of each year, Mj was the concentration of the contaminants at the end of each year.

The contaminant concentration significantly affected removal of diesel fuel, TPHs and AHs ($p < 0.05$). Because diesel fuel consisted of TPHs mainly, thus its removal with 38–70% in the first year, 24–45% in the second year and overall 61–84% in this experiment depended on the removal of TPHs with 45–78%, 16–62% and overall 62–88% respectively. The removal of AHs with high molecular weight always was very difficult, and its highest removal was only 57–70%, although the soil environmental condition had been optimized in this experiment. Although 10 g kg^{-1} diesel fuel of soil was the threshold for the plant growth in this experiment, the microorganism could still survive in 30 g kg^{-1} diesel fuel of soil and cause the removal of the last two treatments (no plant growth) which decreased only 10–20% compared to the No. 4 treatment in the second year. Significant differences were observed between the last two treatments and other treatments. The removal of contaminants was statistically higher than the control among 2–6 treatments.

In this study, the highest removal of TPHs was 88% was similar with the study of Huang et al. (2005), in which they used a multi-process system including land-farming, bioremediation and phytoremediation to remediate the soil from the imperial oil land farm site in an experiment of

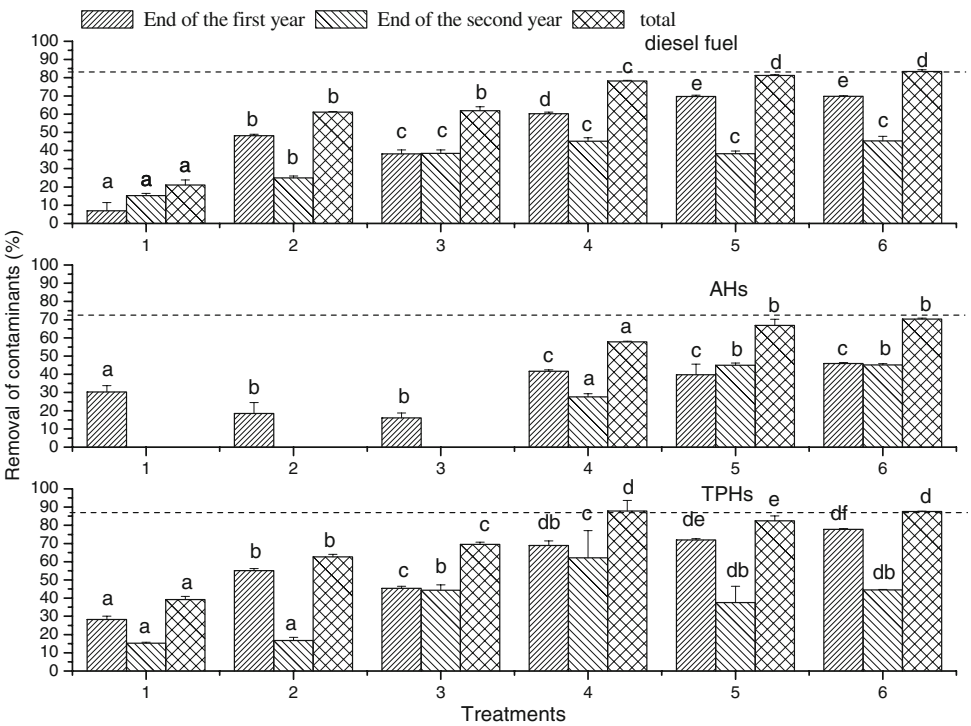

Fig. 2 Removal of diesel fuel, TPHs and AHs in the remediation of plant–microorganism synergy. The error bars are standard errors (n = 3). Labels (**a**)–(**f**) indicate statistically significant differences between the treatment in the same situation ($p < 0.05$). The treatments no. were same as Table 1

8 months. However, the environmental condition of this study was more similar with reality than those of experiment condition (in green house) conducted by Huang et al. For recalcitrant contaminants, AHs, the highest degradation was approximately 70%, lower than that of Huang et al. (2004), but higher than that of Joner et al. (2002). Joner et al. explained the reason of low degradation was absence of mycorrhizal fungi which was from a lack of mineral nutrients, as previously observed in another industrially polluted soil.

The contents of AHs and TPHs in diesel fuel in different periods were presented in Fig. 3, and the contents of TPHs and AHs (D%) were given by the formula: D% = 100Ma/Mt, in which Mt was the concentration of diesel fuel at the initial, end of first or second year in different treatments respectively, Ma was the contemporary concentration of TPHs or AHs. Most of the TPH molecules were of low molecular weight and easily degraded while AHs were the contrary. Therefore, most of the TPHs would be degraded quickly while most of the AHs would be degraded slowly in remediation due to its recalcitration. Therefore for varied initial concentrations and different remediation periods, the changes of TPHs and AHs contents in diesel fuel were not the same. Although the contents of TPHs in the diesel fuel were lower than the initial, most of them were still greater than 70% except the No. 4 treatment in the second year. Compared with the control, the contents of AHs in all five treatments were higher than the initial contents, and increasing 0.5–3% at the end of this experiment.

AHs were always recalcitrant to biodegradation. They would be adsorbed or partitioned into soil organic matter or wrapped into nanopores with the contact time (between them and soil components especially organic matter) increasing (Alexander 2000). The states of these contaminants in soil might be changed due to growth and death of plant roots (Parrish et al. 2005), and soil freeze – thawing (Oztas and Fayetorbay 2003). In our study, the AHs contents in the second year were higher than the initial values in the first three treatments. Similar results were also observed in some other studies (Banks et al. 2003; Denys et al. 2006). We thought that the increase of AHs in this study was probably due to the freeze-thaw process which increased the release of sequestrated AHs, however, this required further studies for detailed explanations.

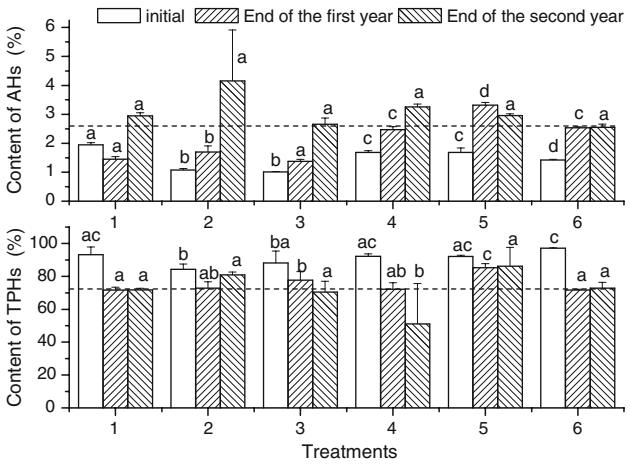

Fig. 3 Contents of AHs and TPHs in diesel fuel in different periods. The error bars are standard errors (n = 3). Labels (**a**)–(**d**) indicate statistically significant differences among these treatments in the same situation ($p < 0.05$). The treatments No. were same as Table 1

The degree of bioremediation of a soil depended not only on the type of the soil, but also on the pollutant's longevity, toxicity, as well as its mobility in the soil. The toxicity of a pollutant to an organism was defined by its chemical composition, its concentration in the environment, the duration of contact, overall environmental conditions, and the organism's physiological condition (Hatfield and Stewart 1994). Our study showed that the 10 g kg^{-1} diesel in soil was the threshold for *A. adsurgens*. This value was higher than that obtained by Huang et al. (2004), in which a multi-process phytoremediation system for removal of PAHs would probably less effective while the creosote concentration was higher than 3 g kg^{-1} in growth chambers (Huang et al. 2004). However, some studies showed that if the contamination was moderate or weak with (10% by weight of oil sediments, many plants still are able to survive and grow in oil-contaminated areas (Radwan et al. 1995). Lapinskien et al. (2006) founded that the diesel fuel was toxic when its concentration in the soil was >3% w/w. Although the matters used in these experiments and the environmental situations were not agreed with each other, these results still benefit us for carrying out the field remediation.

Remediation of plant–microorganism synergy might serve as a good remediation method on the removal of diesel fuel in field. In this study, the effect of plant–microorganism synergy on diesel-contaminated soil was significantly affected by the contaminant concentrations. Compared with lower concentrations, plant growth would be limited while the concentration was higher than 10 g kg^{-1} diesel in soil. Although this experiment was carried out in the field situation, plant-microorganism synergy significantly improved the degradation of diesel fuel. The highest removal of the diesel fuel in the soil was up to 80% during the 2-year period and the removal of the recalcitrant fraction of diesel fuel was 60–70% compared to their initial concentrations in the soil respectively. The degradation of diesel fuel and its components was 13–30% higher than that of control while no plant grew.

Acknowledgements This research was supported by funds provided by National Basic Research Program of China (No. 2004CB418506) and Postdoctoral funds of Liaoning province.

References

Alexander M (1999) Biodegradation and bioremediation. Academic Press Inc., San Diego, USA

Alexander M (2000) Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ Sci Technol 34: 4259–4265. doi:10.1021/es001069+

Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630–2637. doi: 10.1021/es00049a001

Bamforth SM, Singleton I (2005) Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biot 80:723–736. doi:10.1002/jctb.1276

Banks MK, Schwab P, Liu B, Kulakow P, Smith JS, Kim R (2003) The effect of plants on the degradation and toxicity of petroleum contaminants in soil: a field assessment. Adv Biochem Eng Biotechnol 78:75–96

Betancur-Galvis LA, Alvarez-Bernal D, Ramos-Valdivia AC, Dendooven L (2006) Bioremediation of polycyclic aromatic hydrocarbon-contaminated saline–alkaline soils of the former Lake Texcoco. Chemosphere 62:1749–1760. doi:10.1016/j.chemosphere.2005.07.026

Denys S, Rollin C, Guillot F, Baroudi H (2006) In-situ phytoremediation of PAHs contaminated soils following a bioremediation treatment. Water Air Soil Pollut Focus 6, 299–315

Dibble JT, Bartha R (1979) Effect of environmental parameters on the biodegradation of oil sludge. Appl Environ Microbiol 37:729–739

Hatfield JL, Stewart BA (1994) Soil biology: effects on soil quality. Lewis Publishers, Cherry Hill

Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM (2005) A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 81:139–147. doi:10.1016/j.microc.2005.01.009

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004) A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Pollut 130:465–476. doi:10.1016/j.envpol.2003.09.031

Jacks G, Forsberg J, Mahgoub F, Palmqvist K (2000) Sustainability of local water supply and sewage system – a case study in a vulnerable environment. Ecol Eng 15:147–153. doi:10.1016/S0925-8574(99)00038-5

Joner EJ, Corgié S, Amellal N, Leyval C (2002) Nutritional constraints to PAH degradation in a rhizosphere model. Soil Biol Biochem 34:859–864. doi:10.1016/S0038-0717(02)00018-4

Kaimi E, Mukaidani T, Miyoshi S, Tamaki M (2006) Ryegrass enhancement of biodegradation in diesel-contaminated soil. Environ Exp Bot 55:110–119. doi:10.1016/j.envexpbot.2004.10.005

Lapinskien A, Martinkus P, Rebvilija Z (2006) Eco-toxicological studies of diesel and biodiesel fuels in aerated soil. Environ Pollut 142:432–437. doi:10.1016/j.envpol.2005.10.023

Lin X, Li P, Zhou QX, Xu HX, Zhang HR (2004) Microbial changes in rhizospheric soils contaminated with petroleum hydrocarbons after bioremediation. J Environ Sci 16:987–990

Liu WH, Zhao JZ, Ouyang ZY (2005) Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environ Int 31:805–812. doi:10.1016/j.envint.2005.05.042

Lundstedt S, Haglund P, Oberg L (2003) Degradation and formation of polycyclic aromatic compounds during bioslurry treatment of an acid aged gasworks soil. Environ Toxicol Chem 22:1413–1420. doi:10.1897/1551-5028(2003)22<1413:DAFOPA>2.0.CO;2

Mathew M, Tan LR, Su Q, Yang X, Baxter M, Senior E (2006) Bioremediation of 6% [w/w] diesel-contaminated mainland soil in Singapore: comparison of different biostimulation and bioaugmentation treatments. Eng Life Sci 6:63–67. doi:10.1002/elsc.200620119

Mendon CE, Picado A (2002) Ecotoxicological monitoring of remediation in a coke oven soil. Environ Toxicol 17:74–79. doi: 10.1002/tox.10034

Mishra S, Jyot J, Kuhad RC et al (2001) Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. Appl Environ Microbiol 67(4):1675–1681. doi:10.1128/AEM.67.4.1675-1681.2001

Oztas T, Fayetorbay F (2003) Effect of freezing and thawing processes on soil aggregate stability. Catena 52:1–8. doi:10.1016/S0341-8162(02)00177-7

Parrish ZD, Banks MK, Schwab AP (2005) Effect of root death and decay on dissipation of polycyclic aromatic hydrocarbons in the rhizosphere of yellow sweet clover and tall fescue. J Environ Qual 34:207–216

Radwan S, Sorkhoh N, El-Nemr I (1995) Oil biodegradation around roots. Nature 376:302. doi:10.1038/376302a0

Singer AC, Smith D, Jury WA, Khoiviet H, Crowley DE (2003) Impact of the plant rhizosphere and augmentation on remediation of polychlorinated biphenyl contaminated soil. Environ Toxicol Chem 22:1998–2004. doi:10.1897/02-471

Tesar M, Reichenauer TG, Sessitsch A (2002) Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. Soil Biol Biochem 34:1883–1892. doi:10.1016/S0038-0717(02)00202-X

Trindade PVO, Sobral LG, Rizzo ACL, Leite SGF, Soriano AU (2005) Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparison study. Chemosphere 58:515–522. doi:10.1016/j.chemosphere.2004.09.021

Vinas M, Sabaté J, Espuny MJ, Solanas AM (2005) Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. Appl Environ Microbiol 71:7008–7018. doi:10.1128/AEM.71.11.7008-7018.2005