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Revised version
Organic Geochemistry 30, 963-969, 1999. doi:10.1016/S0146-6380(99)00080-7
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Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination.

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ABSTRACT

The phytotoxicity of various contaminated soils was assessed by plant inventories on ancient industrial fields, and by phytotoxicity tests. Industrial fields are well colonised by numerous weedy plants. Phytotoxicity was tested with pure PAHs, ancient industrial soils, soil leaches, liquid tar and tar volatile compounds. Both field studies and toxicity tests show that contaminated samples can be classified into two categories: first, a recently excavated soil/liquid tar that was foul-smelling and phytotoxic, and second, an 'aged', surface soil that was weathered and non-phytotxoic. Plant germination and growth are strongly inhibited by the presence of volatile, water-soluble low molecular-weight hydrocarbons (< 3 rings) such as benzene, toluene, xylene (BTX), styrene, indene, naphthalene and other possibly toxic substances. On the other hand, high molecular weight PAH (3-5 rings) did not show any phytoxicity under the conditions studied. These findings suggest that once low molecular weight aromatic hydrocarbons are removed e.g. by volatilization, biodegradation, weathering, tillage and fertilising, plants should be able to grow.

Key words : plants, contaminated soil, tar, PAH, BTX, phytotoxicity, gaswork soils

INTRODUCTION

The occurrence of Polycyclic Aromatic Hydrocarbons (PAHs) in soils, sediments, aerosols, waters, animals and plants is of increasing environmental concern because some PAHs may exhibit mutagenic and carcinogenic effects (Edwards, 1983, Baek *et al.*, 1991, Lichtfouse *et al.*, 1997 and refs. therein). Industrial soils from oil refineries and ancient gasworks worldwide are potentially contaminated by PAHs. So far, the main remediation strategies include thermal and biological treatments (Wilson and Jones, 1993, Thomas and Lester, 1993, Oudot, 1994). Incineration is rapid but suffers from its cost, its energy demand and its production of hazardous waste. Thermal desorption is less expensive, fast and efficient. It allows the decontaminated soil to be reused, though not for agronomical practices. On the other hand, biological treatments appear as cheap and efficient alternatives to reclame low- to medium contaminated soils. Several recent investigations suggest that plant cropping could be used as an alternative technique to decrease PAHs levels in soils because microbial activity is enhanced in the root zone (Aprill and Sims, 1990, Lee and Banks, 1993, Chaîneau *et al.*, 1997, Leyval and Binet, 1998). For example, Aprill and Sims (1990) observed the apparent disappearance of pure, high-molecular weight

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PAHs (4-5 rings) spiked on soils colonised by prairie grasses. Nonetheless, the presence of low-molecular weight hydrocarbons could inhibit plant growth as suggested by experiments on soils spiked with fuel oil (Chaîneau *et al.*, 1997). Since industrial sites often contain low molecular weight aromatic hydrocarbons and other toxic chemicals such as heavy metals, phenols, and cyanides, experiments must be undertaken to assess the toxicity of contaminated soils toward plants. Here we report the results of seed germination and growth tests in the presence of various media such as pure PAHs, PAH-contaminated field soils, contaminated soil leaches, liquid tar and tar volatile compounds

EXPERIMENTAL

Field studies

We identified the main native plant species growing on French contaminated soils: 1) from ancient gasworks at sites S, G and A, 2) from an ancient coking factory at site H and 3) from a biohillock after treatment with fertilisers (NPK compounds) at site LH.

Germination and growth

Plant seeds ranging in number from 75 to 200 were incubated at 21°C in the dark in 10 cm glass Petri dishes filled with 50 g dry-weight soil wetted at 80% water holding capacity using deionised water (3 replicates). For pure PAH tests, an agricultural soil was spiked with CH_2Cl_2 solutions of pure PAHs then allowed to concentrate 1 h under a ventilated hood. Here, a blank run without PAHs showed the absence of phytotoxicity from possible remaining traces of CH_2Cl_2. Pure PAH tests were also done on cellulose acetate filters using saturated PAH-water solution made by mixing 1 mg of each PAH with 5 ml of water (3 replicates). Tests with naphthalene were incubated alone because of the strong naphthalene smell which may have cross-contaminated other PAH dishes. 5 ml of rain leaches from outdoor boxes filled with ancient gaswork soils were tested on cellulose acetate filters (3 replicates). Viscous tar mixed with the agricultural soil was tested in three ways: in a <u>closed</u> Petri dish at 21°C in the dark; in an <u>open</u> Petri dish under a ventilated hood; and in a special device allowing seed contact solely with volatile compounds. This device is made of an open, small Petri dish fitted into a closed, larger Petri dish. Growth tests were done in a greenhouse at 20°C either by opening Petri dishes from germination tests (1 month) or with pots filled with 1 kg of soil (2 months).

Samples

The following contaminated media were tested for phytotoxicity:

- PURE PAHs: naphthalene, phenanthrene, fluoranthene, chrysene, benzo[a]anthracene and benzo[a]pyrene (> 99 %, Aldrich).

- COKING SOIL refers to an ‘aged’, weathered, 0-20 cm depth surface soil from an ancient coking factory. ‘Aged’ means that the soil has not been moved during the last 20 years, as opposed to ‘recently excavated’. This soil is actually well colonised by weedy plants. Characteristics: 16 EPA PAH content > 150 mg/kg, clay 6% (<2 μm), silt 19% (2-50 μm), sand 75% (50-2000 μm), C 6.8%, N 0.18%, C/N 38, P 0.0090%, pH 8.8.

- GASWORK SOILS refer to several tons of petroleum-smelling soils sampled at various depths between 0 and about 20 m, then well-mixed. <u>Medium polluted soil</u> characteristics : average 16

EPA PAH content 1584 mg/kg, clay 21%, silt 24%, sand 55%, C 4.4%, N 0.13%, C/N 34, P 0.0020%, pH 8.2. Highly polluted soil characteristics: average 16 EPA PAH content 3251 mg/kg, clay 11%, silt 21%, sand 68%, C 5.1%, N 0.12%, C/N 43, P 0.042%, pH 10.4.

- AGRICULTURAL SOIL, used as toxicity blank, refers to a prairie soil from Chenevières. Characteristics: 16 EPA PAH content below detection level, clay 11%, silt 32%, sand 57%, C 1.1%. N 0.11%. C/N 10, P 0.094%, pH 5.9.

- LEACHES refers to the first aqueous rain washings (April 4, 1998) of the recently excavated gaswork soils which were put outdoors on March 27, 1998, into boxes equipped with a tap at the bottom.

- LIQUID TAR refers to a foul smelling, black, viscous liquid sampled in an old tank at an ancient gaswork site.

Identification of tar volatile compounds

The empty barrel of a 1 µl gas chromatographic syringe was hung for 1 hour in the headspace of the viscous tar pot, then plugged. The vapour was then rapidly transferred into a Varian Star 3400 gas chromatograph coupled to a Saturn 2000 ion trap mass spectrometer. Conditions: helium flow 11 psi; on column injector; 30 m x 0.25 mm i.d. fused silica column coated with 5%phenyl-95%methylpolysiloxane phase (0.25 µm thickness); oven temperature : 35-200°C at 4°/min; EI 70 eV, scan range 30-400 amu, solvent delay 0 min.

RESULTS AND DISCUSSION

Field studies

We identified plants growing on soils from three ancient gasworks, from an ancient coking factory and from a prepared decontamination bed filled with gaswork soil. The main species are reported on **Table 1**. All plants are typical pioneer weeds that are commonly found in heterogeneous, low fertility soils. They are well adapted to drought and all showed a dense, deep root network, even through ‘aged’ tars.

Observation of tar shows of weathered soils in industrial fields revealed that the location of the contamination can be very heterogeneous, some areas being highly polluted while others much less. The possible toxic chemicals include PAHs, cyanides, phenols, volatile compounds and heavy metals. Nonetheless, all locations showed a well-flourishing vegetation, somewhat enhanced by fertilisers at the decontamination bed. Noteworthy, at some locations, plants were even able to grow roots through ‘aged’, non-smelling solid tar layers located at about 3-10 cm depth. There were only two exceptions showing the absence of plants: first, a small soil area, e.g. 20 m^2, located at an ancient gaswork field, where high amounts of foul smelling liquid tar occur between 0 and 20 cm depth; and second, a hillock of several tons of ‘benzene smelling’ gaswork soils which had been excavated 6 months ago. Here, it is interesting to note that, during the decontamination bed experiment, contaminated soils have evolved into well vegetated, non-smelling soils by fertilisation and water extraction.

Table 1. Plant species identified on three ancient gaswork sites.

Apiaceae	*Daucus carota*	Loganiaceae	*Buddleia*
Asteraceae	*Artemisia vulgaris*	Onagraceae	*Epilobium angustifolium*
	Cirsum arvensis		*Epilobium perforatum*
	Erigeron canadensis		
	Matricaria perforata	Papaveraceae	*Papaver rhoeas*
	Sonchus oleracerus		
	Tanacetum vulgare	Poeaceae	*Cynodon dactylon*
			Digitaria sanguinalis
Boraginaceae	*Echium vulgare*		*Festuca rubra*
			Poa annua
Brassicaceae	*Diplotaxis tenuifolia*		
	Sinapis arvensis	Ranunculaceae	*Clematis Vitalba*
	Sysimbrium officinale		
		Rosaceae	*Ronce*
Caryophyllaceae	*Silene dioica*		
		Solanaceae	*Solanum dulcamara*
Chenopodiaceae	*Chenopodium hybridum*		*Solanum nigrum*
	Chenopodium murale	Verbasceae	*Verbascum thapsus*
Hypericaceae	*Hypericum perforatum*	Betulaceae	*Betula alba*
Crassulaceae	*Sedum acrum*	Salicaceae	*Populus Tremula*
	Sedum album		

Altogether, these results indicate that contaminated field soils can be classified into two categories. First, non smelling, weathered, ‘aged’ soils and solid tars which apparently do not impede plant growth. This is in agreement with the common occurrence of weeds growing through weathered road asphalt. Second, foul smelling, recently excavated soils and liquid tars which strongly inhibit plant growth. Moreover, the decontamination bed experiment suggest that once the most bioavailable, water-soluble compounds are removed, plants could grow. These observations strongly suggest that the inhibition of plant growth is due to volatile, water-soluble compounds. This finding is strengthened by the germination tests described below.

Germination on pure PAH

PAH aqueous solutions

Seeds of *Zea mays* (maize), *Lupinus albus* (lupin), *Hordeum vulgare* (barley), *Festusca rubra* (fescue), *Brassica napus* (colza), *Lolium perenne* (rye grass), *Medicago sativa* (alfalfa) and *Trifolium pratense* (red clover) were incubated into closed Petri dishes filled with paper filter then wetted with saturated aqueous solutions of naphthalene, phenanthrene, fluoranthene, chrysene or benzo[a]pyrene. All species gave similar germination patterns as shown for colza on **Figure 1a**. During the first days of incubation, the germination of all species was delayed by naphthalene, and to a lesser extent by fluoranthene. On the other hand, the germination was enhanced by benzo[a]pyrene during the same time. Other PAHs did not show any significant differences relative to uncontaminated dishes. After 5 days, all PAH-contaminated and non-contaminated dishes gave the same germination levels.

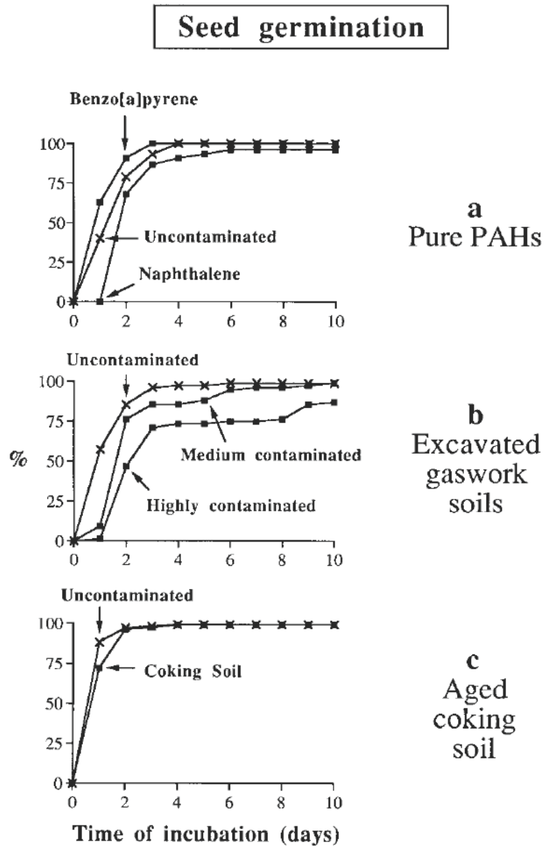

Figure 1. Colza germination tests with a) PAH-spiked soils, b) foul smelling, gaswork soils, and c) aged, weathered coking soil. Germination is expressed in percentage of germinated seeds. On figure 1a, deviations (3replicates) range from ± 1 to ± 10% : results are significantly different on the first 2 days but overlap thereafter. On figures 2b,c, deviations are smaller than the size of the signs (± 2-3%).

Our results indicate a transitory inhibition by naphthalene. Since naphthalene is the most volatile, water soluble (31 mg/l) compound of all tested PAHs, this observation is in agreement with field studies. Moreover, the extent of inhibition by naphthalene could be underestimated because a Petri dish allows some volatilisation, thus decreasing naphthalene concentration with time. On the other hand, surprisingly, we observed an enhanced germination during the first days using benzo[a]pyrene. A similar effect on plant growth has already been observed (Gräf and Nowak, 1966, Borneff *et al.*, 1968, Ewards 1983), suggesting that benzo[a]pyrene and its degradation products could act as growth stimulators. Further, the absence of inhibition observed at the end of germination tests (10 days) indicates that high molecular-weight PAHs are not phytotoxic (for germination). Nonetheless, at 10 days naphthalene had probably been completely volatilized (21°C) from the open Petri dish.

PAH-spiked soils

Lupin seeds were incubated in Petri dishes filled with PAH-spiked agricultural soil at high concentration levels, well above water solubility, ranging from 31 to 155 mg PAH per kg of soil. The compounds tested were benzo[a]anthracene, benzo[a]pyrene and dibenz[a,h]anthracene.

After germination, the Petri dishes were opened and the lupins were allowed to grow for 1 month in a greenhouse. The results showed no inhibition of germination and growth (size, weight), thus confirming the absence of toxicity of high molecular weight PAHs.

Germination on industrial soils

Petri dish tests were run using two kind of soils: first, an 'aged', weathered, surface coking soil where plants were developing well, and second, foul smelling, recently excavated, ancient gaswork soil at medium and high PAH concentrations. Species were *Brassica napus* (colza), *Brassica sinapis* (mustard), *Brassica rapa*, *Raphanus sativus* (turnip), *Helianthus anuus* (sunflower), *Triticum aestivum* (wheat), *Dactylis glomerata* (dactyl), *Festuca rubra* (fescue), *Hordeum vulgare* (barley), *Lolium perenne* (rye grass), *Lupinus albus* (lupin), *Medicago sativa* (alfalfa), *Phacelia*, *Trifolium alexandrii (*clover), *Trifolium repens* (white clover), *Trifolium incarnatum* (clover) and *Trifolium pratense* (clover).

Germination tests using smell, recently excavated gaswork soils showed significant inhibition, especially during the first days of incubation as shown for colza on **Figure 1b**. Nonetheless, the germination level increased with time, reaching sometimes 100% after 6 days for maize, rye grass and barley. Further, 2 months growth tests on gaswork soils showed a strong inhibition as shown by plant height (**Table 2**). Here, the most resistant species appear to be maize and rye grass. Noteworthy, the roots of all plants able to grow on gaswork soils are much smaller and much more branched than those growing on the agricultural soil. This behaviour is a typical indicator of chemical toxicity. Alternatively, germination tests using the 'aged' coking soil did not show any significant inhibition as observed for colza on **Figure 1c**. Further cultivation of lupin, rye grass and wheat during 1 month revealed no growth inhibition.

Table 2. Plant growth on medium- and highly-contaminated gaswork soils. Normalized height percent after 60 days.

	Uncontaminated	**Medium contamination**	**High contamination**
Brassica napus	100 ± 14	45 ± 5	0
Medicago sativa	100 ± 31	18 ± 10	0
Trifolium repens	100 ± 18	29 ± 3	0
Hordeum vulgare	100 ± 6	84 ± 12	9 ± 1
Festusca rubra	100 ± 2	47 ± 3	13 ± 3
Zea mays	100 ± 6	85 ± 4	13 ± 3
Lolium perenne	100 ± 1	64 ± 1	29 ± 2

Our findings demonstrate the absence of phytotoxicity of weathered, ‘aged’, contaminated soils, thus confirming our field observations. On the other hand, germination and growth are partially inhibited on recently excavated, foul smelling soils. Again, this strongly strengthens the hypothesis by which water soluble, volatile compounds are a source of toxicity. It also suggests that cultivation may be possible once soluble, volatile compounds are somehow degraded, e.g. by tillage with fertilisers and water-washing.

Germination on liquid tar

Seeds of colza and barley were incubated in Petri dishes with an agricultural soil spiked with foul smelling, liquid tar at concentrations ranging from 14 to 142 g per kg. **Figure 2** shows that germination levels sharply decreased with increasing tar concentration. Further, since we suspected a possible effect of volatile compounds, we did similar tests without closing the Petri dishes. Such an operation dramatically increased the germination levels. Then, in order to firmly establish the toxic effect of volatile compounds, we designed an apparatus consisting of a small, open Petri dish filled with agricultural soil, placed inside a large, closed Petri dish filled with liquid tar (C. Payet, personal communication). This apparatus thus allowed us to test the toxicity of volatile compounds without direct contact between seeds and tar. Here, the inhibition levels were strikingly similar to those using closed Petri dishes filled with tar-spiked soil at 142g/kg. The main toxicity stems therefore from the presence of volatile compounds.

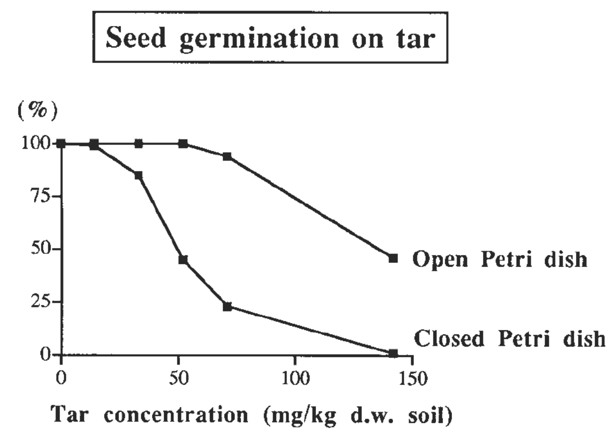

Figure 2. Germination of barley seeds versus tar concentration in soil, after 10 days incubation. Note the decreased inhibition when Petri dishes stay opened. Germination is expressed in percent of germinated seeds.

Germination on gaswork soil leaches

Outdoor boxes (24) each filled with 180 kg of medium- and highly-contaminated gaswork soils were allowed to weather starting in March 1998. After rain, the yellow-coloured, petroleum-like smelling leaches were tested for the germination of seeds using closed Petri dishes. Here, the results were similar to those obtained with Petri dishes filled with gaswork soils. More specifically, the rain water leaches strongly inhibited the germination of seeds. These findings, along with field observations and other germination tests, clearly demonstrate that volatile, water-soluble compounds from smelling soils and tar are the main source of phytotoxicity.

Identification of volatile compounds

The atmospheric constituents of the liquid tar pot were analysed by gas chomatography coupled to mass spectrometry (**Figure 3**). The main volatile compounds are benzene, toluene, xylene isomers, styrene, indene and naphthalene. Therefore, the inhibition of plant germination is mainly due to these low molecular weight, water soluble, aromatic hydrocarbons. The phytotoxicity of fuel oil observed by Chaîneau et al. (1998) can thus be explained, at least partly, by the presence of low molecular weight aromatic hydrocarbons.

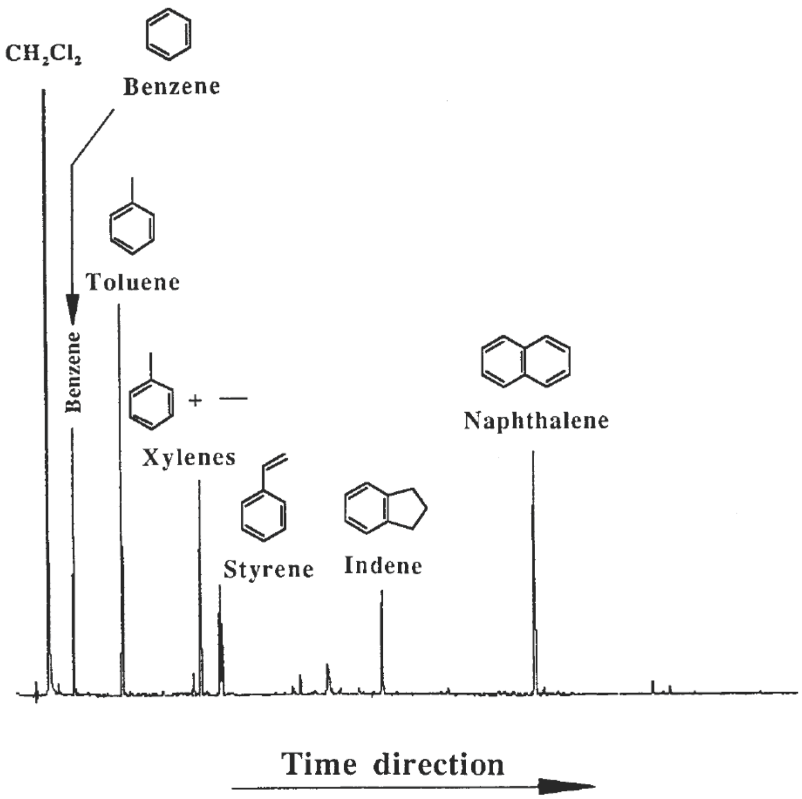

Figure 3. Volatile compounds from the liquid tar pot atmosphere. Methylene chloride peak represents the residual solvent from syringe decontamination. Gas chromatography coupled to mass spectrometry (relative intensity versus time). Conditions : see experimental part.

CONCLUSION

PAH-contaminated samples can be classified into two categories according to their phytotoxicity. First, recently excavated, foul smelling, volatile-rich soils and liquid tar which inhibit plant germination and growth, and second, weathered, aged, plant-colonised, surface soils which do not inhibit plant germination and growth. Under the conditions used, while high molecular weight PAHs do not inhibit plant germination and growth, the plant toxicity is mainly due to volatile, water-soluble substances such as benzene, toluene, xylenes (BTX), styrene, indene, naphthalene and other possible toxic molecules. Since these small aromatic compounds are water soluble and biodegradable, their removal from contaminated soil, e.g. by weathering, tillage and fertilisation, should allow the growth of plants. Further work is in progress to test the ability of plants to improve PAH degradation.

Acknowledgements. We thank Gaz de France and ELF for a PhD studentship (PH), helpful discussions and financial support.

REFERENCES

Aprill, W. and Sims, R. C. (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere* **20**, 253-265.

Baek, S. O., Field, R. A., Goldstone, M. E., Kirk, P. W., Lester, J. N. and Perry, R. (1991) A review of atmospheric polycyclic aromatic hydrocarbons: sources, fate and behavior. *Water, Air, and Soil Pollution* **60**, 279-300.

Borneff J., Selenka F., Kunte H. and Maximos A. (1968) Experimental studies on the formation of polycyclic aromatic hydrocarbons in plants. *Environmental Research* **2**, 22-29.

Chaîneau, C. H., Morel, J. L. and Oudot, J. (1997) Phytotoxicity and plant uptake of fuel oil hydrocarbons. *Journal of Environmental Quality* **26**, 1478-1483.

Edwards, N. T. (1983) Polycyclic aromatic hydrocarbons (PAH's) in the terrestrial environment. A review. *Journal of Environmental Quality* **12**, 427-441.

Gräf W. and Nowak W. (1996) Wachstumsförderung bei niederen und höheren Pflanzen durch kanzerogene polyzyklische aromate. *Archiv für Hygiene und Bacteriologie* **150**, 513-528.

Lee, E. and Banks, M. K. (1993) Bioremediation of petroleum contaminated soil using vegetation: a microbial study. Journal of Environmental Science and Health **A28**, 2187-2198.

Leyval, C. and Binet, P. (1988) Effect of polyaromatic hydrocarbons in soil on Arbuscular Mycorrhizal plants. *Journal of Environmental Quality* **27**, 402-407.

Lichtfouse, E., Budzinski, H., Garrigues, P. and Eglinton, T. I. (1997) Ancient polycyclic aromatic hydrocarbons in modern soils: ^{13}C, ^{14}C and biomarker evidence. *Organic Geochemistry* **26**, 353-359.

Oudot, J. (1994) Soil pollution by petroleum products and treatments. *Analusis* **22**, 16-18.

Thomas, A. O. and Lester, J. N. (1993) The microbial remediation of former gaswork sites: a review. *Environmental Technology* **14**, 1-24.

Wilson, S. C. and Jones, K. C. (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. *Environmental Pollution* **81**, 229-249.