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Original Article

Potential of Butterfly Pea (*Clitoria ternatea*) and Yam Bean (*Pachyrhizus erosus*) Plants for Phytoremediation of Anthracene- and Pyrene-Contaminated Soil

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Abstract

The ability of butterfly pea (*Clitoria ternatea*) and yam bean (*Pachyrhizus erosus*) to stimulate the removal of anthracene and pyrene from contaminated soil was investigated. Of the two plants, only butterfly pea enhanced the removal of anthracene and pyrene from soil on day 30. The initial concentrations in soil of anthracene and pyrene were 86.3 and 74.1 mg/kg, respectively, while their remaining concentrations were 20.1 and 27.1 mg/kg on day 30. In comparison, the respective concentrations in unplanted soil on day 30 were 67.6 and 68.2 mg/kg. Anthracene and pyrene were completely removed from soil planted with butterfly pea and yam bean on day 75. In comparison, 17.8 and 18.2 mg/kg of anthracene and pyrene, respectively, remained in unplanted soil on day 75. Neither anthracene nor pyrene accumulated in any of the plant tissues examined during the study, so the mechanism for enhanced anthracene and pyrene removal was the stimulation by these plants of competent indigenous microorganisms to degrade the PAH compounds.

Keywords: anthracene, butterfly pea, phytoremediation, phytostimulation, pyrene, yam bean

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants, which are widely distributed at sites associated with petroleum, coal, or gas plants, or with wood processing industries. Anthropogenic activities including vehicle emission, accidental spillage of crude oil and incomplete combustion of petroleum, wood and coal are considered major sources of PAH contamination in the environment (Wang *et al.*, 2017). PAHs associated with petroleum sources usually are of LMW, such as phenanthrene and anthracene, while those associated with combustion processes are of HMW such as pyrene (Gao *et al.*, 2007). PAHs are known to possess toxicity, carcinogenicity and teratogenicity (Bansal & Kim, 2015).

PAHs are commonly found in soils and sediments in many regions of the world including Thailand. As an example, the total concentrations of PAHs with 3-7 fused benzene rings have been reported to range within 6-8,400 µg/kg by dry weight in sediment samples from canals, rivers, estuaries and coastal areas in the central part of Thailand (Boonyatumanond *et al.*, 2006). Many PAHs, including anthracene and pyrene that were used as model PAH compounds in this study, are listed as priority pollutants by the US EPA. Anthracene and pyrene have been found together in Thai soil and sediment

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samples. For example, the concentrations of anthracene and pyrene were reported to range within 0.1-5.0 and 0.3-48.3 μg/kg, respectively, in Bangkok surface soil (Wilcke *et al.*, 1999). Anthracene and pyrene were also found in Khao Lak terrestrial soil in Phang Nga province, with concentration ranges 0.006-28.4 and 3.74-350 μg/kg, respectively (Pongpiachan *et al*., 2013). Anthracene and pyrene have toxic equivalency factors of 0.1 and 0.001, respectively. Toxic equivalency factors are used by some researchers to estimate the toxicity or carcinogenicity of PAHs (Bansal & Kim, 2015).

Phytoremediation is an effective method to remove organic pollutants such as PAHs from soils (Somtrakoon *et al*., 2014a; Somtrakoon *et al*., 2014b; Somtrakoon et al., 2015). Cropping of plants in PAH-contaminated soil has been shown to exert beneficial effects on PAH removal by several mechanisms. For example, exudates secreted by plant roots can enhance PAH degradation in soil through the promotion of activity of competent microorganisms (Sun *et al*., 2010; Xu *et al*., 2006). Also, growth of the roots can improve the physico-chemical and biological properties of soil increasing soil aeration and bioavailability of contaminants (Álvarez-Bernal *et al*., 2007; Xu *et al*., 2006). Several crop plants in Thailand, such as waxy corn, sweet corn, long bean, wing bean, mungbean, okra, ridge gourd and cucumber have been shown in pot experiments to have potential to remediate PAH-contaminated soil to varying extents, depending on the type of plants and the PAHs, as well as on soil conditions (Chiapusio *et al*., 2007; Chouychai *et al*., 2016; Somtrakoon *et al*., 2014a; Somtrakoon *et al*., 2014b; Somtrakoon *et al*., 2015). Among these plants, we found that wing bean has a low germination rate in PAH-contaminated soil. While the long bean has a high germination rate in PAH-contaminated soil, most of its seedlings did not develop after germination.

Butterfly pea (*Clitoria ternatea*), an ornamental perennial climber plant, and yam bean (*Pachyrhizus erosus*), a tropical tuberous plant, belong to the Leguminosae family and are widely and easily grown in tropical Asian countries such as Thailand (Mukherjee *et al*., 2008; Ramos-de-la-Peña *et al*., 2013). Both plants have high germination rates and require less care in seedling preparation than wing bean and long bean. Growth of butterfly pea and yam bean is thought to require less nitrogen fertilization because of the presence of nodule-forming bacteria with nitrogen fixing ability in their roots (Castellanos *et al*., 1997; Mukherjee *et al*., 2008). These characteristics should render them good candidates for PAH phytoremediation, as the use of plants with nitrogen fixing ability for phytoremediation could reduce cost of nitrogen fertilizer application and improve soil fertility.

Several plants in Leguminosae have been reported to promote PAH removal from soil. For example, the percentage of 3-ring and 4-ring PAHs remaining in soil planted with clover (*Trifolium repens*) was about 55 %, as compared to 70 % in the unplanted control soil (the initial concentration of all 16 PAHs was 23.1 mg/kg in this soil) (Meng *et al*., 2011). In addition, >99 % of phenanthrene and 77 % of pyrene were degraded in soil planted with *Astragalus membranaceus* and *Aeschynomene indica* after 80 days of experiment, whereas 99 % and 69 % of phenanthrene and pyrene were degraded in unplanted soil (the respective initial concentrations were 87.6 and 98.6 mg/kg) (Lee *et al*., 2008). Cowpea (*Vigna unguiculata*) was found to slightly enhance phenanthrene biodegradation in soil (from initial 100 mg/kg). The amount of phenanthrene removal after 35 days of experiment ranged from 66.3-78.6 % in soil planted with cowpea. In comparison, the extent of phenanthrene removal in soil without cowpea was only 48.4% (Sun *et al*., 2015). Several studies suggested phytostimulation as the main mechanism by which Leguminosae plants can assist with PAH removal from soil (Cheema *et al*., 2009; Meng *et al*., 2011). The present study was undertaken to investigate the growth of two Leguminosae plants, butterfly pea and yam bean, in anthracene- and pyrene-contaminated soil, and to assess their potential to enhance the removal of these PAHs from soil.

2. Materials and Methods

2.1 Soil collection and analysis

Soil with no previous history of PAH contamination was collected from the Saentung subdistrict, Khaosaming district, Trat province, Thailand. Care was taken to ensure the site of soil sampling was far away from the roads, industries or other activities that may result in soil contamination by PAHs. The soil was spread on black plastic bag and left to air-dry at room temperature (28 – 31 °C) for at least 24 h to constant weight before use. One kilogram of the soil was sent to the Central Laboratory (Thailand) Co., Ltd., Bangkok, Thailand for chemical and physical analyses. The soil used in this study was a sandy loam consisting of 70.08 % sand, 10.45 % silt and 19.47 % clay. The pH of the soil was 5.9. The soil contained less than 5,000 mg/kg of total nitrogen and the available phosphorus, exchangeable potassium and organic matter were 5.41, 27.72 and 315,000 mg/kg, respectively.

2.2 Seedling preparation

Commercial seeds of the butterfly pea (*Clitoriaternatea* cv. Pailin) and yam bean (*Pachyrhizus erosus* cv. Saphaokaew) were obtained from East West Seed Ltd., Nonthaburi province and Chuayongseng Co. Ltd. Bangkok, Thailand, respectively. Seedlings of both plants were prepared according to Somtrakoon *et al*. (2014a) by germination of these seeds in moist soil spiked with anthracene and pyrene at room temperature (28 - 31 °C) in a room receiving natural sunlight. Ten-day old seedlings of comparable sizes were transplanted into the experimental pots.

2.3 Experimental setup

Each experimental pot containing 1 kg dry weight of soil spiked with anthracene and pyrene was prepared according to Somtrakoon *et al*. (2015). Briefly, the mixture solution of anthracene (Fluka, USA, purity 98 %) and pyrene (Sigma-Aldrich, USA, purity 98 %) was dissolved in acetone and poured onto 200 g dry weight of soil. To this was added 800 g of soil. The soil was mixed thoroughly and left inside a fume hood until acetone had completely evaporated. After mixing, concentrations of anthracene and pyrene in soil were analyzed by a gas chromatograph (Shimadzu GC AOC-5000) equipped with a mass spectroscopic detector (Shimadzu MS-QP2010). Three replicate soil samples were randomly col-lected for analysis of the initial concentrations of anthracene and pyrene.

The initial concentrations found were 86.3 ± 37.0 and 74.1 ± 17.9 mg/kg dry soil, respectively.

PAH phytoremediation experiments were performed in a plant nursery as described in Somtrakoon *et al*. (2015). Ten-day old seedlings of butterfly pea or yam bean were planted in cylindrical plastic pots, each containing 1 kg dry soil spiked with anthracene and pyrene. Soil without any spiked anthracene and pyrene was planted with butterfly pea or yam bean and these cases served as the controls for plant growth. Another control was soil spiked with anthracene and pyrene, but without any plants, for the determination of abiotic loss of PAHs during the course of the experiment. Each treatment had five independent replicates in a completely randomized design and the total number of pots was 25 for each sampling time. The details and objectives of each treatment are described below.

1) Soil spiked with anthracene + pyrene and planted with butterfly pea - to investigate the ability of butterfly pea to stimulate PAH removal by indigenous soil microorganisms and the possible accumulation of PAHs in plant biomass.
2) Soil spiked with anthracene + pyrene and planted with yam bean - to investigate the ability of yam bean to stimulate PAH removal by indigenous soil microorganisms and the possible accumulation of PAHs in plant biomass.
3) Soil spiked with anthracene + pyrene, but without plant – to investigate abiotic loss of PAHs and the ability of indigenous soil microorganisms to remove PAHs.
4) Soil without anthracene or pyrene and planted with butterfly pea - to investigate the growth of butterfly pea in non-contaminated soil.
5) Soil without anthracene or pyrene and planted with yam bean - to investigate the growth of yam bean in non-contaminated soil.

The experiment was performed for 75 days. Two sampling periods were selected based on similar studies carried out previously using similar PAHs in planted and non-planted soil (Gao *et al*., 2011; Somtrakoon *et al*., 2014a; Somtrakoon *et al*., 2014b; Somtrakoon *et al*., 2015). At the earlier sampling time (30 days), it was expected that PAHs would not be completely removed, but a greater amount of PAHs may be removed in the planted than non-planted soil. At the later sampling time (75 days), it was expected that the bulk of the PAHs may be removed in the planted soil, but not necessarily in the unplanted soil. Thus, these sampling times allowed an assessment of both the rate and extent of PAH degradation.

Parameters for plant growth were also measured. These involved measuring the length, fresh weight and dry weight of root and shoot, chlorophyll a, chlorophyll b and total chlorophyll contents in leaves, leaf morphology and number of nodules formed from plant samples collected on days 30 and 75 of experiment. Contents of various chlorophylls in leaves of these plants were analyzed and calculated using the equations provided in Huang *et al*. (2004).

2.4 PAH extraction and analytical procedures

Collected soil samples were subjected to Soxhlet extraction and analyzed for anthracene and pyrene contents by GC-MS according to Somtrakoon *et al*. (2014a). The shoot and root of each plant were extracted in the same manner as soil and the concentrations of anthracene and pyrene determined also by GC-MS.

2.5 Statistical analysis

Parameters for plant growth and percentages of each PAH remaining are presented as mean ± SD. The statistical significance of differences between treatments at $P < 0.05$ was analyzed with two-way ANOVA for plant health comparison and by one-way ANOVA for comparing PAH remaining in soil. Subsequent multiple comparisons of means were performed using the LSD test.

3. Results and Discussion

3.1 General morphology of plants grown in PAH-contaminated Soil

Butterfly pea and yam bean grew well in both anthracene + pyrene-contaminated soil and in non-contaminated soil, based on plant growth parameters such as shoot growth, root growth, leaf morphology (Figure 1) and chlorophyll content in leaves (Table 1). However, nodule formation was adversely affected by the presence of anthracene and pyrene in soil, as noted from the decrease in the number of nodules formed in butterfly pea. Butterfly pea did not produce any nodules on day 30. On day 75, two nodules per plant and 5.5 nodules per plant were observed in butterfly pea grown in anthracene + pyrene-contaminated and in non-contaminated soil, respectively. Unexpectedly, nodules were not found in yam bean plant grown in either anthracene + pyrene-contaminated or non-contaminated soil, on days 30 and 75. Nodules are expected to form in the yam bean roots (Castellanos *et al*., 1997). The reason for their absence in the roots of yam bean grown in this experiment is unknown. Storage root was formed in yam bean grown in non-contaminated soil, but not in soil contaminated with anthracene and pyrene (Figure 2). To our knowledge, storage root is not known to occur in butterfly pea in nature.

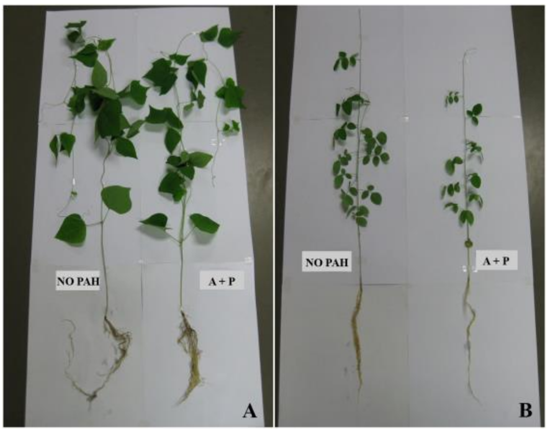

Figure 1. Comparative growth of yam bean (A) and butterfly pea (B) on day 75 of experiment. Abbreviations; A = anthracene, P = pyrene and NO PAH = non-contaminated soil.

Table 1. Chlorophyll contents in leaves of butterfly pea and yam bean grown in anthracene + pyrene-contaminated and non-contaminated control soils for 30 and 75 days. Data are given as mean ± SD.

Plant	Butterfly bean				Yam bean			
	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total chlorophyll (mg/ml)	a/b ratio	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total chlorophyll (mg/ml)	a/b ratio
Day 30								
Control	31.2 ± 2.01a	11.1 ± 1.58a	43.9 ± 3.70a	2.8 ± 0.22a	97.2 ± 7.05a	40.1 ± 6.28a	142.3 ± 13.71a	2.4 ± 0.21a
Anthracene + Pyrene	58.3 ± 14.00ba	23.1 ± 9.07a	84.3 ± 23.79a	2.6 ± 0.42a	111.8 ± 19.20a	53.7 ± 16.61a	171.3 ± 36.85a	2.1 ± 0.30a
Day 75								
Control	70.9 ± 14.28b*	27.3 ± 7.26a	101.8 ± 21.87b	2.6 ± 0.33a	59.3 ± 24.23a	20.2 ± 10.48a	82.4 ± 35.94a	3.1 ± 0.40a
Anthracene + Pyrene	103.6 ± 29.38a*	41.0 ± 13.10a	150.0 ± 43.88a*	2.6 ± 0.23a	39.0 ± 16.85a	13.4 ± 6.56a	54.5 ± 24.26a	3.0 ± 0.15a*

Different lower case letters in the same column denote significant differences (P < 0.05) between PAH contaminated and non-contaminated soil on the same day. *Denotes significant difference (P < 0.05) between values for different days in the same soil.

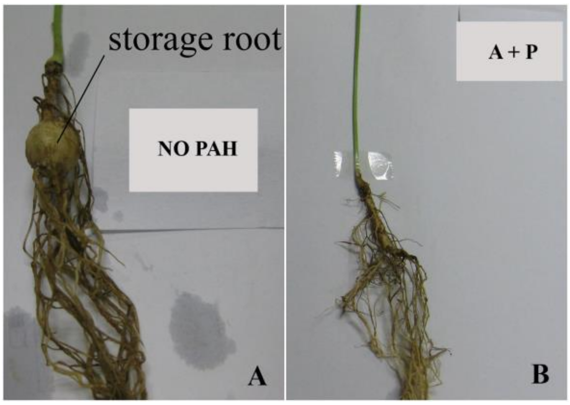

Figure 2. Appearance of storage root in yam bean grown in non-contaminated soil (A) on day 75 of experiment. No storage root was seen in yam bean grown in anthracene + pyrene-contaminated soil (B). Abbreviations; A = anthracene, P = pyrene and NO PAH = non-contaminated soil.

3.2 Growth of shoot and root of butterfly pea and yam bean in PAH-contaminated soil

The presence of anthracene and pyrene in soil did not exert any apparent adverse effect on shoot growth in butterfly pea and yam bean over 75 days. Shoot length, shoot fresh weight and shoot dry weight of yam bean grown in anthracene + pyrene-contaminated soil were not significantly different from those of plants grown in non-contaminated soil. The shoot length, shoot fresh weight and shoot dry weight of yam bean planted in either soil contaminated with anthracene + pyrene or non-contaminated soil were around 77.5-85.8 cm, 2.6-2.7 g and 0.49-0.63 g, respectively. The same trend was observed in butterfly pea, in that the shoot growth parameters of butterfly pea planted in anthracene + pyrene-contaminated soil were not significantly different from those of plants grown in non-contaminated soil (Table 2).

Table 2. Shoot health parameters of butterfly pea and yam bean grown in anthracene + pyrene-contaminated and non-contaminated control soils for 30 and 75 days. Data are given as mean ± SD.

Plant	Butterfly pea			Yam bean		
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Day 30						
Control	12.8 ± 1.9a	0.4 ± 0.2a	0.02 ± 0.01a	60.7 ± 11.4a	1.2 ± 0.2a	0.16 ± 0.02a
Anthracene + Pyrene	14.7 ± 2.9a	0.4 ± 0.1a	0.03 ± 0.00a	44.2 ± 19.7a	0.9 ± 0.3a	0.11 ± 0.04a
Day 75						
Control	41.1 ± 7.5a*	0.8 ± 0.25a*	0.16 ± 0.06a*	85.8 ± 25.8a	2.7 ± 0.86a*	0.63 ± 0.20a*
Anthracene + Pyrene	34.9 ± 8.0a*	0.6 ± 0.15a	0.08 ± 0.02a	77.5 ± 25.3a*	2.6 ± 1.21a*	0.49 ± 0.21a*

Different lower case letters in the same column denote significant difference (P < 0.05) between PAH contaminated and non-contaminated soil on the same day. *Denotes significant difference (P < 0.05) between values for different days in the same soil.

Similar to the shoots, the presence of anthracene and pyrene in soil did not affect the root length, root fresh weight or root dry weight of yam bean, relative to the control plants grown in non-contaminated soil over 75 days. In contrast, the root fresh weight of butterfly pea was lower than that of plants grown in non-contaminated soil (Table 3). The root fresh weight of butterfly pea grown in anthracene + pyrene-contaminated soil was only 0.3 on day 75 day of experiment. In comparison, root fresh weight of butterfly pea in non-contaminated soil was 0.48 g. Thus, based on root growth characteristics alone, yam bean seemed to be more tolerant to anthracene and pyrene than butterfly pea.

3.3 Leaf morphology and chlorophyll content of butterfly pea and yam bean grown in PAH-contaminated soil

Leaf morphology parameters, such as leaf length and leaf width of both butterfly pea and yam bean grown in anthracene + pyrene-contaminated soil, were not significantly different from those of plants grown in non-contaminated soil on day 30 or 75 (Table 4). The leaf chlorophyll a, chlorophyll b and total chlorophyll contents of yam bean did not exhibit significant changes when the plants were grown in the presence of anthracene and pyrene in soil (Table 1). Interestingly, chlorophyll a and total chlorophyll contents in leaves of butterfly pea grown in anthracene + pyrene-conta-minated soil were higher than those grown in non-contaminated soil on day 75. The reason for this observation is unknown. Increased chlorophyll contents in plants grown in the presence of PAHs have been described. In one study, Huang et al. (2004) reported higher chlorophyll contents in the leaves of wild rye (*Elymus canadensis*) planted in soil contaminated with 50-200 mg/kg creosote. In another study, Somtrakoon *et al.* (2014b) observed increased chlorophyll content in the leaves of sweet corn grown in soil contaminated with 139 mg/kg anthracene and 96 mg/kg fluorene. In other studies, the chlorophyll content either remained unchanged in leaves of cucumber or winged bean in the presence of anthracene and fluorene (Somtrakoon *et al*., 2014b) or decreased in leaves of Kentucky bluegrass (*Poa pratensis*) and Tall fescue (*Festuca arundinacea*) in the presence of creosote (Huang *et al*., 2004). Thus, the effects of organic pollutants on chlorophyll content vary by the case studied.

Table 3. Root health parameters of butterfly bean and yam bean grown in anthracene + pyrene-contaminated and non-contaminated control soils for 30 and 75 days. Data are given as mean ± SD.

Plant	Butterfly bean			Yam bean		
	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Day 30						
Control	6.7 ± 2.5a	0.04 ± 0.02a	0.004 ± 0.002a	8.2 ± 5.7a	0.30 ± 0.12a	0.021 ± 0.005a
Anthracene + Pyrene	4.9 ± 2.6a	0.02 ± 0.00a	0.002 ± 0.001a	12.0 ± 4.7a	0.24 ± 0.14a	0.025 ± 0.017a
Day 75						
Control	21.7 ± 2.7a*	0.48 ± 0.12a*	0.03 ± 0.01a*	24.0 ± 12.4a*	1.01 ± 0.46a*	0.08 ± 0.04a*
Anthracene + Pyrene	21.1 ± 9.1a*	0.30 ± 0.11b*	0.02 ± 0.00a*	20.2 ± 4.7a	1.29 ± 0.41a*	0.11 ± 0.04a*

Different lower case letters in the same column denote significant difference ($P < 0.05$) within the same plant. *Denotes significant difference ($P < 0.05$) between values for different days in the same soil.

Table 4. Leaf morphology of butterfly pea and yam bean grown in anthracene + pyrene-contaminated and non-contaminated control soil for 30 and 75 days. Data are given as mean ± SD.

Plant	Butterfly bean		Yam bean	
	Leaf length (cm)	Leaf width (cm)	Leaf length (cm)	Leaf width (cm)
Day 30				
Control	2.0 ± 0.3a	1.3 ± 0.3a	5.9 ± 1.4a	5.1 ± 1.5a
Anthracene + Pyrene	2.3 ± 0.3a	1.4 ± 0.4a	4.6 ± 0.8a	4.0 ± 1.1a
Day 75				
Control	2.3 ± 0.28a	1.8 ± 0.37a*	6.1 ± 0.53a	5.4 ± 0.62a
Anthracene + Pyrene	2.4 ± 0.28a	1.6 ± 0.19a	7.3 ± 0.92a*	6.6 ± 1.22a*

Different lower case letters in the same column denote significant difference ($P < 0.05$) within the same plant.
*Denotes significant difference ($P < 0.05$) between values for different days in the same soil.

3.4 Removal of anthracene and pyrene by butterfly pea and yam bean from soil

The ability of butterfly pea and yam bean to remove both anthracene and pyrene from contaminated soil was assessed, and the results are shown in Table 5. In unplanted control soil, the amounts of anthracene and pyrene remaining on day 30 in soil were 67.6 and 68.2 mg/kg, respectively. Butterfly pea could enhance PAH removal compared to unplanted control; this was observed on day 30 when only 20.1 and 27.1 mg/kg of anthracene and pyrene remained in planted soil. Soil planted with yam bean did not exhibit improved anthracene and pyrene removal on day 30 compared to unplanted control. The amounts of anthracene and pyrene remaining in soil planted with yam bean were not significantly different from those remaining in unplanted control soil on day 30. By day 75, both butterfly pea and yam bean could remove anthracene and pyrene completely from contaminated soil. In contrast, the amounts of anthracene and pyrene remaining in unplanted soil on day 75 were 17.8 and 18.2 mg/kg, respectively.

Anthracene and pyrene were not found in any of the plant tissues collected from either plant (data not shown). The detection limit was 2 mg/kg. This suggests that the PAHs were removed from the soil by competent indigenous microorganisms.

Table 5. Percentages of anthracene and pyrene remaining in soil planted with butterfly pea and yam bean for 30 and 75 days. Data are given as mean ± SD.

Plant	Amount of PAH (mg/kg)	
	Anthracene	Pyrene
Day 0	86.3 ± 37.0a	74.1 ± 17.9a
Day 30		
No plant	67.6 ± 19.9ab	68.2 ± 9.7a
Butterfly pea	20.1 ± 8.9c	27.1 ± 23.1b
Yam bean	48.4 ± 22.8bc	43.9 ± 24.8ab
Day 75		
No plant	17.8± 8.0c	18.2 ± 3.8b
Butterfly pea	B.D.	B.D.
Yam bean	B.D.	B.D.

Different lower case letters show significant differences ($P < 0.05$) between treatments. Abbreviations: B.D. = below detection limit (2 mg/kg of each PAH).

Butterfly pea and yam bean are commonly grown in tropical countries (Mukherjee *et al*., 2008; Ramos-de-la-Peña *et al*., 2013). The results of this study suggest that both plants have the potential to be used in PAH phytoremediation. Butterfly pea and yam bean seemed to tolerate the presence of 86.3 mg/kg of anthracene and 74.1 mg/kg of pyrene in soil, as shown by a lack of overt signs of toxicity except for decreased nodule formation. While significant amounts of anthracene and pyrene were removed from both planted and unplanted soil, the rate and extent of PAH removal from planted soil were greater than from unplanted control soil. In unplanted soil, anthracene and pyrene were degraded by competent indigenous soil microorganisms. In planted soil, the rhizosphere not only provided a hospitable environment for competent microorganisms, but also enhanced their ability to degrade the PAHs. The lack of accumulation of any detectable PAHs in plant tissues during the course of this study provides further evidence of this phytostimulation mechanism mediated by butterfly pea and yam bean.

Butterfly pea and yam bean belong to the Leguminosae family and several plants in this family have been shown to be capable of stimulating PAH removal from soil. For example, white clover (*Trifolium repens*) could remove 77 % pyrene from spiked soil on day 60 (initial concentration of pyrene ranged within 4.22-365.38 mg/kg); this was 31 % higher than removal from unplanted spiked soil by indigenous microbes (Xu *et al*., 2009). Several plants in the Leguminosae family have been tested for PAH phytoremediation in Thailand. For example, winged bean was reported to remove fluorene and anthracene in rhizospheric soil. The percentages of fluorene and anthracene remaining in soil after 30 days were 7.8 % and 24.2 %, respectively (initial concentrations were 138.9 mg/kg and 95.9 mg/kg, respectively). In comparison, the percentages of fluorene and anthracene remaining in unplanted soil were 60.8 % and 76.1 %, respectively (Somtrakoon *et al*., 2014b). Long bean was also found to decrease fluoranthene in bulk soil to 10.2 % of the initial 150 mg/kg concentration after 30 days, whereas the amount of fluoranthene remaining in unplanted soil was 98.4 % (Somtrakoon *et al*., 2015). In another study, mungbean was reported to enhance phenanthrene and anthracene degradation in soil. The amounts of phenanthrene and anthracene remaining in mungbean planted soil were only 2.8 and 6.6% after 30 days of experiment (initial concentration of each PAH was 100 mg/kg) (Chouychai *et al.*, 2016). The current study adds to the growing body of literature on the use of plants in the Leguminosae family, in particular butterfly pea and yam bean that are easily grown in Thailand, for PAH phytoremediation. More studies are warranted to assess their performance in the field.

4. Conclusions

Butterfly pea and yam bean, two plants in the Leguminosae family commonly found in Thailand, were found to stimulate the removal of anthracene and pyrene from spiked soil. Thus, they appear to possess potential for PAH phytoremediation. Given that PAHs were not accumulated in the plant tissues, we reasoned that phytostimulation is the most likely mechanism by which these plants enhanced the degradation anthracene and pyrene in contaminated soil.

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References

Álvarez-Bernal, D., Contreras-Ramos, S., Marsch, R., & Dendooven, L. (2007). Influence of catclaw *Minosa monancistra* on the dissipation of soil PAHs. *International Journal of Phytoremediation, 9*, 79-90.

Bansal, V., & Kim, K. H. (2015). Review of PAH contamination in food products andtheir health hazards. *Environment International, 84*, 26-38.

Boonyatumanond, R., Wattayakorn, G., Togo, A., & Takada, H. (2006). Distribution and origins of polycyclic aromatic hydrocarbons (PAHs) in riverine, estuarine, and marine sediments in Thailand. *Marine Pollution Bulletin, 52*, 942-956.

Castellanos, J. Z., Zapata, F., Badillo, V., Peña-Cabriales, J. J., Jensen, E. S., & Heredia-Garcia, E. (1997). Symbiotic nitrogen fixation and yield of *Pachyrhzzus erosus* (L) urban cultivars and *Pachyrhzzus ahzpa* (WEDD) parodilandraces as affected by flowing pruning. *Soil Biology and Biochemistry, 29*, 973-981.

Cheema, S. A., Khan, M. I., Tang, X., Zhang, C., Shen, C., Malik, Z., . . . Chen, Y. (2009). Enhancement of phenanthrene and pyrene degradation in rhizosphere of tall fescue (*Festuca arundinacea*). *Journal of Hazardous Materials, 166*, 1226-1231.

Chiapusio, G., Pujol, S., Toussaint, M. L., Badot, P. M., & Binet, P. (2007). Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (*Lolium perenne* L. and *Trifolium pretense* L.) in three spiked soils. *Plant and Soil, 294*, 103-112.

Chouychai, W., Paemsom, T., Pobsuwan, C., Somtrakoon, K., & Lee, H. (2016). Effect of indole-3-acetic acid-producing bacteria on phytoremediation of soil contaminated with phenanthrene and anthracene by mungbean. *EnvironmentAsia, 9*, 128-133.

Gao, S., Sun, C., & Zhang, A. (2007). *Pollution of polycyclic aromatic hydrocarbons in China In Persistent Organic Pollutant in Asia: Sources, Distributions, Transport and Fate.* New York, NY: Elsevier Publications.

Gao, Y., Li, Q., Ling, W., & Zhu, X. (2011). Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene. *Journal of Hazardous Materials, 185*, 703-709.

Huang, X. D., El-Alawi, Y., Penrose, D. M., Glick, B. R., & Greenberg, B. M. (2004). Responses of three grass species to creosote during phytoremediation. *Environmental Pollution, 130*, 453-463.

Lee, S. H., Lee, W. S., Lee, C. H., & Kim, J. G. (2008). Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. *Journal of Hazardous Materials, 153*, 892-898.

Meng, L., Qiao, M., & Arp, H. P. H. (2011). Phytoremediation efficiency of a PAH-contaminated industrial soil using ryegrass, white clover, and celery as mono- and mixed cultures. *Journal of Soils and Sediments, 11*, 482-490.

Mukherjee, P. K., Kumar, V., Kumar, N. S., & Heinrich, M. (2008). The Ayurvedic medicine *Clitoria ternatea* - From traditional use to scientific assessment. *Journal of Ethnopharmacol, 120*, 291-301.

Pongpiachan, S., Tipmanee, D., Deelaman, W., Muprasit, J., Feldens, P., &Schwarzer, K. (2013). Risk assessment of the presence of polycyclic aromatic hydrocarbons (PAHs) in coastal areas of Thailand affected by the 2004 tsunami. *Marine Pollution Bulletin, 76*, 370-378.

Ramos-de-la-Peña, A. M., Renard, C. M. G. C., Wicker, L., & Contreras-Esquivel, J. C. (2013). Advances and perspectives of *Pachyrhizus* spp. in food science and biotechnology. *Trends in Food Science and Technology, 29*, 44-54.

Somtrakoon, K., Chouychai, W., & Lee, H. (2014a). Phytoremediation of anthracene and fluoranthene co-contaminated soils by *Luffa acutangula*. *Maejo International Journal of Science and Technology, 8*, 221-231.

Somtrakoon, K., Chouychai, W., & Lee, H. (2014b). Comparing anthracene and fluorene degradation in anthracene and fluorene-contaminated soil by single and mixed plant cultivation. *International Journal of Phytoremediation, 16*, 415-428.

Somtrakoon, K., Chouychai, W., & Lee, H. (2015). Removal of anthracene and fluoranthene by waxy corn, long bean and okra in lead-contaminated soil. *Bulletin of Environmental Contamination and Toxicology, 95*, 407-413.

Sun, T. R., Cang, L., Wang, Q. Y., Zhou, D. M., Cheng, J. M., & Xu, H. (2010). Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. *Journal of Hazardous Materials, 176*, 919-925.

Sun, R., Belcher, R. W., Liang, J., Wang, L., Thater, B., Crowley, D. E., & Wei, G. (2015). Effects of cowpea (*Vigna unguiculata*) root mucilage on microbial community response and capacity for phenanthrene remediation. *Journal of Environmental Sciences, 33*, 45-59.

Wang, C., Wu, S., Zhou, S., SHI, Y., & Song, J. (2017). Characteristics and source identification of polycyclic aromatic hydrocarbons (PAHs) in urban soils: A review. *Pedosphere, 27*(1), 17-26.

Wilcke, W., Muller, S., Kanchanakool, N., Niamskul, C., & Zech, W. (1999). Polycyclic aromatic hydrocarbons in hydromorphic soils of the tropical metropolis Bangkok. *Geoderma, 91*, 297-309.

Xu, S. Y., Chen, Y. X., Wu, W. X., Wang, K. X., Lin, Q., & Liang, X. Q. (2006). Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plant cultivation. *Science of the Total Environment, 363*, 206-215.

Xu, S. Y., Chen, Y. X., Lin, K. F., Chen, X. C., Lin, Q., Li, F., & Wang, Z. W. (2009). Removal of pyrene from contaminated soils by white clover. *Pedosphere, 19*, 265-272.