Phytotoxicity Assay of Crop Plants to Phenanthrene and Pyrene Contaminants in Acidic Soil

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ABSTRACT: Four selected plants (corn, groundnut, cow pea, and mungbean) were tested for their ability to germinate and grow in an acidic soil contaminated with phenanthrene or pyrene, two typical polycyclic aromatic hydrocarbons (PAHs). The growth of corn root was the least sensitive to, but its germination rate was the lowest in the presence of, contaminants. Among the legumes, the growth of groundnut root was better than others. Corn and groundnut were selected to further test their ability to tolerate a mixture of phenanthrene and pyrene in the acidic soil. The presence of both PAHs led to a greater decrease in the lengths of shoot and root of groundnut than phenanthrene or pyrene alone, but the lengths of shoot and root of corn were decreased to a similar extent as when phenanthrene or pyrene was present alone. The growth of corn root was also better than that of groundnut root when they were grown in oil-contaminated soil. Based on these results, we conclude that corn is the most suitable to be grown in PAH-contaminated acidic soil. © 2007 Wiley Periodicals, Inc. Environ Toxicol 22: 597–604, 2007.

Keywords: acidic soil; corn; PAH; phenanthrene; phytotoxicity; pyrene

INTRODUCTION

The extensive and widespread use of petroleum has led to serious contamination of our environments worldwide, including Thailand. Petroleum is composed of varying concentrations of many hydrocarbon compounds. The most recalcitrant of these are the polycyclic aromatic hydrocarbons (PAHs), some of which are also the most difficult to degrade and may persist for a long period of time in the environment. Because of their hydrophobic nature, PAHs have the potential to be bioaccumulated and biomagnified to higher trophic levels. Sixteen PAHs are listed as priority pollutants by the U.S. Environmental Protection Agency (USEPA) (Harvey et al., 2002).

Acidity is one of the important soil quality problems in Southeast Asia, especially, in Vietnam, Indonesia, Malaysia, and Thailand. Soil in those areas is mainly acidic with high

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sulfur content. The pH of this soil type is generally less than 3.5. This low pH phenomenon is believed to arise from oxidized pyrite being deposited at the time when this area was the sediment before land formation (Shamshuddin et al., 2004). The eastern Provinces of Rayong and Chonburi are known to have sites contaminated with petroleum hydrocarbons, due to the presence of petrochemical industries and sea transportation of crude or refined oil products (Duangkaew, 2003; Boonyatumanond et al., 2006). The presence of petroleum hydrocarbon contamination in acidic soil may pose a unique problem in efforts to remediate such sites. Soil pH is an important factor that can limit plant growth and this may in turn limit the efficiency of phytoremediation through plant-induced rhizosphere biodegradation. To date, most of the pot studies on phytoremediation of PAHs were done in soil with pH values ranging from 5.9–10.4 (Reilly et al., 1996; Henner et al., 1999; Binet et al., 2000; Liste and Alexander, 2000; Huang et al., 2004; Kirk et al., 2005). How plants may respond in acidic soil contaminated with PAHs is not known and should be investigated.

Plants intended for use to support bacterial biodegradation of PAHs in acidic soil should grow well and be tolerant to low soil pH and to PAHs at levels found in the field. In this study, four plants species (corn, groundnut, cow pea, and mungbean) were tested for their ability to germinate and grow in soil contaminated with phenanthrene and pyrene. These plants were selected for two reasons. First, they are grasses or legumes which have been reported as being beneficial for use in PAH phytoremediation (Reilley et al., 1996; Binet et al., 2000). Second, these plants are easily grown in Thailand. Such initial phytotoxicity bioassays can be a useful and effective screening tool to eliminate plants which are sensitive to the contaminants found in soil and reduce the number of plants for pot or greenhouse study (Kirk et al., 2002). Phenanthrene and pyrene were selected for testing, as they are components of PAHs normally found in petroleum. For example, phenanthrene and pyrene ranging in concentrations from 17.0–1100 and 0.6–97 mg/kg, respectively, have been reported in 22 types of oil (Wernersson, 2003). Both compounds have been shown to be biodegradable (Kanaly and Harayama, 2000).

MATERIALS AND METHODS

Phytotoxicity Testing

Noncontaminated soil was collected from forested area in Trat Province, Thailand. The soil had no previously known history of contamination with PAHs. The soil was kept at room temperature (28–31°C) in black plastic bag. The soil was air-dried at room temperature (28–31°C) for at least 24 h to constant weight before use. A sample of the soil was sent to the Department of Land Development, Ministry of Agriculture and Cooperatives, Chonburi Branch, Thailand for chemical and physical characterization.

The general phytotoxicity assay described by Henner et al. (1999) and Kirk et al. (2002) was followed, with a few modifications. For each experiment, 50 g of dried soil were added to a glass Petri dish in triplicate. Phenanthrene (Carlo Erba Reagent, solid, 99.5%, code number 449984 6C116288L) and pyrene (Fluka Chemie GmbH, CH-9471, Buches, Switzerland, solid, 97.0% purity by GC) were weighed individually and dissolved in acetone. Each PAH solution was transferred to a glass sprayer and spiked to soil to final concentrations of 5, 10, 20, 40, 80, 100, 150, and 200 mg/kg dried soil for phenanthrene, and 5, 10, 20, 40, 80, and 100 mg/kg dried soil for pyrene. Soil in each dish was thoroughly mixed with a digger. The spiked soil was air-dried at room temperature (28–30°C) for more than 24 h or until the smell of acetone had disappeared.

Seeds of sweet corn (*Zea mays*) (commercial seeds of P.A. Seeds, Ltd.,Bangkok, Thailand), mungbean (*Vigna radiata*) (commercial seeds of Thai Cereal World, Bangkok, Thailand), groundnut (*Arachis hypogea* L.) (collected from a farm in Udornthani Province, Thailand), and cow pea (*Vigna sinensis* (L.) Saviex-Hass) (commercial seeds of Chia Tai, Bangkok, Thailand) were used in this study. Seeds were immersed in water for 3 h and then inoculated into PAH-spiked soil at 5–7 seeds (total 15–21 seeds per treatment) per Petri dish. The dishes were kept at 29°C in a room which received natural sunlight from ~06.00 to 18.00 h. To make up for the water loss, each plate received 10–20 mL of water at daily intervals. After 10 days, the number of seeds germinated for each treatment was counted. Six plants were randomly removed for measurement of their shoot and root length.

In nature, PAHs, such as phenanthrene and pyrene, are found in combination with other hydrocarbons. Therefore, plants to be used for the phytoremediation of petroleum-contaminated soil should be able to tolerate a mixture of hydrocarbons. In this study, selected plants found to be the most tolerant of phenanthrene and pyrene individually in the initial phytotoxicity assay were tested for their tolerance to combinations of the two PAHs and engine oil which contained a mixture of aliphatic and aromatic hydrocarbons. The toxic effect of PAH mixture was tested by spiking into 1 kg of dried soil to final concentrations of 0, 40, 100, and 200 mg phenanthrene plus 0, 20, 40, and 100 mg pyrene in different combinations. Engine oil (Pennzoil®, Pennzoil – Quaker State Company) was spiked to the soil to 1, 1.5, 2, 2.5, and 3% (volume/dry weight soil). To each plate, five seeds were added and the phytotoxicity test was done as described above.

Statistical Analysis

ANOVA was used to test for significant differences between treatments. One-way ANOVA was used to examine PAH and engine oil toxicity. Two-way ANOVA was

used to examine the toxicity of a combination of two PAHs on corn and groundnut followed by Tukey's test.

RESULTS

Phenanthrene or Pyrene Phytotoxicity

The soil used in this experiment was relatively acidic (pH 3.3), with a low organic content (0.99% w/w). The soil contained 0.049% nitrogen, 12 ppm phosphorus, and 22 ppm potassium.

In initial phytotoxicity tests, the effect of either phenanthrene or pyrene alone on individual plant was assessed by measuring the germination rate, as well as shoot and root length. For mungbean, cow pea, and groundnut, the percent of seeds germinated was not affected in the presence of 5–200 mg/kg of phenanthrene (Table I) or 5–80 mg/kg of pyrene (Table II). The percent germination decreased when the concentration of pyrene was more than 100 mg/kg (Table II). The response of corn seeds to phenanthrene and pyrene differed from the other three plants. The percent of corn seeds germinated decreased with increasing concentration of phenanthrene up to 80 mg/kg, and then increased at 100–200 mg/kg phenanthrene (Table I). Similarly, the percent of corn seeds germinated was relatively lower at low concentration (0–10 mg/kg) of pyrene, but increased as pyrene concentration increased from 20 to 100 mg/kg soil (Table II). The reason for this anomaly is not known.

The shoot lengths of control corn and mungbean plants in the absence of phenanthrene or pyrene were uncharacteristically shorter than when the plants were grown in the presence of the PAHs (Tables I and II). Other than this anomaly, the shoot lengths of all four plants were not affected at low phenanthrene concentrations up to 80 mg/kg. Above this concentration, the shoot lengths declined gradually. The same trend was seen in the shoot lengths of the four plants in the presence of pyrene, in that at low concentrations (up to 20–80 mg/kg), the shoot lengths remained relatively unaffected. The shoot lengths declined gradually above 80 mg/kg pyrene (Table II).

The root lengths of the four plants were also affected by increasing concentrations of PAHs. For mungbean, cow pea, and groundnut, their root lengths generally decreased progressively (up to 100 mg/kg pyrene or 200 mg/kg phenanthrene) with increasing phenanthrene or pyrene concentrations (Tables I and II). In contrast, the root lengths of corn increased slightly relative to the control at lower PAH concentrations, but then decreased with higher PAH concentrations (Tables I and II).

TABLE I. Shoot length, root length, and seed germination of four plants (mean ± S.D.) grown in varying concentration of phenanthrene-contaminated acidic soil for 10 days

Phenanthrene Concentration (mg/kg dry soil)	Shoot Length (cm)	Root Length (cm)	Percent Seed Germination
Corn			
0	12.6 ± 6.0b[a]	17.4 ± 5.1bc	57.1
5	21.5 ± 4.8ab	16.7 ± 5.6bc	71.4
10	23.4 ± 4.9ab	21.7 ± 6.2ab	42.9
20	23.5 ± 6.4ab	28.4 ± 8.5a	71.4
40	24.2 ± 5.0a	16.8 ± 4.5bc	38.1
80	21.9 ± 9.2ab	14.4 ± 3.6bc	28.6
100	18.8 ± 6.3ab	12.1 ± 17c	100
150	16.2 ± 4.7ab	12.6 ± 2.9bc	86.7
200	16.2 ± 4.3ab	10.6 ± 2.3c	80.0
Groundnut			
0	9.4 ± 1.7a	18.6 ± 3.1a	100
5	10.5 ± 0.9a	14.6 ± 0.9ab	100
10	8.6 ± 3.1a	13.2 ± 4.5abc	100
20	9.5 ± 2.1a	13.7 ± 3.7abc	100
40	10.3 ± 3.4a	14.3 ± 2.2abc	100
80	8.1 ± 3.0a	13.6 ± 2.0abc	100
100	5.2 ± 1.6b	9.5 ± 3.4bc	100
150	7.0 ± 2.0ab	10.1 ± 2.9bc	93.3
200	3.7 ± 1.6b	8.7 ± 2.9c	93.3
Cow pea			
0	21.4 ± 1.6a	16.3 ± 1.9a	100
5	21.5 ± 2.4a	11.1 ± 5.4a	100
10	18.6 ± 4.5ab	13.6 ± 3.6a	100
20	20.2 ± 5.1ab	13.6 ± 5.1a	100
40	21.6 ± 3.5a	12.5 ± 3.5a	100
80	21.3 ± 2.2a	12.6 ± 2.1a	93.3
100	18.1 ± 2.8ab	12.3 ± 3.8a	100
150	16.8 ± 2.7ab	12.2 ± 3.9a	100
200	14.2 ± 3.0b	9.2 ± 3.5a	100
Mungbean			
0	12.7±1.8d	16.9 ± 1.1a	95.2
5	19.5 ± 1.9a	9.2 ± 3.9bc	85.7
10	18.6 ± 1.2ab	12.2 ± 3.2abc	100
20	19.5 ± 0.8a	13.1 ± 4.9ab	95.2
40	19.1 ± 2.2ab	6.6 ± 2.0d	95.2
80	16.8 ± 0.6abc	6.1 ± 3.7d	100
100	15.4 ± 0.8cd	8.2 ± 0.6bc	100
150	16.3 ± 2.8bc	8.0 ± 1.4bc	100
200	15.4 ± 1.0cd	7.2 ± 1.0cd	100

[a] Values followed by different letters are statistically different ($P < 0.05$).

Effect of Engine Oil on Plant Toxicity

PAH-contaminated soils likely contain a complex mixture of many other hydrocarbons. Therefore, plants to be used for the phytoremediation of petroleum-contaminated soil should also be tolerant to various other components found in oil. For this reason, the four plants which could germinate and grow in PAH-contaminated acidic soil were tested for their ability to grow in the same acidic soil contaminated with various concentrations of engine oil.

TABLE II. Shoot length, root length, and seed germination of four plants (mean ± S.D.) grown in varying concentration of pyrene-contaminated acidic soil for 10 days

Pyrene Concentration (mg/kg) dry soil	Shoot Length (cm)	Root Length (cm)	Percent Seed Germination
Corn			
0	12.7 ± 6.0b[a]	17.4 ± 5.1ab	57.1
5	23.2 ± 8.0a	22.8 ± 7.5a	38.1
10	23.6 ± 4.7a	17.6 ± 2.9ab	47.6
20	23.3 ± 7.2a	23.2 ± 6.3a	61.9
40	23.5 ± 2.2a	18.8 ± 6.6ab	42.9
80	17.8 ± 4.1ab	14.4 ± 4.5ab	66.7
100	13.6 ± 3.7ab	11.4 ± 5.0b	93.3
Groundnut			
0	9.4 ± 1.7a	18.6 ± 3.1a	100
5	10.9 ± 2.2a	13.7 ± 2.5b	86.7
10	9.3 ± 4.0a	13.6 ± 2.4b	93.3
20	10.3 ± 2.2a	11.7 ± 3.2bc	93.3
40	10.2 ± 1.4a	10.6 ± 2.1bc	100
80	7.5 ± 3.5ab	9.6 ± 2.5bc	100
100	4.1 ± 0.3b	8.3 ± 2.2c	86.7
Cow pea			
0	21.4 ± 1.6a	16.3 ± 1.9a	100
5	22.4 ± 1.5a	13.4 ± 3.4ab	93.3
10	18.9 ± 2.5ab	9.8 ± 3.1b	100
20	22.6 ± 2.0a	12.2 ± 3.5ab	100
40	18.3 ± 4.5ab	14.5 ± 3.8ab	93.3
80	16.7 ± 5.6ab	9.8 ± 3.4b	100
100	15.2 ± 3.3b	10.7 ± 3.3ab	80.0
Mungbean			
0	12.7 ±1.8c	16.9 ± 1.1a	95.2
5	19.3 ± 1.3a	11.9 ± 6.0ab	100
10	19.1 ± 0.9a	6. 8 ± 5.1b	95.2
20	19.4 ± 1.1a	7.8 ± 2b	100
40	18.4 ± 1.1a	7.3 ± 3.6b	90.5
80	16.8 ± 0.6ab	7.8 ± 3.0b	85.7
100	14.0 ± 4.4ab	8.2 ± 1. 7b	86.7

[a] Values followed by different letters are statistically different ($P < 0.05$).

Engine oil up to 3% (volume/g dry soil) did not affect the germination of mungbean seeds (Table III). In contrast, the germination of other plant seeds was inhibited at high engine oil concentrations (more than 2.5% engine oil/g soil). In particular, corn seeds germinated poorly in the presence of engine oil (Table III). The presence of 1.0% engine oil led to decreases in the root lengths of all three legumes. The decreases in root lengths at higher engine oil concentrations were statistically significant ($P < 0.05$), with the exception of corn. The decreases of shoot lengths of four plants were not statistically significant ($P > 0.05$) at all concentrations of engine oil tested.

Phenanthrene Plus Pyrene Phytotoxicity

PAH-contaminated soils in the environment typically contain more than one PAH compound. Since our goal is to select plants suitable for use in the phytoremediation of phenanthrene- and pyrene-contaminated acidic soil, we felt it was necessary to directly test the ability of the two plants selected above, corn and groundnut, to tolerate a mixture of phenanthrene and pyrene.

Corn and groundnut responded to phenanthrene plus pyrene contaminated acidic soil in different ways. The presence of both phenanthrene and pyrene led to greater decreases in the lengths of shoot and root of groundnut than phenanthrene or pyrene alone (Tables IV and V). This suggested a significant combined effect of phenanthrene and pyrene on groundnut. The longest shoot was found in the presence of 20 mg/kg pyrene and 40 mg/kg phenanthrene, while the longest root was found in control plants in the

TABLE III. Shoot length, root length, and seed germination (mean ± S.D.) of four plants grown in varying concentrations of engine oil-contaminated acidic soil for 10 days

Engine Oil Concentration (%)	Shoot Length (cm)	Root Length (cm)	Percent Seed Germination
Corn			
0	14.4 ± 0.3a[a]	13.3 ± 1.8a	60.0
1.0	5.5 ± 4.7a	7.6 ± 6.6a	33.3
1.5	12.2 ± 5.0a	10.5 ± 4.5a	60.0
2.0	12.1 ± 7.6a	8.1 ± 3.5a	33.3
2.5	12.6 ± 7.4a	11.1 ± 3.4a	33.3
3.0	8.0 ± 2.1a	7.3 ± 2.8a	26.7
Groundnut			
0	5.9 ± 0.7a	8.9 ± 3.0a	93.3
1.0	3.8 ± 1.36a	4.7 ± 0.8b	100
1.5	4.0 ± 0.8a	5.0 ± 1.1b	86.7
2.0	3.8 ± 1.4a	3.9 ± 0.5b	80.0
2.5	5.1 ± 1.7a	4.9 ± 1.5b	80.0
3.0	5.3 ± 1.0a	3.4 ± 0.7b	80.0
Cow pea			
0	13.4± 2.0a	9.3 ± 0.7a	100
1.0	12.9 ± 1.9a	6.7 ± 1.8b	93.3
1.5	13.8 ± 1.0a	4.8 ± 1.4ac	100
2.0	13.9 ± 2.3a	4.4± 1.2c	10
2.5	14.0 ± 3.1a	4.2 ± 0.7c	93.3
3.0	10.9 ± 1.9a	3.6 ± 0.9c	86.7
Mungbean			
0	16.1 ± 2.6a	10.2 ± 2.2a	100
1.0	14.0 ± 3.2a	5.1 ± 0.9b	100
1.5	14.1 ± 2.1a	4.2 ± 0.6b	100
2.0	13.6 ± 1.7a	4.0 ± 0.5b	100
2.5	13.3 ± 1.8a	3.9 ± 1.0b	100
3.0	13.4 ± 2.4	3.7± 1.1	100

[a] Values followed by different letters are statistically different ($P < 0.05$).

TABLE IV. Shoot length of groundnut grown in varying concentrations of phenanthrene plus pyrene contaminated acidic soil for 10 days

Pyrene Concentration (mg/kg)	Shoot Length (cm)			
	Phenanthrene Concentration (mg/kg)			
	0	40	100	200
0	9.4 ± 1.7Aa[a]	10.3 ± 3.4Aa	5.2 ± 1.6Ab	3.7 ± 1.6Ab
20	10.3 ± 2.2Aa	4.3 ± 1.2Bb	3.3 ± 1.2Ab	1.9 ± 0.5Ab
40	10.2 ± 1.4Aa	4.4 ± 1.2Bb	3.6 ± 1.2Ab	2.9 ± 1.1Ab
100	4.1 ± 0.3Ba	3.4 ± 1.4Ba	3.6 ± 1.3Aa	3.2 ± 0.7Aa

[a] Values in columns followed by different capital letters are statistically different, and values in rows followed by different lower-case letters are statistically different ($P < 0.05$).

absence of any PAHs. The shortest shoot and root were found in the presence of 200 mg/kg phenanthrene plus 20 mg/kg pyrene. The presence of both phenanthrene and pyrene also decreased the seed germination rate of groundnut when the concentration of phenanthrene was 200 mg/kg. The lowest seed germination rate was found in the presence of 200 mg/kg phenanthrene plus 40 mg/kg pyrene.

In contrast, in the presence of both phenanthrene and pyrene, the shoot and root lengths of corn decreased to a similar extent as when phenanthrene or pyrene was present alone (Tables VI and VII). The effect seemed to depend on the total concentration of the two PAHs. There was a significant combined effect of phenanthrene and pyrene on shoot length, but this combined effect was not found for root length. The longest shoot was found in the presence of 40 mg/kg phenanthrene, while the longest root was found in the presence of 20 mg/kg pyrene. The shortest shoot was found in the presence of 100 mg/kg pyrene, while the shortest root was found in the presence of 200 mg/kg phenanthrene plus 100 mg/kg pyrene. The presence of both phenanthrene and pyrene increased the seed germination rate of corn more than the presence of 0–40 mg/kg phenanthrene or pyrene alone in acidic soil. The lowest seed germination rate was found in the presence of 40 mg/kg phenanthrene.

DISCUSSION

The phytotoxicity of PAHs seems to differ from some other low MW hydrocarbons, such as benzene, xylene, or styrene. PAHs generally do not kill plants, but would slow down or inhibit growth by decreasing plant biomass or elongation. The toxicity of PAHs may be exerted in part by their ability to damage cell membranes, thereby reducing nutrient or metabolite transport. These effects are relatively nonspecific, and dependent on the water solubility of the particular PAH compound. PAHs are also known to induce genetic mutation, retard growth, and increase the sensitivity of the plant to other stresses (Maliszewska-Kordybach and Smreczak, 2000).

The myriad effects of PAHs on plant cells are reflected in the wide assortment of plant- and contaminant-specific phytotoxic effects of PAH being reported in the literature. For example, phenanthrene at concentrations greater than 0.05 mM was reported to adversely affect the growth of *Arabidopsis thaliana* in vitro, as reflected by shoot and root growth reduction, deformed tricomes, reduced root hairs, chlorosis, late flowering, and the appearance of white spots on leaves (Alkio et al., 2005). In another study, phenanthrene, pyrene, fluoranthene, and fluorine were found to

TABLE V. Root length of groundnut grown in varying concentrations of phenanthrene plus pyrene-contaminated acidic soil for 10 days

Pyrene Concentration (mg/kg)	Root Length (cm)			
	Phenanthrene Concentration (mg/kg)			
	0	40	100	200
0	18.6 ± 3.1Aa[a]	14.3 ± 2.2Aab	9.5 ± 3.4Abc	8.7 ± 2.9Ac
20	11.7 ± 3.2Ba	5.1 ± 2.3Bb	5.9 ± 0.9Ab	3.5 ± 1.8Bb
40	10.6 ± 3.2Ba	5.5 ± 1.6Bb	5.5 ± 2.5Ab	3.8 ± 2.6Bb
100	4.1 ± 2.2Ca	4.4 ± 2.0Ba	4.2 ± 2.0Ba	3.6 ± 1.3Ba

[a] Values in columns followed by different capital letters are statistically different, and values in rows followed by different lower-case letters are statistically different ($P < 0.05$).

TABLE VI. Shoot length of corn grown in varying concentrations of phenanthrene plus pyrene-contaminated acidic soil for 10 days

Pyrene Concentration (mg/kg)	Shoot Length (cm)			
	Phenanthrene Concentration (mg/kg)			
	0	40	100	200
0	12.6 ± 6.0Bb[a]	24.2 ± 5.6Aa	18.8 ± 6.3Aab	16.2 ± 4.3Aab
20	23.3 ± 7.2Aa	18.8 ± 5.8Aa	17.3 ± 4.5Aa	18.0 ± 7.8Aa
40	23.5 ± 2.2Aa	21.7 ± 4.9Aa	21.1 ± 6.8Aa	18.5 ± 7.3Aa
100	13.6 ± 3.7ABa	15.8 ± 3.8Aa	21.0 ± 5.2Aa	14.7 ± 4.4Aa

[a] Values in columns followed by different capital letters are statistically different, and values in rows followed by different lower-case letters are statistically different ($P < 0.05$).

reduce the fresh and dry weights of *Lolium perene Trifolium pratense* and *Sinapsis alba*, with the former plant being affected more than the latter (Sverdrup et al., 2003). In contrast, the shoot and root dry weights of *Trifolium repens* L. were not affected when grown in benzo[a]pyrene- or naphthalene-contaminated soil. However, these two PAHs were able induce DNA polymorphism in *T. repens*. Benzo[a]pyrene induced greater DNA polymorphism than naphthalene, suggesting that the former may be more genotoxic than the latter (Aina et al., 2006). It has been reported that high MW PAHs (benzo[a]pyrene, benzo[a]antracene, chrysene, fluoranthrene, each tested alone) are not toxic to the germination of *Festusca rubra, Zea mays, Lolium perenne* (Henner et al., 1999). Similarly, Ren et al. (1996) reported that PAHs (antracene, benzo[a]pyrene, and fluoranthrene, each tested alone) had little impact on the fresh shoot weight of *Brassica napus* L. but markedly inhibited fresh root weight. The variable nature of some of the phytotoxic effects of PAHs may also be attributed to differences in experimental conditions which in turn can influence their bioavailability and volatilization rates (Smith et al., 2006).

In this study, phenanthrene and pyrene did not inhibit the germination of three legume seeds. Seedlings of these plants only produced shorter shoot or root. Corn seeds exhibited the lowest percentage of germination but their root was the least sensitive to the presence of the test PAHs. Thus, the effect of PAH on percent seed germination may not correlate with subsequent plant growth. This is not surprising since germination and seedling growth represent different endpoints which may be affected to different extents by the PAHs. Smith et al. (2006) also reported the differential sensitivity between germination and seedling growth of grasses and legumes exposed to PAHs. In their study, germination of seven plants (*Dactylis glomerata, Festuca arundinaceae, F. rebra, L. perenne, Lotus corniculatus*, and *T. pretense*) were not affect by PAH contamination in soil, but dry foliage yield was significantly reduced. More recently, Sverdrup et al. (2007) reported that benzo[a]pyrene did not affect seed germination of *L. perenna T. pretense* and *Brassica alba*, but subsequent growth of these plants, as measured by plant dry weight, were reduced. Huang et al. (1996) reported that PAHs (anthracene, benzo[a]pyrene, phenanthrene and pyrene) induce chlorosis in *Brassica napus* L., but this symptom was not found in this study.

In this study, we measured the extent of seed germination and assessed seedling growth over a relatively short period of time (10 day). Seed germination is obviously important for effective phytoremediation. If seeds do not germinate, then seedlings won't emerge to grow in PAH-contaminated soils. Young seedlings may be particularly susceptible to PAH contaminants as early periods of

TABLE VII. Root length of corn grown in varying concentrations of phenanthrene plus pyrene-contaminated acidic soil for 10 days

Pyrene Concentration (mg/kg)	Root Length (cm)			
	Phenanthrene Concentration (mg/kg)			
	0	40	100	200
0	17.4 ± 5.1ABa[a]	16.8 ± 4.5Aa	12.7 ± 1.7Aa	10.6 ± 2.3Aa
20	23.2 ± 6.3Aa	16.2 ± 4.5Aa	11.5 ± 2.5Ab	10.5 ± 4.6Ab
40	18.8 ± 6.6ABa	15.7 ± 3.3Aa	12.2 ± 1.8Ab	9.5 ± 2.9Ab
100	11.4 ± 5.0Ba	13.0 ± 3.7Aa	10.6 ± 4.7Aa	7.7 ± 2.3Aa

[a] Values in columns followed by different capital letters are statistically different, and values in rows followed by different lower-case letters are statistically different ($P < 0.05$).

seedling growth are especially important for root development which is vital for phytoremediation. Thus, a comparison of how well the young seedlings grow in PAH-contaminated soils may provide a good indication of the potential value of the plant in phytoremediation.

For effective phytoremediation, it is desirable to have petroleum-tolerant plants which have healthy roots that distribute well in soil. Thus, root lengths are used as a parameter to assess plant species. Longer root may increase the rhizosphere area, thereby enhancing the ability to support soil microorganisms as compared to shorter roots (Harvey et al., 2002). Based on the above consideration, corn was judged to have the potential to be the most petroleum-tolerant plant among the four plants tested. When grown in the presence of phenanthrene and pyrene, their shoot and root were the longest and they were healthy. Mungbean may be the least tolerant plant. When grown in the presence of phenanthrene and pyrene, their shoot and root were the shortest and their shoots were more fragile than those of control plants grown in the absence of the PAHs. The shoot and root lengths of cow pea and groundnut were slightly different. However, groundnut seemed to be better than cow pea because their roots were more healthy and robust. The shoots of groundnut were shorter than those of cow pea but they were thicker and not fragile.

The results of engine oil toxicity showed that all four plants could germinate and grow in engine oil-contaminated acidic soil. Among the four plants, mungbean was the most sensitive to engine oil based on root length measurements, despite the fact that it germinated well and its shoot length was little affected in the presence of engine oil or PAHs. Despite its poor germination ability, the root length of corn was the least affected in the presence of engine oil or PAHs. Corn grew well both in PAH- and engine oil-contaminated acidic soils. The responses of cow pea and groundnut were in between those of corn and mungbean, although groundnut seemed to be slightly better because of their robust, healthy, and hairy root. Considering that an extensive root system is preferred for plants destined for use in phytoremediation, we selected corn and groundnut for further test in the next experiment using combined phenanthrene and pyrene.

The result of the combined toxicity of phenanthrene and pyrene further confirmed that corn was the most tolerant of the four plants tested. The toxicity to corn did not depend on combined effect of the two PAHs. The shoot and root growth of corn grown in acidic soil containing a mixture of phenanthrene and pyrene were not more sensitive than those of corn grown in acidic soil containing phenanthrene or pyrene alone. For groundnut, the shoot and root lengths were more sensitive to soil containing phenanthrene and pyrene more than soil containing only phenanthrene or pyrene alone. Groundnut seemed to be affected more by the combined effect of PAH than individual PAH. The addition of pyrene accentuated the inhibitory effect of phenanthrene on root growth. For example, the root length of groundnut grown in 100 mg/kg phenanthrene plus 100 mg/kg pyrene (total = 200 mg/kg, root length = 4.25 cm) is shorter than that grown in 200 mg/kg phenanthrene (total = 200 mg/kg, root length = 8.72 cm). Similarly, the addition of phenanthrene exacerbated root growth inhibition in 20–40 mg/kg pyrene contaminated acidic soil but the effect was less apparent in 100 mg/kg pyrene contaminated acidic soil, mainly because root growth was already inhibited to a large extent at that pyrene concentration.

The differences between corn and groundnut in their responses maybe due to their differing capacity for PAH uptake or transformation. For example, Harvey et al. (2002) examined the distribution of radioactivity in two types of plant cells after exposure to radiolabelled benzo[a]pyrene. For soybean cells, they found that 16.2% of radioactivity was retained inside the cells as polar metabolite. However, 48.6% of the radioactivity was found as unchanged benzo[a]pyrene inside wheat cells (Harvey et al., 2002). Whether there are differences between corn and groundnut in their ability to take up and metabolize phenanthrene and pyrene remains to be investigated.

CONCLUSION

Plants to be used for the remediation of PAH-contaminated acidic soil should grow and tolerate well to low soil pH and PAHs. In this study, corn was found to be the most suitable plant with the longest root length when grown in PAH-contaminated acidic soil. Corns also grew well in engine oil contaminated acidic soil. The general response of all plant species to PAHs is similar in that the shoot and root growth decreased with increasing PAHs concentration in the acidic soil. Phenenthrene and pyrene did not exert a combined toxic effect on the growth of corn in the acidic soil, in contrast to that of groundnut. Plant growth in PAH-contaminated acidic soil depends on the plant's ability to grow in acidic soil and to tolerate to PAHs.

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